



**ASSESSMENT OF VITAMIN D LEVELS IN SUDANESE PATIENTS WITH SICKLE CELL DISEASE AND ITS IMPACT ON SICKLE CELLS COMPLICATIONS-KHATOUM-2017**

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**ABSTRACT**

Sickle cell disease SCD is a common reason urging patients of African descent to seek emergency medical care.<sup>[1]</sup> This study was done in Khartoum state in a period between March 2015 until July 2015. This study was done to assess vitamin D levels in Sudanese patients with sickle cell disease and study its impact on sickle cells complications. This study was included 200, 100 patients with SCD as case group and 100 apparently healthy subjects as a control group with matched age and gender. The results were as follow: The mean of RBC, WBCs, and platelets among case were  $(3.41 \pm 0.96, 10.28 \pm 2.66 \times 10^3/\mu\text{l}, 420 \pm 113.22 \times 10^3/\mu\text{l})$  in comparison to control  $(4.41 \pm 0.96, 5.72 \pm 1.44 \times 10^3/\mu\text{l}, 222 \pm 56.00 \times 10^3/\mu\text{l})$ , respectively). Hemoglobin (Hgb), PCV, MCV, MCH, MCHC in cases were  $(8.9 \pm 1.49 \text{ g/dl}, 27.12 \pm 4.60\%, 89.75 \pm 6.64\text{fl}, 25.56 \pm 2.47\text{pg}, 29.58 \pm 1.00 \text{ g/dl})$  in comparison to control  $(14.11 \pm 3.32\text{g/dl}, 40.45 \pm 3.45\%, 82.36 \pm 5.82\text{fl}, 28.20 \pm 2.42\text{pg}, 32.79 \pm 1.21 \text{ g/dl})$  respectively. The serum levels of vitamin D in cases were significantly lower than in controls  $(14.6 \pm 3.6 \text{ vs. } 27.4 \pm 4.2 \text{ ng/ml})$ , respectively). SCD patients have lower values of Hemoglobin concentration, packed cell volume, red cell indices, but higher values of white cell count and platelets compared to controls. Vitamin D deficiency and insufficiency are predominant in SCD.

**KEYWORDS:** Vitamin D, sickle cells complications SCD, Sudan.

**INTRODUCTION**

Sickle cell disease (SCD) is an autosomal recessive genetic disease that results from the substitution of valine for glutamic acid at position 6 of the  $\beta$ -globin gene, leading to the production of a defective form of hemoglobin, hemoglobin S (Hb S).<sup>[2]</sup> Scanning electron micrograph showing a mixture of red blood cells, some with round normal morphology, some with mild sickling showing elongation and bending. The loss of red blood cell elasticity is central to the pathophysiology of sickle-cell disease. In sickle-cell disease, low-oxygen tension promotes red blood cell sickling and repeated episodes of sickling damage the cell membrane and decrease the cell's elasticity. These cells fail to return to normal shape when normal oxygen tension is restored.<sup>[3]</sup>

**1. MATERIAL AND METHODS**

**Study area and population**

This study was conducted in Khartoum state during a period between March 2015 until July 2015. We excluded patients on vitamin D supplementation drug affecting vitamin D levels and patients admitted with terminal illness due to other causes. The questionnaire

was used to collect data regarding name, age, gender, residence, presence of skeletal complications, history of autoimmune disease, presence of systemic disease and medication.

**Sample collection**

This study was included 200 peoples, 100 patients with SCD as case group and 100 apparently healthy subjects as a control group. From patients were taken 5ml of venous blood from both cases and controls, 3 ml of sample were added into EDTA anti-coagulated. Gentle mixing of the anti-coagulated specimens was achieved to avoid hemolysis, clotting or platelet aggregation. The rest of sample was drawn into plain container allowed to clot and then centrifuged to get sera. Sera were preserved in  $2-6^{\circ}\text{C}$  and tested for vitamin D within 14 days after collections.

**Statistical analysis**

All data included in questionnaire was coded and listed in the table sheet and after that computerized SPSS (Statistical package for social science program Version

20, P value of < 0.05 was considered statistically significant).

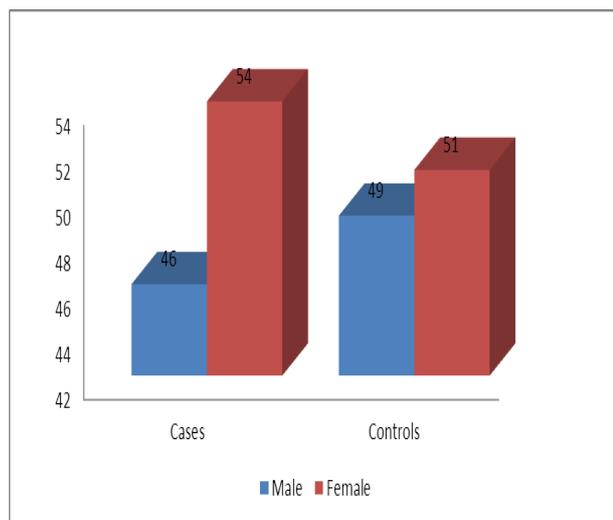
**2. RESULTS**

Two hundred blood samples were included in this study, 100 from patients with SCD as case group and 100 apparently healthy subjects as a control group.

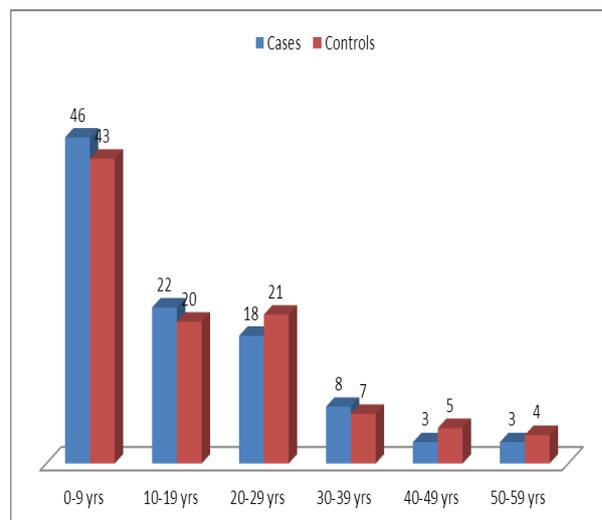
The case group was composed of 46 (46%) males and 54(54%) females and the control group was composed of 49 (49%) males and 51(51%)females. The mean age of patients was  $18.62 \pm 6.93$  (years) and the mean age of the control group was  $21.74 \pm 7.47$  (years) [table and Fig (1)].

**Table 1: The distribution of gender/ age (case/control).**

Parameters	Cases		Controls	
	100		100	
	Males	Females	Males	Females
Frequency	46(46%)	54(54%)	49(49%)	51(51%)
Mean age $\pm$ SD	$18.62 \pm 6.93$		$21.74 \pm 7.47$	



**Figure 1: Frequency distribution of male and female among the case and control groups.**



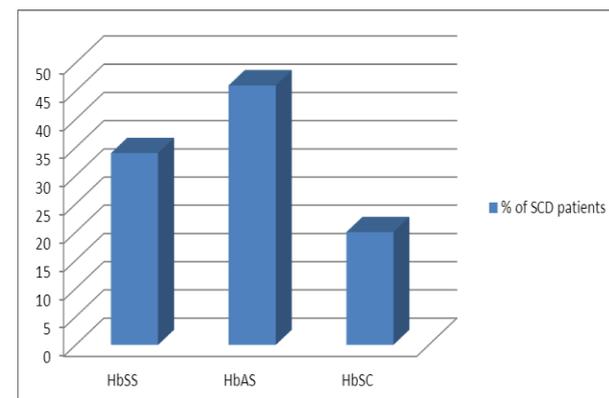
**Figure 2: Frequency of cases and controls according to age groups.**

The distribution of case and control according to age were categorized into 6 groups as follow: 0-9,10-19, 20-29, 30-39, 40-49, 50-59 yrs [table and figure(2)]

The distribution of SCD patients was divided into three groups according to Hb differentiation HbSS 34(34%), 46(46%) HbAS and 20(20%) HbSC [ Fig 3].

**Table 2: The distribution of cases and controls according to age.**

S. No	Age Grp.	Cases	Controls
1	0-9 yrs	46	43
2	10-19 yrs	22	20
3	20-29 yrs	18	21
4	30-39 yrs	8	7
5	40-49 yrs	3	5
6	50-59 yrs	3	4



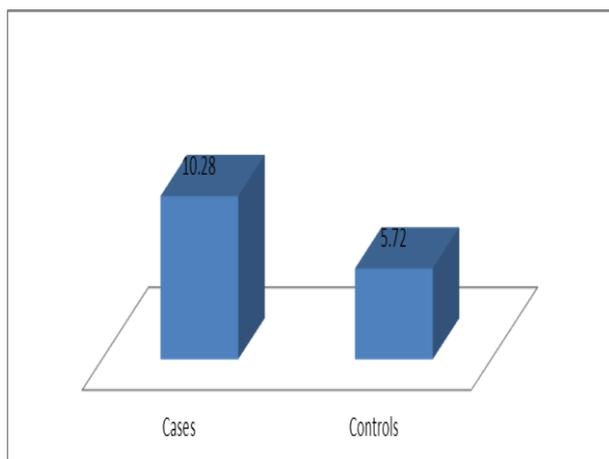
**Figure 3: The distribution of patient according to type of Hb differentiation.**

The RBC count, Hb, PCV, MCH, MCHC in SCD patients were significant slightly lower than in control group with p.value (0.008, 0.003, 0.005, 0.005, 0.009, respectively) and significant within normal MCV with p.value (0.008)[table 3].

**Table 3: Mean values of full blood count parameters.**

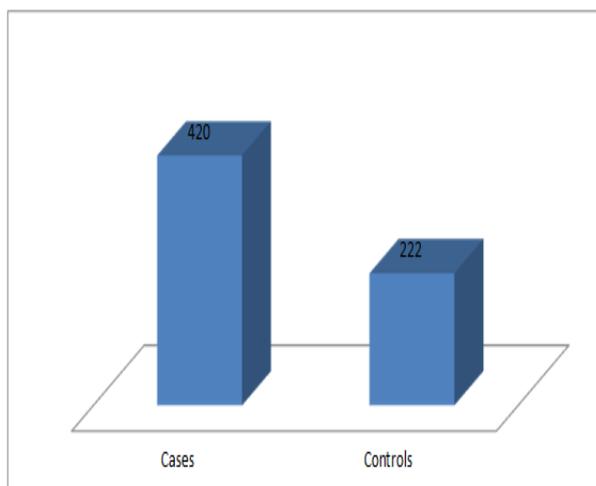
Parameters	Cases	Controls	P value
	No (100)	No (100)	
RBC (mill/mm <sup>3</sup> )	3.41±0.96	4.41±0.96	0.008
Hgb g/dl	8.9 ± 1.49	14.11 ± 3.32	0.003
PCV%	27.12 ± 4.60	40.45 ± 3.45	0.005
MCV fl	89.75 ± 6.64	82.36 ± 5.82	0.008
MCH pg	25.56 ± 2.47	28.20 ± 2.42	0.005
MCHCg/dl	29.58 ± 1.00	32.79 ± 1.21	0.009

The average WBC count among cases ( $10.28 \pm 2.66 \times 10^3/\mu\text{l}$ ) was significantly raised than among controls ( $5.72 \pm 1.44 \times 10^3/\mu\text{l}$ ) [figure 4].



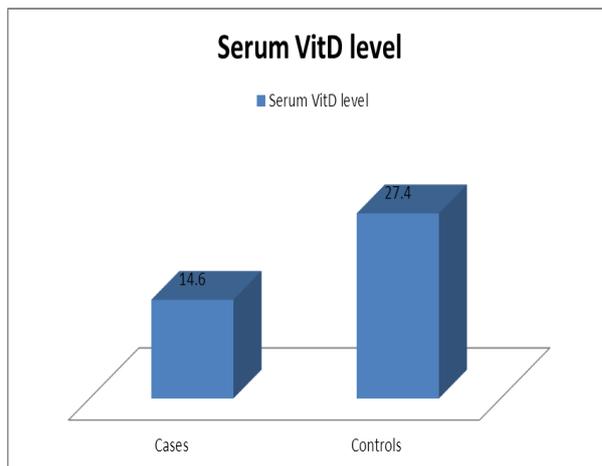
**Figure 4: The average WBC count  $\times 10^3/\mu\text{l}$  among cases and controls.**

There was a significant elevation in platelet count in cases ( $420 \pm 113.22 \times 10^3/\mu\text{l}$ ) compared to controls ( $222 \pm 56.00 \times 10^3/\mu\text{l}$ ) [figure 5].



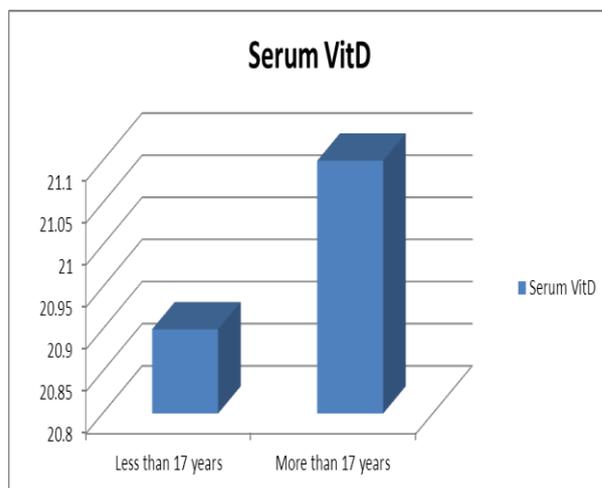
**Figure 5: The average platelet count  $\times 10^3/\mu\text{l}$  among cases and control.**

The serum levels of vitamin D in cases were significantly lower than in controls ( $14.6 \pm 3.6$  vs.  $27.4 \pm 4.2$  ng/ml respectively) [figure 6].



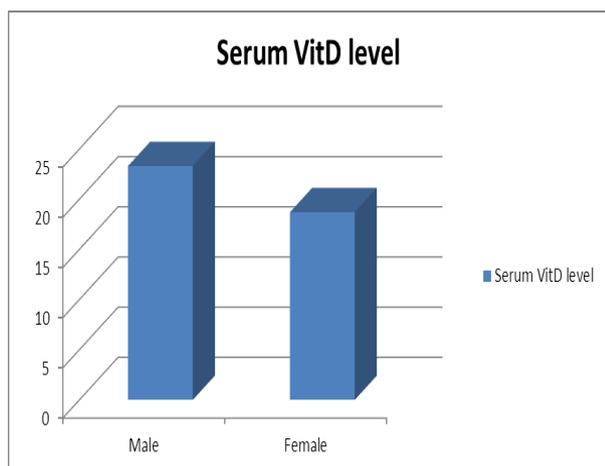
**Figure 6: The mean serum VitD levels in case and control groups.**

The average serum vitD levels in cases aged above and those below 17 were  $20.9 \pm 3.6$  ng/ml and  $21.1 \pm 5.2$  ng/ml respectively, this difference was statistically insignificant [figure 7]



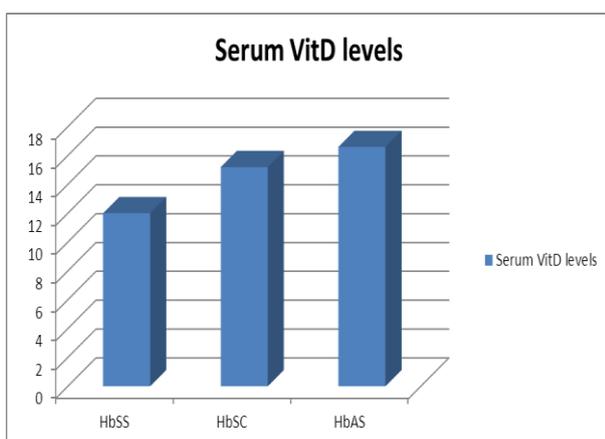
**Figure 7: The averages serum VitD levels in subjects below and above 17 year.**

The difference in serum vitD levels in males and females was statistically insignificant. The mean values of serum vitD in male and female were  $23.3 \pm 5.5$  ng/ml and  $18.7 \pm 3.3$  ng/ml respectively [figure 8].



**Figure 8: Serum vitD levels in males and females.**

Analysis of variance (ANOVA) showed that the levels of serum vitD were more deficient in HbSS (12.0ng/ml) patients followed by those with HbSC (15.2ng/ml) and HbAS (16.6ng/ml) ( $P=0.001$ ) [figure 9].



**Figure 9: The mean values of serum VitD according to hemoglobin differentiation.**

## DISCUSSIONS

The study evaluated the prevalence of vitamin D deficiency in 100 patients with SCD and 100 controls. Hematological profiles of all studied subjects were reported. SCD causes a chronic form of anemia which can lead to fatigue. The sickle red blood cells are prone to breakage (membrane rupture) which causes a much shorter lifespan of these cells. Anemia and splenomegaly were common findings in our cases. Some of them were splenectomized. Hematological indices revealed that individuals with SCD have low Hb, RBC count, PCV, MCH, MCHC and high MCV values compared with normal individuals. Total hemoglobin is significantly low in SCD patients as compared to controls. It may be due to chronic hemolytic process, blood loss due to hematuria.<sup>[4]</sup> Higher MCV values were found in patients compared to control, although this difference is statistically insignificant. While the red cell indices were generally lower in sickle cell patients than controls, the white blood cells and platelet counts were higher than control values.

Our finding showed that SCD was significantly associated with raised WBC count as compared to controls. White blood cells are now well known to be involved in the pathophysiology of SCD; similar findings are noted by Belcher *et al.*<sup>[5]</sup> Other authors have also shown the importance of leukocytosis to clinical outcomes of early SCD related death, clinically overt stroke and acute chest syndrome.<sup>[6]</sup>

These results were expected considering the degree of chronic hemolysis, higher risk of infections and chronic pain in sickle cell patients. Similarly lower values were obtained by Omoti in Benin City, Nigeria.<sup>[7]</sup> In our results elevated platelet count was associated with SCD; this is in agreement with previous reports.<sup>[8]</sup> Our cross-sectional analysis indicated that vitD deficiency is related to SCD. This is consistent with literatures.<sup>[9][10][11]</sup> Children with SCD-SS were considered to be at greater risk for vitamin D deficiency than healthy children.<sup>[12]</sup> The previous study found that 96.4 % of the patients had values below 20 ng/mL.<sup>[10]</sup>, which by the Institute of Medicine has been considered a level that confers increased risk for osteomalacia in the general population of adolescents and young adults.<sup>[13]</sup> Low bone mineral density (BMD), a feature found in sickle cell disease is associated with SCD.<sup>[14]</sup> Vitamin-D levels were found to be lower in older patients in a previous report.<sup>[10]</sup> No association between Vit D and age exist in our study. Investigators in Atlanta reported complete resolution of chronic pain in a 16-year-old female with SCD who had severe osteoporosis with vitamin D supplementation.<sup>[15]</sup> VitD levels were associated with the hemoglobin variants (HbAS, HSS, and HbSC). This is disagreement with previous reports.<sup>[16]</sup> In our study, no difference found between levels of serum vitD in men and women. The prevalence of vitamin D deficiency was being as high as 32% among women and 46% among men.<sup>[17]</sup>

## 3. CONCLUSION

Sickle cell disease (SCD) patients have lower values of Haemoglobin concentration, packed cell volume, red cell indices, but higher values of white cell count and platelets compared to controls. Vitamin D deficiency and insufficiency are predominant in SCD.

## 4. ACKNOWLEDGEMENTS

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