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Full Length Research Article

HbF STATUS OF TRIBAL INDIVIDUALS WITH SICKLE CELL ANEMIA IN MELGHAT REGION OF AMRAVATI DISTRICT, MAHARASHTRA, INDIA

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ABSTRACT

Sickle cell disease is a major genetic disorder among the tribal population. Fetal hemoglobin is a major contributor to the remarkable phenotypic heterogeneity of sickle cell disease and it helps reduce the disease severity. Its level varies dramatically in concentration in the blood of these patients. And the level of fetal hemoglobin is not yet studied among the tribal individuals of Melghat. Hence the objective of the present study was to determine the Fetal Haemoglobin (HbF) level in SCD patients (SS), carriers (AS) and normal individuals (AA) in the tribal people of Melghat Region of Amravati District, Maharashtra, India. In the population under study, it was found that the status of HbF is highest in SS followed by AS individuals. A slightly higher HbF level was observed in SS females than in their male counterparts. Among different age groups the highest HbF% was found in the age group of 11-20 years.

INTRODUCTION

Fetal hemoglobin (HbF) is the main hemoglobin component throughout fetal life and at birth, accounting for approximately 80% of total hemoglobin in newborns. After birth, HbF synthesis rapidly declines and HbF is gradually substituted by HbA in the peripheral blood, so that within the first two years of life, the characteristic hemoglobin phenotype of the adult with very low levels of HbF (less than 1%) is found (Weatherall and Clegg, 1981; Birgens and Ljung, 2007). In normal adults, HbF is heterogeneously distributed among erythrocytes though its synthesis is restricted to a small population of cells, termed F-cells. Approximately 3–7% of red blood cells are F-cells, containing 20–25% of HbF (Franco *et al*., 2006). The gradual replacement of HbF ($\alpha 2\gamma 2$) by adult haemoglobins A ($\alpha_2\beta_2$) and A2 ($\alpha_2\delta_2$) is essentially complete 150 days after birth (Schechter, 2008), although levels of 1-3% are observed during the first 3 years of life (Wilson *et al*., 1968). Functionally, HbF differs mostly from HbA because it has a slightly higher oxygen affinity, explained by the low interaction of HbF with 2,3-DPG. This characteristic makes the delivery of oxygen through placenta easer, giving fetus better access to oxygen from the mother's bloodstream (Schechter, 2008). Sickle cell disease (SCD) individuals produce haemoglobin SS (HbSS) due to a mutation in the β-globin gene cluster. This mutation results in the production of an abnormal version of the beta chain of haemoglobin (HbS), which has difficulty in carrying oxygen properly through the body. However, this disease has been associated with a great phenotypic heterogeneity and clinical variability (Sebastiani *et al*., 2008). This β-globin chain structure disorder comprises a heterogeneous group of conditions, in which HbF production persists through adult life in the absence of haematological abnormalities called hereditary persistence of fetal hemoglobin (HPFH) (Kan *et al*., 1975). Clinical severity and hematological characteristics of SCA are variable and are influenced by a number of factors including the co-inheritance of α-thalassemia, variation in fetal hemoglobin level and the haplotype background that is linked to the β-globin gene (Awaad *et al*., 1993 and Miller *et al*., 2003). Persistent production of variable levels of HbF into childhood and adult life is a characteristic finding in sickle cell anaemia and more

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Table 1. Showing the values of Mean±SE of different Hb variants in SS, AS and AA individuals

Parameters	Sickle cell patient (SS) n=32		Sickle cell gene carrier (AS) n=48		Normal (AA) n=20	
	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range
Hb A	1.74±0.76	0.3-4.2	61.09±0.72	52.8-68.0	97.3±0.11	96.6-97.8
Hb F	21.63±1.51	9.4-33.2	1.45±0.11	0.9-1.8	0.03±0.1	0.01-0.2
Hb S	75.155±1.41	65.1-87.8	35.81±0.76	27.1-44.7	0	0
Hb A_2	2.6±0.26	1.4-4.8	2.77±0.06	2.2-3.4	2.65±0.10	2.2-3.4

Table 2. Showing comparison of Mean±SE of different Hb variants of SCD males and females compared with that of normal males and females

Parameter	Sickle cell patient (SS)				Normal (AA)			
	Males n=15		Females n=17		Males n=10		Females n=10	
	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range
Hb A	0.6±0.6	0-4.2	1.14±0.66	0-4.1	97.08±0.19	96.6-97.5	97.45±0.10	96.9-97.8
Hb F	21.32±2.47	9.4-33.2	21.95±1.88	14.3-31.3	0	0	0.03±0.1	0.02-0.2
Hb S	75.52±2.42	65.8-87.8	74.79±1.60	67.3-83.7	0	0	0	0
Hb A_2	2.74±0.39	1.6-4.8	2.46±0.37	1.4-4.1	2.92±0.19	2.5-3.4	2.47±0.05	2.2-2.6

Table 3. Showing comparison of Mean±SE of different Hb variants of SCD patients belonging to different age groups. (n=08)

Parameter	<10 yrs		11-20 yrs		21-30 yrs		>31 yrs	
	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range
Hb A	0.04±0.04	0-3.3	1.18±0.76	0.4.2	0	0	0.51±04	0-3.3
Hb F	23.05±0.45	22.6-24.5	25.22±2.43	18.3-33.2	19.42±5.22	9.4-26.4	19.45±2.15	13.4-31.9
Hb S	74.35±0.55	73.8-74.9	70.7±1.53	65.1-73.9	78.86±5.08	71.1-87.8	76.73±2.14	66.2-84.6
Hb A_2	1.45±0.05	1.4-1.5	2.36±0.57	1.4-4.5	2.46±0.20	2.1-2.8	2.99±0.41	1.4-4.8

severe forms of β-thal. HbF levels are also useful for predicting the clinical severity of sickle cell disease (SCD) (Kotila *et al*., 2000). The varying levels of foetal haemoglobin in erythrocytes account for a larger part of clinical heterogeneity observed in patients with sickle cell anaemia (Bailey *et al*., 1992). As this hemoglobin variant has not been previously studied in the tribes of Melghat Region Of Amravati district. Hence, this study deals with the status of HbF level in SCD patients (SS) and sickle cell carriers (AS) of tribal people belonging to this particular region.

MATERIALS AND METHODS

Collection of Blood Samples: In the selected tribal villages of Melghat region of Amaravati district, 1000 individuals belonging to 7 different tribal castes were screened for SCD. Blood samples were collected either by door to door screening or organizing screening camps in co-ordination with the officials from Primary Health Centers as well as Sub-district and Rural Hospitals, with prior written consent of tribal people.

Solubility Test: The solubility test (Bernard and Webber, 1979) was performed prior to the blood collection. The samples found to be positive for solubility test were preferred for collection and further analysis.

Sebia Capillary Electrophoresis (CE): It is the approved method which offers quantitation and detection of normal and abnormal haemoglobins, as an aid in the diagnosis of hemoglobinopathies. CE also provides much enhanced resolution and foculisation in the separation of HbA_2, HbF, HbA and HbS especially useful in Sickle Cell anaemia diagnosis (Chen *et al*., 1991; Ishioka *et al*., 1992; Gulbis *et al*., 2003). CE has been demonstrated to be comparable to high-pressure liquid chromatography (HPLC) in the detection of hemoglobin variants; furthermore, it has proven superior to HPLC in the measurement of HbA_2 in the presence of HbE (Keren *et al*., 2008; Cotton *et al*., 1999; Jenkins *et al*., 1997). Hence, Sebia Capillarys Electrophoresis was performed in alkaline buffer, pH 9.4, provided by the manufacturer (Sebia), with separation primary by pH of the solution and endosmosis. The hemoglobins were measured at 415 nm wavelength. Electrophoretograms were recorded with the location of specific hemoglobins in specific zones.

RESULTS

Capillary Electrophoresis results confirmed the presence of SCD homozygous (SS), heterozygous (AS) and HbS with β Thalassemia individuals (HbS β0/+ thalassemia). Out of the total 80 solubility positive tests, 32 individuals were found to be homozygous (SS) and 48 were heterozygous (AS). However, 2 homozygous (SS) and 2 heterozygous (AS) SCD individuals were showing HbS β0/+ thalassemia status. And no individuals were recorded for α thalssemia. The status of Hb F was found to be highest in SS individuals (21.63±1.51) followed by AS individuals (1.45±0.11) and negligible in AA (0.03±0.1). Similarly, the highest level of Hb S was seen in SS individuals (75.15±0.41), moderate in AS (35.81±0.76) and not seen in AA individuals. However, the level of Hb A was found to be highest in AA individuals (97.3±0.11), moderate in AS (61.09±0.72) and negligible in SS individuals (1.74±0.76). Whereas, Hb A_2 was observed in minute quantity in SS (2.6±0.26), AS (2.77±0.06) and AA (2.65±0.10) individuals (Table 1.). When the level of Hb F was compared between SS male and female individuals, it was found that, Hb F was slightly higher in female (21.95±1.88) than their male counterparts (21.32±2.47). However, the level of Hb S was more in male (75.52±2.42) than their female counterparts (74.79±1.60) (Table 2.). In different age groups differing level of Hb F was observed. In the age group <10 yrs level of Hb F

was found to be (23.05±0.45), slightly higher in 11-20 yrs (25.22±2.43). In the 21- 30 yrs of age group it was (19.42±5.22) and slightly lower in >31 yrs (19.45±2.15). It was also observed that the mean HbF level appears to be declining as age advances. Similarly, the level of Hb S varies according to level of Hb F (Table 3).

DISCUSSION

From Previously published findings the range of different hemoglobin variants was known, for SS individuals HbS up to 74.85%, HbA-0.89-1% or more, HbF up to 21.76%, and HbA_2- 2.2%; for AS individuals HbS up to 34.57%, HbA-61.67% or more, HbF up to 1.02% and HbA_2-2.2% to 3.2%; for AA individuals HbA-96.8% or more; HbF-less than 0.5%; and HbA_2-2.2% to 3.2% and for HbS-β0/+ thalassemia HbF is less than 30% and HbA_2 is elevated (Wilson *et al*., 1968; Kan *et al*., 1975; Kotila *et al*., 2000). In the present study the level of Hb F and Hb S were found to be highest in SS individuals moderate in AS and negligible in AA individuals. A study performed in Calabar, Nigeria, reported that the mean HbF value in HbSS subjects was higher (3.05±1.61%) than in HbA and HbAS subjects, i.e. 0.20±0.25% and 1.07±0.98%, respectively (Uko *et al*., 1997). In three different studies conducted at Nigeria, a mean foetal haemoglobin level of (5.16±4.04) (Olaniyi *et al*., 2010), (6.4±4.0) (Enosolease *et al*., 2005) and (7.4±3.6) (Uda *et al*., 2008) was reported in SS individuals. The variations in the HbF levels in HbSS patients from different localities could be due to common single-nucleotide polymorphisms at the BCL11A and HBS1L-MYB loci, which have been implicated previously in HbF level variation in non-anemic European populations (Kotila *et al*., 2000). An association between a BCL11A SNP and HbF levels in a SCD cohort study in the USA has also recently been demonstrated. A report on human HbF expression also supports this claim, suggesting that the BCL 11A gene is a potential regulator of HbF expression (Sankaran *et al*., 2008). This increased HbF level is a compensatory mechanism for sickling in SS subjects (Wood *et al*., 1993).

The HbF level in SS females was recorded slightly higher as compared to SS males and the difference was statistically significant ($p<0.001$). However, another study showed statistically higher value of HbF in males than in females (Falusi and Esan, 1989). A study showing that, after the age of 10, HbF levels were consistently higher in females than in males (Maude *et al*., 1987). The difference between males and females was suspected to be due to the hormonal effects of puberty. In a study estimating HbF levels in SCD, male sickle cell patients were found to have significantly lower levels of HbF than their female counterparts (Mason *et al*., 1982). Gender influences the expression of HbF in normal individuals and HbSS patients, implying that X-chromosomal genes or hormonal factors might be operative (Miyoshi *et al*., 1988; Dover *et al*., 1992). When the level of HbF was compared among different age groups, highest value was found in the age group 11-20 year (26.22±2.43) followed by 21-30 year (19.66±5.22) and then >30 years(19.45±2.15). When age is considered, the 1-10-year age group had the slightly lower mean HbF level (24.05±0.45) than the age group 11-20 year. The 21-30-year age range had the lowest HbF levels among all hemoglobin genotypes and the relationship was statistically significant ($P<0.05$). The mean HbF level appears to be declining as age advances (Uko *et al*., 1997). This increased HbF level is a compensatory mechanism for sickling in SS subjects (Wood, 1993). This highlights the need to determine HbF along with HbA2 in assisting to differentiate HbSS, HbS-beta-thalassemia and HbS-HPFH and hence determination of HbA2 and HbF should graduate from research activity to routine tool in order to project the management of SCA to a level where the clinical course among others could be easily predicted at diagnosis. Genetic studies have established that increased HbF level may result from rare deletions within the beta globin gene cluster or from point mutations in the promoters of the fetal gamma-globin genes (hereditary persistence of fetal haemoglobin, HPFH), however, additional loci are known to increase HbF levels in adult life, which has been identified using combination of genome-wide analysis within a large kindred (Thein *et al*., 1994).

Conclusion

When the HbF status was observed in the study population it was found that, its level is highest in SCD patients (SS) as compared to SCD carriers (AS) and normal individuals (AA). However the higher status was seen in SS females compared to that of SS males. Whereas the highest level of HbF was recorded for the age group of 11-20 years when compared with different age groups. It is highly imperative to always estimate not only the levels of HbF, but also of HbA2 so as to be able to clearly define the clinical course of every sickle cell disease patient.

Ethical standards

All human studies have been approved by the appropriate ethics committee, in collaboration with Anthropological survey of India, Nagpur Central Regional Centre, Nagpur. 440002, Maharashtra. All persons had given their informed consent prior to their inclusion in the study.

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