ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4

Journal of Coastal Life Medicine

Influence of Haemoglobin Variants in Sickle Cell Anaemic Children Between 1 to 12 Year Ages from Western Maharashtra

Received: 15 February 2023, **Revised:** 22 March 2023, **Accepted:** 24 April 2023

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Keywords
Sickle cell Anaemia, HPLC, Chromatography, Haemoglobin, HbF, Maharashtra.

Abstract
Introduction: Sickle cell anaemia is a molecular disease. WHO recognise it as a global public health problem. In India it is common among tribal community. HbA0, HbS, HbF and HbA2 are common Hb variant found in blood. Therapeutic research is focused on maintaining high level of HbF. Naturally compensated Hb variants may have influence in SCA. Aim: To assess the role of naturally available variants in blood of SCA patients. Result: We observed low level of Total Hb, and elevated levels of HbF, HbA2 along with decrease level of HbA0. Higher than normal level of HbF in SCA patient have lowered osmotic fragility and less frequency of clinical crises. Conclusion: Low HbA0 is compensated by other haemoglobin variants and particularly high level of naturally compensated HbF and HbA2 in sickle cell anaemic patients have beneficial influence.

1. Introduction

Sickle cell anaemia [SCA] is characterized by an abnormal haemoglobin structure due to replacement of adenine by thymine on the β-globin gene. As an effect valine on the 6th position of the β globin chain is replaced by glutamic acid giving abnormal haemoglobin Hb-Sickle structure.[1] In 1983 report, the World Health Organization [WHO] states that 60 million new cases of sickle cell carriers and 120,000 sickle cells homozygous are added to the population every year.[2] In 2006, the WHO recognized SCA as a global public health problem.[3] SCA is one of the common inherited life-threatening disorders in human, it predominantly affects people of African, Indian and Arab ancestry.[4-6] The first description of sickle haemoglobin among tribal populations of the Nilgiri hills in south India was reported by Lehman and Cutbush in 1952.[7] In the same year, Dunlop and Mazumder also reported cases of sickle haemoglobin among the tea garden workers of Upper Assam who were then recognised as migrant labourers from tribal groups of Bihar and Odisha.[8] Further studies found that, there are few pockets in India where sickle cell anaemia is more prevalent. Tribal population of Maharashtra, Gujarat and Madhya Pradesh show higher prevalence.

Homozygous sickle cell anaemia [HbSS] is generally considered the most severe form of Sickle Cell Disease [SCD]. People with SCD are at higher risk of certain infectious diseases including pneumonia, blood stream infections, meningitis, and bone infections. Sickled RBC can clog blood vessels in the spleen, leading to damage and increased susceptibility to infection at early age.[9] The clinical complications of SCD include chronic haemolytic anaemia, painful episodes of Vaso-occlusion, high risk of infections, acute chest syndrome and cumulative damage of multiple organs.[10,11]

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The functional property of haemoglobin is determined by characteristics folds of their peptide in the globin chain i.e. Seven stretches of α-helix in the α globin chains and eight stretches of β-helix in the β globin chains.[12] The reversible binding of O_2, CO and NO to the four ferrous iron atoms of the heme is responsible for transportation of these gases by haemoglobin.[13] Combinations of α gene with β, δ and γ genes give rise to haemoglobins variants in adult. Haemoglobin structure having $\alpha_2\beta_2$ is known as haemoglobin A_0, it makes 95-97% of total haemoglobin. Haemoglobin A_2 consists of $\alpha_2\delta_2$ accounts for ≤2% and ≤1% of adult haemoglobin formed by $\alpha_2\gamma_2$ is haemoglobin F.[14]

Among different haemoglobin variants HbS has lowest affinity for Oxygen as compared to HbA_0 and HbF.[15] Sickle haemoglobin [HbS] has tendency to polymerise under hypoxia and stress; whereas higher level of HbA_2 can inhibit the polymerization of HbS [10-13] which might therefore be of benefit in sickle cell anaemia.[16,17] Also HbF has anti-polymerization effect on HbS and prevents erythrocyte sickling.[18]

Though higher than normal HbF levels are associated with milder form of sickle cell anaemia [19] SCD in India is not uniformly mild despite high fetal hemoglobin levels. In addition, HbS itself has resistance against malaria.[20] Though there is no cure for this disorder but much of the therapeutic research is focussed on maintaining higher level of HbF and HbA_2.[21,22]

Therefore, in the present study levels of Haemoglobin variants in 1 to 12 years age group children from tribal belt of western Maharashtra having sickle cell anaemia was analysed. The aim of study is to assess beneficial effect of naturally present HbA_2 and HbF in sickle cell anaemia by comparing their levels with osmotic fragility of erythrocytes and frequency of clinical crises observed among SCA patients and normal healthy children of same age group by HPLC method.

Objectives

To assess the levels of different Haemoglobin variants naturally present in 1 to 12 years normal healthy children and children having sickle cell disease. Correlate osmotic fragility of erythrocyte and frequency of clinical crises among SCA patients at different levels of HbA_2 and HbF. To assess the beneficial effect of naturally present HbA_2 and HbF in Sickle cell patients.

2. Materials and Method

Present case control study was carried out in Department of Biochemistry of D Y Patil Medical College, Navi Mumbai with collaboration of Department of Paediatrics, Department of Medicine of DYPMC and Vedanta Institute of Medical Sciences, Dahanu.

Selection of Cases:

Study comprised 200 children of both gender fitting in 1 to 12 year age group. Participants were randomly selected from Outpatient Department of Medicine and Paediatric clinics of Vedanta Institute of Medical Sciences, Dahanu. They were divided in to two groups. Group-I comprised known cases of sickle cell disease and Group-II included healthy looking children; free of any haemoglobinopathies.

Exclusion criterion: Children below 1 year and above 13 years of age, children having haemoglobinopathies other than Sickle cell and Children having history of any metabolic disorder were excluded from study. Participants whose parent did not agree to give consent were also excluded.

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 sample was mixed gently by inversion to ensure proper mixing of EDTA anticoagulant with blood. Whole blood samples were stored at -80^0C until processed on D-10 HPLC machine [supplied by Bio-Rad Company] for assessment of haemoglobin variants.

All samples were first screened for sickling by Dithionate qualitative solubility test NESTROF method. The levels of HbA_0, HbF, HbS and HbA_2 were measured on D-10 High Performance Liquid Chromatography (HPLC) machine supplied by Bio-Rad Company.

Total haemoglobin level was evaluated by cyanmethemoglobin method. Osmotic fragility test was performed with 4.5gm/L NaCl solution.[23] Data on

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
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frequency of clinical crises was recorded during history taking.

The results obtained from D-10 and osmotic fragility test were analysed using the statistical package for the social sciences (SPSS) version 20 and presented as Mean ± SD. Student unpaired t- test was employed to assess the significance of the differences between two groups. The differences with p-values < 0.05 were considered statistically significant.

Ethical approval:

The study protocol was approved by the Institutional Ethical Committee for Biomedical Health Research from D Y Patil Medical College, Navi Mumbai (No. IEC/ DYP/IECBH/2021/271

3. Results:

Table 1: Haemoglobin Variants in Sickle cell disease and Control group among tribal children in 01 to 12 years of age from north western Maharashtra.

Parameters	SCD Mean ± SD (n = 100)	Healthy Control Mean ± SD (n = 100)	p Value
Total Haemoglobin gm / dl	9.2 ± 1.8	13.4 ± 0.6	P < 0.001
HbA_0 %	6.1 ± 5.9	96.3 ± 1.05	P < 0.001
HbF %	14.5± 6.7	1.2 ± 0.52	P < 0.001
HbS %	75.2 ± 8.2	0 ± 0	P < 0.001
HbA_2%	3.9 ± 1.7	2.3 ± 1.1	P < 0.001
Osmotic fragility of erythrocyte	0.30 ± 0.08	0.42 ± 0.02	P < 0.001

A total 200 subjects were included in present study among which 100 subjects were confirmed cases of homozygous sickle cell anaemia and 100 control subjects. We measured the levels of Total Hb, HbA_0, HbA_2, HbF and HbS for study population. Osmotic fragility of red cell and frequency of clinical crises was recorded to assess beneficial effect of naturally present haemoglobin Variants in SC anaemic patients.

As shown in **Table No.1** Mean total haemoglobin (THb) levels in sickle cell anaemia is 9.2 gm/dl which is lower as compared to healthy control subjects showing 13.4 gm/dl.

HbA_2, HbS, HbF levels in SCA are 3.9 %, 75.2 % and 14.5 % respectively; these variants of haemoglobin in control group are 2.3 %, 0% and 1.2%. Naturally present Hb variants HbF, HbS and HbA_2 levels in SCA group were significantly higher than control group ($p < 0.001$) whereas mean HbA_0 levels in SCA group [6.1%] is significantly reduced [$p<0.001$] compared to control group [96.3%]. Mean Osmotic fragility test value for erythrocyte in SCA is 0.30 ± 0.08 which is lower than 0.42 ± 0.02 value obtained for control group.

Table:2 Comparison of Hb variant level and clinical condition of SCA patient

Hb Variant	SCA group	Clinical condition	Control group	P value

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
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		Steady state	Clinical crises		
HbF	14.5 + 6.7	10.9+5.48	4.38+2.98	1.2+0.52	<0.001
HbA_2	3.9+1.7	4.02+1.61	2.94+1.22	2.3 +1.1	<0.03

Table No: 2 shows clinical crises in SCA patients related with level of HbF and HbA_2. Our observation is that frequencies of clinical crises were low when naturally present HbF and HbA_2 levels were high among SCA patients.

4. Discussion:

Sickle cell disease refers to all disease genotype including sickle cell anaemia and compound heterozygous disorders such as HbSC, HbSβthal and other less common variants.[24] Sickle cell anaemia is a significant and under recognised global health problem responsible for high mortality of children below 5 years of age.[25]

Sickle cell disease is molecular defect. 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 resulting in early destruction and intermittent vaso-occlusion causing tissue damage and pain crises.[26] This watershed arising from sickled haemoglobin is inhibited by elevated HbF in erythrocyte. Although all patients with SCA have exactly the same molecular defect, there is considerable clinical variation ranging from death in early childhood [27] to a normal life span with few complications.[24] The variation in severity of disease is due to the influence of genetic modifiers of SCA like co-existence of β –thalassemia [29] and increased levels of naturally available HbF and HbA_2. Fetal haemoglobin (HbF, $\alpha_2\gamma_2$) can inhibit the polymerization and reduce events of clinical complications. [30] The shifting of HbF to HbS in sickle cell anaemia (homozygosity for the HbS gene) is delayed, and stable levels of HbF are not reached until 5 to 10 years age which may be the cause of high mortality under 5 year age.[31] Our results of HbA_0, HbA_2, HbS and HbF among healthy control group were consistent with other studies.[32] In the present Study levels of naturally available haemoglobin variants among homozygous (HbSS) sickle cell cases were compared with haemoglobin of phenotypes AA. Total haemoglobin level among SCA patients is low; the plausible cause may be chronic haemolysis due to stressed erythrocytes. Our results of THb are lower as compared to a study performed in Gadhchiroli, the same area of north western Maharashtra.[33] We detected clinically moderate anaemia in first five years among both genders. Along with haemolysis, additional contributing factor for low Hb may be lack of good nutrition, recurrent infection in tribal children of this region.

We also observed significantly high level of naturally present HbA_2 (3.9 ± 1.7), HbS (75.2 ± 8.2) in sickle cell anaemia patients and significantly lower level of HbA_0 (6.1 ± 5.9) as compared to control group. Our findings are in accordance with studies done by Shirley L et al and Eman A et al.[34,35] Shu DD and Head CE also reported high levels of HbS in SCA patients along with significantly increased levels of HbA_2.[36,37] Some earlier studies reported 6.4±4.0% and 7.4±3.6% HbF concentration in SCA[38,39] whereas our results of HbF are higher (14.5 ± 6.7) than both earlier reports. Platt OS reports higher expression of anti-sickling HbF in adulthood ameliorates morbidity and mortality in SCD and increases life expectancy.[40]

Significant lowering in HbA_0 level in homozygous SCA is compensated by natural production of other haemoglobin variants HbF, HbA_2 and HbS which may provide benefiting effect to sickle cell anaemic patient. Our thought is supported by present therapeutic research which aims to keep elevated level of HbF and HbA2 for minimising the clinical crises. [41,42]

We correlated the beneficial effect by analysis of osmotic fragility and clinical crises history data obtained for SCA group. Normally erythrocytes show 5 to 45% haemolysis with 4.5gm/L NaCl solution. In present study we observed SCA erythrocytes have higher percentage of haemolysis when HbF is < 2%. But, when HbF% levels are on higher side percentage

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of haemolysis was lower. Our result is in congruence with Coleman E (2007) reports. [43] similarly clinical conditions of patients were free of crisis when HbF and HbA2 levels were on higher side of normal. Overall observation in our study is elevated level of naturally synthesized HbF and HbA_2 among the SCA patients of north western Maharashtra region. This may be providing protection against crisis and they remain asymptomatic with average life expectancy. Our result is similar to Caroline EO study.[44]

5. Conclusion

Our study observed low level of total haemoglobin among sickle cell anaemia patient. Low HbA_0 is compensated by other haemoglobin variants and particularly high level of naturally compensated HbF and HbA_2 in sickle cell anaemic patients have beneficial influence.

Funding Support: The author did not receive any funding, grant or financial support from organisation.

Conflict of interest: None of the authors or person connected with this study raised conflict of interest.

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