

## Study of Biochemical Parameter in Children with Sickle Cell Anemia from Western Maharashtra (India)

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### Abstract

**Introduction:** Sickle-Cell Anemia (SCA) is a genetically transmitted hemoglobinopathy, around 60 million carrier and 1,20,000 sickle cells suffer added every year in the world. In India the SCA predominantly seen in tribal region. Nutrition is a major health concern. Therefore, present study was carried out to measure serum Total protein, Albumin, Globulin, A/G Ratio, Vitamin B<sub>12</sub>, Vitamin D<sub>3</sub>, Calcium in SCA and healthy children.

**Aim & Objectives:** To evaluate and compare biochemical parameter in SCA and healthy children's.

**Material & Methods:** In this comparative analytical study from total 230 subjects, 130 were SCA and 100 control were recruited after obtaining written inform consent. All subject visiting OPD between the age group of 1-12 yrs. were selected. We used whole EDTA and plain blood sample for analysis. Serum was used to measured Total Protein by Biuret method, Vitamin B<sub>12</sub> by Electrochemiluminescence immunoassay method, Vitamin D<sub>3</sub> by Enzyme immunoassay competition method, Calcium by Arsenazo III method.

**Results:** It was observed that there was no significant difference in the serum Total protein levels ( $p > 0.05$ ). The significant decreased levels of albumin and increased levels of globulin, A/G ratio is decreased in SCA than control. The mean of Vitamin B<sub>12</sub> and Vitamin D<sub>3</sub> was significantly lower in SCA when compared with control.

**Conclusion:** In the present studynormal levels of Total protein, hypoalbuminemia and hyperglobulinemia found in SCA. Low levels of vitamin D<sub>3</sub> and hypocalcaemia, hypocalcaemia was observed in SCA. In toto by measuring biochemical parameter, poor nutritional status was found in SCA.

**Keywords:** Sickle Cell Anemia, Total protein, Vitamin B<sub>12</sub>, Vitamin D<sub>3</sub>, Calcium, Nutrition

## 1. Introduction

Sickle Cell Anemia (SCA) is a homozygous disorder of hemoglobin synthesis in which individual has inherited two mutant globin genes (HbSS) one from each parent. This mutation occurs at 6<sup>th</sup> position, where glutamic acid is replaced by valine on chromosome number 11 [1]. It is estimated that about 4% of the world population carry an abnormal haemoglobin gene, with SCA being the most common form of haemoglobinopathy[2]. The prevalence of sickle cell disease (SCD) varies from 0-45% from different tribes of Maharashtra region [3].

In these genetically transmitted diseases, mutation occurs at DNA levels which leads to formation of abnormal hemoglobin called sickle hemoglobin (HbS). In deoxygenated phase, RBC leads to polymerization, crystallization that causes sickle shape RBC. The sickle RBC unable to transfer oxygen properly. HbS in decreased oxygen state tend to block capillary by occluding micro vasculature. There is a disturbance in the supply of hemoglobin to various parts of the body. Blockade in blood capillaries are main reason for severe pain in the SCA / homozygous Sickle Cell Disease. Pain is the most important sign of SCA. Vaso-occlusive crises, increased erythropoiesis, hemolysis, anemia, and further associated health complications are seen in SCA [4].

Nutrition plays a crucial role in growth and development of children [5]. Some important biochemical parameter or nutrients like Protein, Albumin, Globulin, Vitamin B<sub>12</sub>, Vitamin D<sub>3</sub>, Calcium help to detect nutritional status of SCA/ homozygous SCD[6].

Protein is the linear chain of amino acid which are link together by peptide bond. Total protein is the addition of albumin and globulin. It has hormone, enzymatic function, helps in maintenance of immune system, acid-base balance and fluid-electrolyte balance, give structure to body, important nutrient for growth and development, help in transportation of substance in the body [7]. Baron *et al.*, stated that the measurement of total protein in plasma may be used to assess degree of hydration of patient [8]. According to Edozien, variation in protein levels has been attributed to factors such as diet, immunity, genetic and disease of the liver. Patient with SCA is prone to infection probably due to reduced immune status [9].

Vitamin B<sub>12</sub> is also called as cobalamin. It is a water-soluble vitamin that has a vital role in the normal functioning of the brain and nervous system, RBC production. It is involved in the various metabolic process and help in the synthesis of DNA, amino acid and fatty acid metabolism. Reduced dietary intake, decreased intestinal absorption, folic acid deficiency are the main reason for low levels of vitamin B<sub>12</sub> in SCA [10]. By exposure of sunlight, Vitamin D synthesized in the skin, play an important role in calcium homeostasis, helps in normal bone formation and mineralization. Also helps in maintain normal physiological concentrations of calcium in blood [11].

It is remarkable to note that SCA is characterised with varied biochemical abnormalities. Nutrition is major concern in SCA, many studies estimated the biochemical parameter of SCT or SCD but very few references of research studies carried out with same limitation on SCA and their nutritional health problems. Severity of Nutritional health problem and severity of SCA have been markedly observed in tribal western Maharashtra region.

Therefore, the present work was undertaken to evaluate and compare biochemical parameter in SCA / homozygous SCD and control from western Maharashtra. Biochemical parameter is crucial for the management and monitoring the progressed of SCA and also helps in treatment strategy.

## 2. Aims and objectives

**Aim:** To evaluate, compare biochemical parameter in SCA children of western Maharashtra.

**Objective:**i) To estimate and compare the levels of serum Total proteins, Albumin, Globulin and A/G ratio in SCA and control.

ii) To measure and compare the levels of serum Vitamin B<sub>12</sub>, Vitamin D<sub>3</sub> and Calcium in SCA and control

## Material and Method

A comparative analytical study was carried out in Department of Biochemistry of D Y Patil School of Medicine and Research centre, Navi Mumbai in association with Department of Biochemistry, Vedanta Institute of Medical Sciences, Dahanu. Total 230 children between the age group of 1 to 12 years were enrolled prior obtaining written informed consent. SCA (130) and healthy control (100) was enrolled in the study.

## Sample Collection

All the subjects were educated about current study and given a patient information sheet. After taking informed consent from patient's parents or guardian with antiseptic precaution 3 mL venous blood sample was collected from subject and volume divided into EDTA and plain vacutainer. By the centrifugation from plain tube serum sample was separated. All samples were first screened for sickling test by Dithionate qualitative solubility test by NESTROF method and confirmed by HPLC method. Sample further used for biochemical analysis.

## Ethical Approval

The study protocol was approved by the Institutional Ethical Committee for Biomedical Health Research from D Y Patil School of Medicine, Nerul, Navi Mumbai. Institutional Ethics committee letter No.:DYP/IECBH/2021/271.

## Statistical Methods

A Case record form (Questionnaire) was formed and data were entered in MS excel-2019, results were analysed using the statistical tool-SPSS version 2023, Graph pad to obtain mean  $\pm$  standard deviation and *p* Value for significant results. Student t-test was employed to assess the significant differences between two groups. The differences were considered to be statistically significant with *p*-values < 0.05.

## Observations and Results

In the present study total 230 subject comprising 130 SCA (89 male and 41 female) and 100

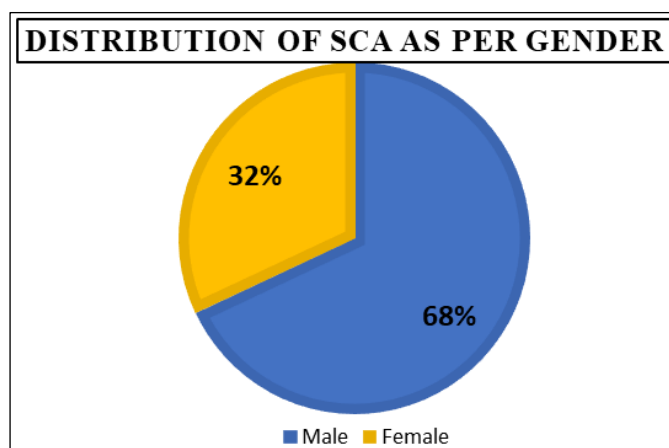
control (69 male and 31 female) were assessed. Biochemical parameter such as Total proteins, Albumin, Globulin and A/G ratio, serum Vitamin B<sub>12</sub>, Vitamin D<sub>3</sub>, and Calcium were evaluated and compared in SCA and control. The Mean and Standard deviation values of biochemical parameter were obtained in this research study.

**TABLE1: DISTRIBUTION OF SCA AND CONTROL GROUP ACCORDING TO GENDER**

Factor	SCA(n = 130)	Control(n = 100)	Total(n = 230)	Percentage
No. of Cases	130	100	230	100%
Male	89	69	158	68 %
Female	41	31	72	32 %

Table No. 1 – Showed the distribution of gender in SCA group, 68% males and 32% females.

That indicate SCA is more prone in male than females.



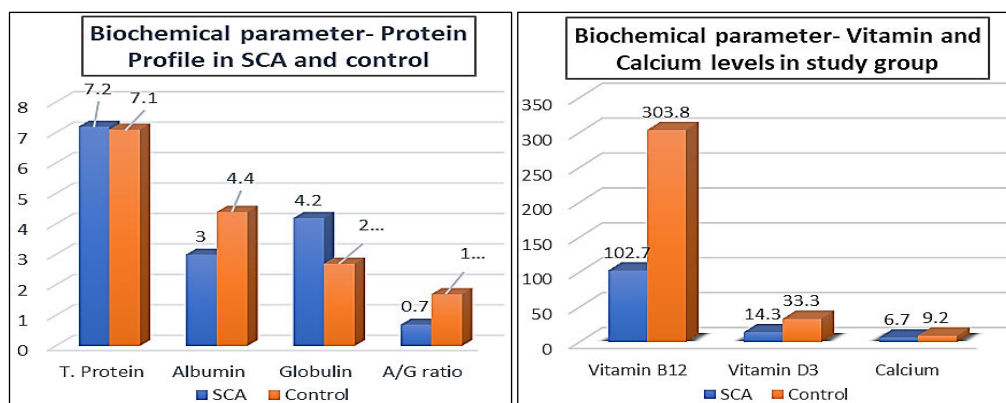
**FIG1: DISTRIBUTION OF SCA AS PER GENDER**

**TABLE2: BIOCHEMICAL PARAMETER IN SCA AND CONTROL**

Parameter	SCA (n = 130) Mean ± SD	Control (n = 100) Mean ± SD	p Value
Total Protein (gm/dL)	7.2 ± 0.4	7.1 ± 0.3	$p > 0.05$
Albumin (gm/dL)	3 ± 0.4	4.4 ± 0.4	$p < 0.001$
Globulin (gm/dL)	4.2 ± 0.6	2.7 ± 0.5	$p < 0.001$
A/G Ratio	0.8 ± 0.2	1.7 ± 0.4	$p < 0.001$
Vitamin B <sub>12</sub> (pg/mL)	102.7 ± 26.1	303.8 ± 62.4	$p < 0.001$
Vitamin D <sub>3</sub> (ng/mL)	14.3 ± 3.1	33.3 ± 2.2	$p < 0.001$
Calcium (mg/dL)	6.7 ± 0.9	9.2 ± 0.7	$p < 0.001$

$p < 0.05$  = significant;  $p > 0.05$  = non-significant.

Table no. 2 represent significant decreased levels of albumin, A/G ratio, vitamin B<sub>12</sub>, vitamin D<sub>3</sub> and calcium ( $p < 0.001$ ) in SCA than control. High levels of globulin ( $p < 0.001$ ) found in SCA. There is no statistically significant difference, as normal levels of total Protein ( $p > 0.05$ ) in observed SCA than control.



**FIG2: BIOCHEMICAL PARAMETER-TOTAL PROTEIN, VITAMINS AND CALCIUM IN SCA AND CONTROL**

### 3. Discussion

SCA / homozygous SCD is recognised as genetic disorder of abnormal hemoglobin synthesis [12]. In general, very few SCA patient attend sickle cell clinic mainly in tribal region. It mostly happens due to low socioeconomic status, backwardness, cultural belief, psychosocial stigma, lack of awareness about health issues [13].

Protein, Vitamin B<sub>12</sub>, Vitamin D<sub>3</sub>, calcium are important nutrients of body. For normal functioning and for the maintenance of homeostasis of the body required these nutrients. Deficiency of these nutrient cause's alteration in several metabolic reactions, disturb nutritional status thereby causing abnormalities such as nutritional disorders. The current study observed hyperglobulinemia-high levels of globulin ( $p < 0.001$ ), hypoalbuminemia-low levels of albumin ( $p < 0.001$ ) and the levels of serum total protein ( $p < 0.05$ ) were slightly normal. Our study results are in accordance with study done by Isichei UP *et al.*, [14]. But these results are in contrast with Tripathi *et al.*, studies they stated low protein, albumin in SCD patient [15]. The hypoalbuminemia in SCA may be due increased consumption of amino acid, decreased synthesis of protein from liver or low intake of protein from diet. Due to the range of antigenic stimulation coming from the environment the gamma ( $\gamma$ ) globulin fraction rises especially in chronic infection [13].

Vitamin B<sub>12</sub> is the utmost prevalent nutritional deficiencies found in SCA. Deficiency of vitamin B<sub>12</sub> causes tingling sensation, fatigue, weakness. Hypocobalaminemia observed in SCA children as lower levels of vitamin B<sub>12</sub> found in SCA than control ( $p < 0.001$ ). Similar study was carried out by Ahmed *et al.* showed decreased levels of vitamin B<sub>12</sub> in SCA patient [16]. Lower levels of cobalamin may be due to poor dietary consumption of nutrients or higher demand of nutrient like vitamin B<sub>12</sub> may leads to deficiency of vitamin B<sub>12</sub> in SCA; however, others aspect in deficiency of vitamin B<sub>12</sub>, such as diminished absorption of vitamin B<sub>12</sub> from the ileum due to the crisis in SCA [10]. Hypocalcaemia in addition with hypovitaminosis D found in SCA that may be due to the elevated calcium influx into the intracellular space as an effect of decreased in blood pH thereby causing VOC chiefly in deoxygenated state. Particularly during VOC, it was found that vitamin D levels were lower than control. Vitamin D is helps in the regulation of calcium in the blood. Poor vitamin D diet leads to vitamin D deficiency as those subsequent reasons of hypocalcaemia [17].

Overall observation in current study showed that there is derangement in biochemical parameter in SCA/homozygous SCD. In toto elevated levels of globulin and significant decreased levels of albumin, vitamin B<sub>12</sub>, Vitamin D<sub>3</sub>, calcium and normal levels of T. Protein found in SCA than control group. Diminished levels of albumin (protein), vitamin B<sub>12</sub>, Vitamin D<sub>3</sub>, calcium leads to nutritional deficiency in SCA children. As these are the most essential nutrient required for various metabolic process and normal functioning of body

#### 4. Conclusion

In conclusion the finding of the study highlights protein, vitamin and calcium are nutritional screening tool which help to identify individuals are at risk of nutritional health disorder (Malnutrition) in the context of SCA. There is a need of proper nutritional therapy and antioxidant support to improve nutritional status of SCA in tribal region of Western Maharashtra.

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