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Original Research Article

Elevated foetal haemoglobin in tribal sickle cell anaemic patients: Blessing in disgust?

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ABSTRACT

Background: Sickle cell anaemia is a molecular disease. WHO recognises it as a global public health problem. In India, it is common among tribal communities. Increased HbS is a culprit. Therapeutic research is focused on maintaining high levels of HbF and decreasing 2,3, BPG to target disease.
Aim: To assess the role of naturally compensated haemoglobin variants in tribal Sickle cell anaemic patients of North East Gujarat.
Settings and Design: Prospective, analytical, case control study conducted on randomly selected fifty tribal Sickle cell anaemic patients having disease for more than 5 years. Fifty age and sex matched, healthy control subjects.
Materials and Methods: Each fifty Tribal sickle cell anaemic patients and healthy control were included in the study. Total Haemoglobin level, Sickling test by NESTROFT method and Haemoglobin variants were analysed by alkaline haemoglobin electrophoresis. Frequencies of clinical crises were recorded by oral questioning. The results were analysed using SPSS version 20. Student unpaired t- test was employed to assess the significance of the differences. P-values < 0.05 considered statistically significant.
Results: We observed decreased levels of total haemoglobin, high levels of HbF and HbA2 along with reduced HbA0. Compensatory increase of HbF in tribal SCA patients have shown lower frequency of clinical crises.
Conclusion: Reduced HbA0 in sickle cell anaemia is compensated by naturally elevated HbF and HbA2 in tribal patients which has a beneficial influence on their general health.

1. Introduction

Sickle cell anaemia [SCA] is characterised by presence of an abnormal haemoglobin HbS. It is due to genetic replacement of valine from the 6^{th} position in the β globin chain by glutamic acid.[1] The World Health Organization accepted SCA as a global public health problem in 2006.[2] As per WHO report[3] there is unceasing addition of new cases of sickle cell carriers and sickle cell homozygous every year. Globally India accounts for 14.5% of the total new-borns with SCD.[4] SCD is the inherited disorder; largely found in people of African, Indian and Arab ancestry.[5–7] First Indian case of sickle cell was detected in the Nilgiri hills of south India by Lehman and Cutbush in 1952.[8] Later, Dunlop and Mazumder reported cases of sickle haemoglobin among the tribal tea garden workers of Upper Assam.[9] Further studies conducted in India found that prevalence of sickle cell anaemia is higher among tribal populations particularly from a few pockets of Maharashtra, Gujarat and Madhya Pradesh.[10]

Major function of Haemoglobin is transportation of gases. Haemoglobin is a conjugated protein having four

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globin chains. Combinations of α gene with β, δ and γ genes give rise to the formation of various types of haemoglobins. Association of $\alpha 2$ β2 forms haemoglobin A_0 [HbA_0] which makes 97% of total haemoglobin in adult, $\alpha 2$ $\delta 2$ forms Haemoglobin A_2 [HbA_2] accounts for 2% and haemoglobin F formed by $\alpha 2$ $\gamma 2$ is less than 1% in adult hemoglobin.[11] Normally HbF is 95 to 98% at birth which gradually decreases to less than 1% at the end of one year of age.[12] Similarly HbS is absent unless individual has sickle cell disease.[13] Sickle cell anaemia homozygous HbSS is severe and express Sickle Cell Disease [SCD]. SCD patients are at risk of infectious diseases including pneumonia, blood stream infections, meningitis, and bone infections. Sickled cells can clog blood vessels leading to damage and increase susceptibility to cardiovascular diseases at an early age.[14] Chronic haemolytic anaemia, painful episodes of Vaso-occlusion, risk of infections, acute chest syndrome and damage of multiple organs are clinical complications.[15,16] Among haemoglobins, HbS has the lowest affinity for Oxygen as compared to HbA and HbF.[17] Sickle haemoglobin [HbS] has tendency to polymerise under hypoxia and stress whereas higher levels of HbA_2 and HbF can inhibit the polymerization of HbS.[18,19] Therefore higher concentrations are beneficial in sickle cell anaemic individuals. Also HbF prevents erythrocyte sickling by exclusion from polymer formed by HbS. Present therapeutic research is aiming to maintain high levels of HbF, reduce 2,3 BPG and increase oxygen to reduce ill effect.[20] Naturally elevated levels of HbF are reported in tribal communities having sickle cell anaemia.[21] Elevated levels of HbF (30%) in Hereditary persistence of HbF (HPFH) eliminates symptoms of sickle cell disease and haemolytic anaemia.[22] In contrast to the high polymerization tendency, HbS shows a protective role against malaria.

Therefore, the present study was performed on patients of sickle cell anaemia from tribal regions of North East Gujarat to assess the beneficial effect of HbA_2 and HbF levels on general health by comparing with normal healthy individuals of the same age group.

2. Aim

To assess beneficial effect of higher than normal level of HbF and HbA2 in tribal patients of SCA from North East Gujarat.

3. Objectives

To evaluate levels of different Haemoglobin fractions in normal healthy Individuals and tribal patients having sickle cell anaemia for more than 5 years. To correlate levels of HbA_2 and HbF in Sickle cell anaemia patients with their general health status.

4. Materials and Methods

Present prospective, analytical, case control study was carried out at Central Clinical Biochemistry lab of Zydus Medical College and Hospital, Dahod.

Participant registration: Random sampling method was adopted for selection of participants for the present study. The study population comprised 50 Sickle cell anaemia patients of both genders having sickle cell anaemia for more than 5 years. Participants having records of tribal community and presence of sickle cell anaemia were randomly selected from the Outpatient department. Fifty healthy subjects devoid of any type of hemoglobinopathies were randomly picked up as controls. Study population was divided into two groups. Group-I comprised known cases of sickle cell anaemia patients and Group-II was control subjects.

4.1. Exclusion criterion

Patients having history of less than 5 years of SCA, having hemoglobinopathies other than Sickle cell and patients having history of any other metabolic disorder were excluded from study. Participants not belonging to the tribal community of North East Gujarat were also excluded.

4.2. Sample collection

After obtaining informed written consent from patients, 1.8 ml of venous blood was collected in EDTA vacuette by taking proper antiseptic precaution. The blood was mixed with EDTA anticoagulant to avoid clotting.

Whole blood samples were first screened for sickling test by Dithionate qualitative solubility test [NESTROFT method][23] and total haemoglobin concentration by Cyanmethemoglobin method. Presence of any haemolysis was recorded.

This was followed by the assessment of HbA0, HbF, HbS and HbA2 levels by preparing haemolysate and subjecting it on cellulose acetate paper for alkaline haemoglobin electrophoresis.[24] Bands obtained were quantified using densitometry software from Helena Company.

Detailed clinical history about frequency of clinical crises (painful episodes), hospitalisation, blood transfusion, frequency of cold and cough, fever, feeling of exhaustion, any gastric trouble, Vaso-occlusion, acute chest symptoms was taken on record by yes /No answer to oral questionnaire to assess general health status.

The results obtained 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.

5. Results

A total of 100 subjects were registered for present study. Among which 50 subjects were from tribal communities and had sickling tests positive. They had this disease for more than 5 years. Another 50 subjects taken as a control group exhibited sickling test negative and had no symptoms of anaemia.

Data from 50 tribal sickle cell anaemia patients of both genders shows 16 males and 34 females with a mean age of 11.3 Years (range 6 to 37 years, male mean age 9.8years and female mean age 13.4years). Among 50 control subjects of both gender shows 21 males and 29 females with mean age of 15.9years (range 5 to 40 years, male mean age 16.2 years and female mean age 15.1 years).

Table 1: Haemoglobin fractions in sickle cell disease and control group

Parameters	Healthy Control Mean ± SD (n = 50)	SCA Mean ± SD (n = 50)	p Value
Total Haemoglobin gm / dl	13.51 ± 0.44	8.62 ± 1.28	P < 0.001
HbA0%	95.59 ± 1.05	7.28 ± 6.42	P < 0.001
HbF%	1.41 ± 0.51	12.80 ± 6.76	P < 0.001
HbS%	0 ± 0	72.70 ± 8.43	P < 0.001
HbA2%	2.69 ± 1.26	5.14 ± 1.68	P < 0.05

As shown in Table 1 Mean total haemoglobin (THb) levels in sickle cell anaemia is 8.62 ± 1.28 gm/dl which is lower as compared to 13.51 ± 0.44gm/dl in healthy control.

HbF, HbS and HbA_2 levels in the SCA group are significantly elevated ($p < 0.001$) when compared with the control group. Whereas mean HbA0 levels in the SCA group [7.28 ± 6.42%] are significantly reduced (P<0.001) compared to the control group [95.59 ± 1.05%].

Concentration of HbA2, HbS and HbF in SCA are 5.14 ± 1.68%, 72.70 ± 8.43% and 12.80 ± 6.76% respectively; these values of haemoglobin fractions are found elevated when compared with respective values of control group 2.69 ± 1.26%, 00 ± 00% and 1.41 ± 0.51%.

A total of 18 sickle cell anaemia tribal patients (12 male and 6 female) reported clinical concern whereas 32 sickle cell anaemia tribal patients either did not have any clinical alerts or it was very minor. Among 18 patients reported clinical concern 03 needed repeated hospitalisation, 08 patients had high frequency of cold and cough [reduced immunity], 03 patients expressed chest pains, 07 patients expressed occasional painful episodes, 02 patients had haemolytic anaemia and only one individual had vaso-occlusion findings. When this sick group of patient's HbF fraction was correlated it was found that their HbF concentration was less than 3% whereas those who did not show clinical complains have HbF levels more than 5%, Among 32 non complaining group, 42.6% patient's HbF levels were more than 9%.

6. Discussion

Sickle cell anaemia is a noteworthy but less recognised global health problem which takes away life of young ones.[25] Sickle cell disease is a molecular defect where sickle haemoglobin polymerizes at reduced oxygen tensions and deforms red cells into the characteristic rigid sickle shape. Such inflexible red cells cannot pass through the microcirculation resulting in early destruction and intermittent vaso-occlusion resulting in tissue damage and pain.[13] This crunch arising from sickled haemoglobin is inhibited in patients with elevated HbF level. Although all patients with SCA have the same molecular defect, there is considerable clinical variation seen among them ranging from deaths at early childhood[25] to a normal life span with few complications.[26,27]

Foetal haemoglobin (HbF, $\alpha_2\gamma_2$) can inhibit the deoxygenation induced polymerization of sickle haemoglobin (HbS, $\alpha_2\beta S_2$) which drives the pathophysiology of sickle cell disease.[28] Patients with increased levels of HbF often tend to have a relatively mild clinical course as HbF reduces the tendency of HbS to polymerize within the red cell. This highlights the need to maintain high HbF levels along with HbA2 in SCA.

The switch from HbF to HbS in sickle cell anaemia is delayed, and stable levels of HbF are not reached until age 5 to 10 years. Our results of HbA, HbA2, and HbF among healthy control groups were consistent with other studies.[29] In the present Study levels of haemoglobin variants among sickle cell anaemia cases are compared with haemoglobin of normal healthy individuals. Total haemoglobin level among SCA patients is low, plausible cause may be chronic haemolysis. Our Hb levels are lower than similar studies performed by Kohchale SR(2015) in western Maharashtra.[30] We observed clinically moderate anaemia in patients with SCA. Along with haemolysis, additional contributing factors may be poor nutrition, recurrent infection in tribal children.

Present study also observed a significantly elevated level of HbS along with HbA_2 and HbF in SCA patients and significantly decreased level of HbA0 as compared to the control group. Our results are in agreement with studies done by Ajjack EA(2014) and Shirley L (2009).[31,32] Earlier reports show 6% to 7.4% HbF concentrations among SCA our results of HbF are higher (12.80% ± 6.76%) than earlier reports. Tribal communities with SCA have expressed higher levels of HbF. Higher expression of HbF in adulthood amends morbidity and mortality in SCA. High concentration of HbF in HPFH nullifies the effect of sickle anaemia. Thus looking at the longevity of

life and less clinical crises among tribal sickle anaemia patients substantiates the ameliorating effect on general health status.

7. Conclusion

Decreased level of HbA0 compensated by increased concentration of HbF and HbA2 among SCA indicates beneficial effect on general health of tribal sickle cell anaemic patients of North East Gujarat. Though the sample size was small, a similar study with a large sample size is desired.

8. Source of Funding

None.

9. Conflict of Interest

None.

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