Section A-Research paper

The percentage levels of haemoglobin variants in Sickle Cell Anemia children from Western Maharashtra

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Abstract

Introduction: Sickle-cell anemia (SCA) is the first monogenic genetic disorder in the world, affecting 20 – 25 million people with SCA worldwide. Present work was undertaken to measure the levels of Hb, HbF, HbA_0, HbA_2 and HbS in sickle cell anemia and healthy children.

Aim & Objectives: Study was undertaken to determine the percentage levels of Hb Varients in children's with SCA and Healthy Children's.

Material & Methods: In this case control study a total 200 subjects were recruited after obtaining written inform consent. All subject visiting OPD between the age group of 1-12 yrs. were randomly selected and grouped as healthy (control group) (n = 100), HbSS positive (n = 100) (Study group). Healthy subjects were age and sex matched with study HbSS patients. We used whole EDTA blood, the levels of HbF, HbA_0, HbA_2 and HbS were determined by ion-exchange High Performance Liquid Chromatography (HPLC) method.

Results: SCA patients had significantly increased levels of HbS ($p<0.001$), HbF ($p<0.001$), HbA_2 compared to control. It was observed that the mean levels of HbA_0 were significantly less than control Group.

Conclusion: High percentage of HbS, HbF HbA_2 and the low percentage of HbA_0 found in sickle cell Anemic children's of Maharashtra, thus in toto due to high level of naturally compensated HbF and HbA_2 leads to benefit the longevity of life of HbSS Children.

Keywords: Sickle Cell Anemia, HPLC, HbF, HbS, HbA_0, HbA_2,

Introduction

SCA is characterized by an abnormal hemoglobin structure consequent on replacement of adenine with thymine on the β-globin gene resulting in valine replacing glutamic acid on the β chain of the hemoglobin structure. [1] The World Health Organization recognized SCA as a global public health problem in 2006. [2] Homozygous SS (sickle cell anaemia) is generally considered the most severe form of SCD. The clinical complications of SCD include chronic haemolytic anaemia, painful episodes of Vaso occlusion, permanent risk of infections, acute

chest syndrome and cumulative damage of multiple organs [3,4]. As per WHO report (7), 60 million carriers of sickle cell and 1,20,000 sickle cells homozygotes are added every year in the world.) SCD is one of the most common inherited life-threatening disorders in human, it predominantly affects people of African, Indian and Arab ancestry (8,9,10). In India, sickle cell anemia is more prevalent in tribal population. The first description of sickle haemoglobin in India was by Lehman and Cutbush in 1952 in the tribal populations in the Nilgiri hills in south India. (11) In the same year, Dunlop and Mazumder also reported the presence of sickle haemoglobin in the tea garden workers of Upper Assam who were migrant labourers from tribal groups in Bihar and Odisha (12)

The functional properties of hemoglobin are determined by their characteristics folds of amino acid chains of the globin proteins including seven stretches of the polypeptide α helix in the α chains and eight β helix in the β chains.[5] The reversible binding of O2 , CO, and NO to the four ferrous iron atoms of the heme is responsible for transportation of these gases by hemoglobin.[6] Normal adult hemoglobin structure of α2 β2 is known as hemoglobin A, it accounts for 97% of total hemoglobin. Other hemoglobin types present in adult are hemoglobin A2 which is α2 δ2. It accounts for 2% and hemoglobin F α2 γ2 which accounts for 1% of adult hemoglobin.[13] Hb F is the best-suited hemoglobin in-utero because it has a slightly higher oxygen affinity than hemoglobin A and a much higher oxygen affinity than hemoglobin S being able to bind 2,3 bi-phosphoglycerate less strongly. Combinations of α gene with β,δ and γ genes give rise to the formation of 97% normal adult hemoglobin A (α2 β2), 2% hemoglobin A2 (α2 δ2) and 1% of hemoglobin F (α2 γ2) by the end of the first year of life.(14,15) HbA2 can inhibit the polymerization of sickle hemoglobin (HbS) and it has a pancellular distribution.[3-6] High HbA2 might therefore be of benefit in sickle cell anemia.[16) fetal hemoglobin (HbF) concentration are differentially distributed among populations,[17] and higher than normal HbF levels are characteristic of sickle cell anemia.[18] Stress erythropoiesis might also be partly responsible for increased HbF levels. A reciprocal relationship between HbA2 and HbF levels is present in acquired disorders where HbF levels are increased [19] People with SCD have an increased risk of developing certain infections including pneumonia, blood stream infections, meningitis, and bone infections. Early in life, sickled cells can clog blood vessels in the spleen, leading to damage and increased susceptibility to infection. (20) HbS polymerizes under low oxygen conditions (e.g., stress, hypoxia, or acidosis), resulting in deformed and fragile RBCs that have a characteristic sickle (half-moon) shape and a reduced lifespan (from 120 days to 10–20 days)

We have measured the levels of HbF, HbA_0, HbA_2 and HbS in children. Higher expression of HbF in adulthood ameliorates morbidity and mortality in SCD, [21,22] as increased level of HbF has been observed to have beneficial effect in sickle cell anemia due to the inhibition of polymerization of HbS which results in erythrocyte sickling. [23,24] Hemoglobin A2 forms less than 3% of total hemoglobin in normal adults [25] and it has a pan cellular distribution, and ability to inhibit the polymerization of sickle hemoglobin (HbS) [26,27]. Therefore, the present work was undertaken to evaluate Hb electrophoretic profile in sickle cell Anemic and healthy children from western Maharashtra region.

Aims and Objectives

Study was undertaken to evaluate, compare Hb electrophoretic profile among children with Sickle cell Anemia and Healthy children.

Material and Method

Present case control study was carried out under Department of Biochemistry of D Y Patil School of Medicine and Research centre, Navi Mumbai in collaboration with department of paediatrics, department of medicine of Vedanta Institute of Medical Sciences, Dahanu.

Study comprised 200 children from 01 to 12-year age group. The children were divided into two groups: In group I known cases diagnosed with sickle cell anemic and Group-II: healthy looking children as control group were enrolled in the study after obtaining written informed consent.

Sample Collection

After obtaining informed written consent from patient's parents or relative, 1.8 ml of venous blood was collected in EDTA vacutainer by taking proper antiseptic precaution. The samples were mixed gently by inversion to ensure proper mixing of EDTA anticoagulant with blood. Whole blood samples were stored at -80^0C untill processed on HPLC [D10- BioRad].

All samples were first screened for sickling test by Dithionate qualitative solubility test [NESTROF method] and confirmed by HPLC method. The levels of HbF, HbS, $HbA_{0,}$ HbA2 were measured by HPLC ion exchange chromatography method using D10- BioRad

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 (No. IEC/ DYP/IECBH/2021/271)

Statistical Methods

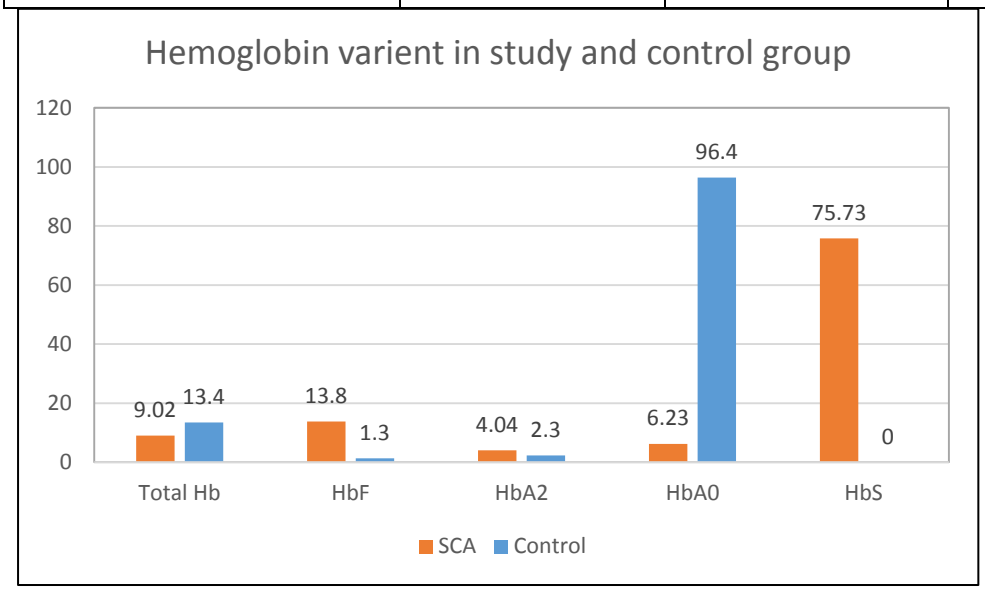
Results were analysed using the statistical package for the social sciences (SPSS) version 20, Graph pad and the descriptive data were given as mean ± standard deviation. Student unpaired t- test was employed to assess the significance of the differences between two groups. The differences were considered to be statistically significant with p-values < 0.05.

Results

Evaluation and Comparison of Hb electrophoretic profile which include HbF, HbS, $HbA_{0,}$ HbA2 in healthy control and study groups. We have demonstrated statistical differences between control and SCA. patients had significantly increased levels of HbF, HbS and HbA_2 ($p < 0.001$), compared to control. We determine very low level of Hb in this type anemia. It was observed that the mean value of HbA_0 ($p < 0.001$) of homozygous Sickle cell patients were significantly less than Healthy subjects.

Table Showing hemoglobin variants of SCA and healthy control group.

Parameter	SCA Mean ± SD (n = 100)	Control (Healthy) Mean ± SD (n = 100)	p Value
Total Hemoglobin (Hb) Gm / dL	9.02 ± 1.80	13.41 ± 0.64	P < 0.001
HbF	13.88 ± 6.56	1.31 ± 0.51	P < 0.001
HbA_2	4.04 ± 1.78	2.29 ± 1.10	P < 0.001
HbA_0	6.23 ± 6.22	96.39 ± 1.05	P < 0.001
HbS	75.73 ± 8.30	0 ± 0	P < 0.001



Graphical presentation of Hemoglobin variant in Study and control group.

A total 200 subjects were included in this study in which 100 were suffering from homozygous sickle cell anemia and 100 were Healthy subjects. As seen in Table No.1 Hemoglobin (Hb) levels in sickle cell anemia are 9.02 Gm/dL which was lesser when compared to control found 13.41 Gm/dL in control. HbF, HbA_2, HbS, levels of SCA were significantly higher than control group ($p < 0.001$) as well as HbA0 levels in study group are significantly lesser than control group. The mean levels of HbA_0 in SCA is 6.23 % found lesser values when compared to control 96.39 % and also HbA_2, HbS, HbF levels in SCA are 4.04% ,75.73 %, 13.88% respectively found increased levels than control 2.29%,0% and 1.31% respectively.

Discussion-

SCA is genetic disorder to be characterized at the molecular level, it's a contributor to mortality and morbidity across the worldwide and it is of high prevalence in Middle-east, Mediterranean region, Southeast Asia, and Sub-Sahara Africa. Sickle cell diseases consist of a group of disorders characterized by the presence of sickle hemoglobin. (28)

It results from the substitution of glutamic acid by valine at position 6 of the beta-chain of haemoglobin. The clinical manifestations of SCD arise from the tendency of sickle haemoglobin to polymerize at reduced oxygen tensions and deform red cells into the characteristic rigid sickle cell shape. Such inflexible red cells cannot pass through the microcirculation efficiently, and this results in anaemia (due to destruction of the red cells) and intermittent vaso-occlusion causing tissue damage and pain (29) This watershed arising from sickled haemoglobin is inhibited in patients with elevated HbF level

Although all patients with SCA have exactly the same molecular defect, there is considerable clinical variation, ranging from death in early childhood (30), to a normal life span with few complications (31) due to the influence of genetic modifiers of SCA like co-existence of á – thalassemia (32). Therefore, patients with increased levels of HbF often tend to have a relatively mild clinical course (33) because HbF reduces the tendency of HbS to polymerize within the red cell. This highlights the need to determine HbF along with HbA2 in SCA.

Fetal hemoglobin (HbF, $\alpha 2\gamma 2$) can inhibit the deoxygenation induced polymerization of sickle hemoglobin (HbS, a2bS 2) that drives the pathophysiology of sickle cell disease. (33) The switch from HbF to HbS in sickle cell anemia (homozygosity for the HbS gene) is delayed, and stable levels of HbF are not reached until age 5 to 10 years. (34) In this study, HPLC results obtained from normal healthy control group revealed that means of HbA, HbA2, and HbF were consistent with other studies. (35) and we found 100 homozygous sickle cell Anemic patient by detection of abnormal hemoglobin (HbS)

In the present case control study Electrophoretic profile of homozygous sickle cell cases (HbSS) compared with normal controls with hemoglobin phenotypes AA. Total hemoglobin is low in all SCA patients, possibly on account of chronic hemolysis. In comparison to other studies in this region, the average value is less than that reported in Gadhchiroli region, (36) By 10 to 12 weeks of age mild haemolytic anemia is apparent in SCA. (37,38) Here, in the first five years of life clinically moderate anemia was seen in both genders. Lower level of hemoglobin seen in age upto 12 years could be due to additional nutritional requirement in childhood and recurrent infections along with pre-existing mild haemolysis

Our study reflected that in sickle cell anemia patients, there is a significantly higher level of HbA2 (4.04 ± 1.78). HbS was only found in patient group, Was significantly higher in HbSS group than control group. The mean value of HbS (75.73 ± 8.30) and significantly lower level of HbA0 (6.23 ± 6.22) as compared to control group.. This is in accordance with studies done by Shirley L et al and Eman A et al. (39,40) Earlier studies obtained a mean foetal haemoglobin level (HbF) of 6.4±4.0% (41) and 7.4±3.6% (42) but the mean HbF (13.88 ± 6.56) of in this study is slightly higher than both earlier values. The difference in HbF levels were statistically significant in our study. HbA2 does not differentiate between patients and controls; however, changes in levels of HbA, HbS, and HbF are important for diagnosis and treatment strategies. HPLC is a rapid, sensitive and reliable test for determining the presence of Hb patterns phenotype within a sample.

Conclusion

This case series shows high percentage of HbF along with HbS in patients with sickle cell disease and this is very common among the rural triable are of western Maharashtra region

population. The HbF level is higher in SS subjects compared with subjects with other hemoglobin variants. This increased HbF level is a compensatory mechanism for sickling in SS subjects, due to high level of naturally compensated HbF and HbA_2 leads to benefit the longevity of life of HbSS Children. As determined by the study, there's a considerable chance that the patients presenting with moderate to severe anemia can have some underlying genetic/hereditary disorder instead of nutritional causes of anemia. Therefore, steps are needed to be taken for proper diagnosis and management of patients with anemia especially in rural areas in order to reduce burden of the disease as well as cost of treatment and general outcome of the patient.

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