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STATUS OF HB F IN SICKLE CELL ANAEMIC INDIVIDUALS OF YAVATMAL DISTRICT, MAHARASHTRA.

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Abstract
Sickle cell disease (SCD) is a major gene disorder among the tribal population of central India. Fetal Haemoglobin (HbF) is the best-known genetic modulator of sickle cell anaemia, which varies dramatically in concentration in the blood of these patients. The patients with SCA display a remarkable variability in the disease severity. 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). Studied population shows the highest HbF level in SS followed by AS individuals and a slightly higher HbF level 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.

Key Words: Sickle cell disease (SCD), Fetal haemoglobin Hb F, Tribals, Yavatmal district.

Introduction

Sickle-cell anemia is a hereditary disease produced by haemoglobin S in its homozygous form, (Hb^sHb^s). It causes a translocation of the amino acid in position 6 of a normal beta globin, transforming glutamic acid into valine, and thus diminishing protein solubility. Haemoglobin is a tetrameric protein compound made up of a complex of four polypeptide chains and four heme groups. It is the oxygen-carrying molecule of RBC, and it makes up approximately 95% of the RBC proteins (Harmening *et al.,* 1999). In normal adults this protein is composed of 96-98% of HbAA ($\alpha_2\beta_2$), up to 3-5% of HbA_2 ($\alpha_2\delta_2$) and less than 1% of HbF ($\alpha_2\gamma_2$) (Cheesbrough, 2006). In sickle cell disease (SCD) patients 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 (Steinberg, 2005). HbF is the most powerful modulator of the cilinical and haematological features of sickle cell anaemia (Sebastiani *et al.,* 2008). To protect against various complications of disease, different concentrations of HbF were postulated to be required, although any increment in HbF had a beneficial effect on mortality (Powars *et al.,* 1984 and Platt *et al.,* 1994).

SCA patients with high HbF levels not only have less severe clinical course, but also have mild clinical complications because an increase in haemoglobin F inhibits polymerization of sickle haemoglobin. Persistent production of variable levels of HbF into childhood and adult life is a characteristic finding in sickle cell anemia and more severe forms of β-thal. HbF levels are also useful for predicting the clinical severity of sickle cell disease (SCD) (Kotila *et al.,* 2000). HbF and S are heterogeneously distributed within the RBC population of patients with HbSS disease, and their transfusion studies indicated that those RBCs with higher proportions of HbF had longer life spans (Singer and Fisher,1952) and distribute the HbF heterogeneously with some red cells (F cells) expressing more HbF than others (Vrudhula, 1981). The strong relationship existing between HbF level and disease severity in SCA suggests that baseline measurement of percentage HbF is paramount in predicting important aspects of clinical course. Hence, this study deals with the status of HbF level in SCD patients (SS), sickle cell carriers (AS) and normals (AA) of tribal individuals belonging to Yavatmal district.

Materials And Methods

A total of 100 individuals belonging to 7 different tribal castes were screened for SCD in some tribal villages from Yavatmal district from February 2013 to June 2013. Blood samples were collected from SCD patients, carriers and controls into Ethylene Diamene Tetraacetic Acid (EDTA) anticoagulant bottle along with written consent forms by organizing screening camps in co-ordination with the officials from Primary Health Centers as well as Sub-district and Rural Hospitals. Sebia Capillary Electrophoresis (CE) is the approved method offers quantitation and detection of normal and abnormal haemoglobins, as an aid in the diagnosis of hemoglobinopathies. CE also provide very enhanced resolution and foculisation in the separation of Hb A_2, F, A and S especially useful in Sickle Cell anaemia diagnosis (Chen *et al.,* 1991; Ishioka *et al.,* 1992 and Gulbis *et al.,* 2003). Sebia Capillarys Electrophoresis was used for

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detecting the levels of Hb A, Hb A_2, Hb S and Hb F of all the individuals studied in the laboratory of Anthropological survey of India, Nagpur regional centre, Nagpur.

Results

Level of Hb F was found to be highest in SS individuals (22.15±1.162), negligible in AS (1.01±0.32) and not found in AA individuals. Similarly, the highest level of Hb S was seen in SS individuals (74.81±0.97), moderate in AS (34.78±0.98) and not seen in AA individuals However, the level of Hb A was found to be highest in AA individuals (97.14±0.13), moderate in AS (61.18±1.20) and negligible in SS individuals (1.94±0.65). Whereas, Hb A_2 was observed in minute quantity as SS(2.48±0.26), AS(2.84±0.06) and AA(2.74±0.13) individuals (Table1.).

Table1. Showing values of Mean±SE of different Hemoglobin variants in SS, AS and AA individuals.

Parameters	Sickle cell patient (SS) n=40		Sickle cell gene carrier (AS) n=30		Normal (AA) n=30	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Hb A	1.94±0.65	0.3-3.5	61.18±1.20	26-71.1	97.14±0.13	94.6-97.9
Hb F	22.15±1.162	10.7-37.4	1.01±0.32	0.3-1.9	0	0
Hb S	74.81±0.97	61.1-85.8	34.78±0.98	26.7-40.8	0	0
Hb A_2	2.48±0.26	0.3-5.8	2.84±0.06	1.8-3.5	2.74±0.13	1.9-5.4

When the level of Hb F was compared between SS male and female individuals, it was found that, Hb F was higher in female (22.85± 1.62) than their male counterparts (20.76± 1.24). However, the level of Hb S was more in male (76± 1.16) than their female counterparts (74.21± 1.33) (Table2.).

Table2. Showing comparison of Mean±SE of different Hemoglobin variants of SCD males and females compared with that of normal males and females.

Parameters	Sickle cell patient (SS)				Normal (AA)			
	Males n=20		Females n=20		Males n=15		Females n=15	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Hb A	0.47± 0.47	0.3.3	1.47± 0.66	0-3.5	97.03± 0.23	94.5-97.7	97.21± 0.16	94.6-97.9
Hb F	20.76± 1.24	16-27.3	22.85± 1.62	11.4-37.4	0	0	0	0
Hb S	76± 1.16	70.5-81.2	74.21± 1.33	61.1-85.8	0	0	0	0
Hb A_2	2.87± 0.428	1.5-5.5	2.29± 0.34	0.7-5.5	2.85± 0.25	1.9-5.5	2.67± 0.15	2.1-5.4

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 (21.55±1.80), slightly higher in 11-20 YRS (23.6±3.39). In the 21-30 YRS of age group it was (22.63±3.75) and slightly lower in >31 YRS (21.6±1.79). Similarly, the level of Hb S varies according to level of Hb F (Table3 and Fig1.).

Table3. Showing comparison of Mean±SE of different Hemoglobin variants of SCD patients belonging to different age groups. (n=10).

Parameters	<10 YRS		11-20 YRS		21-30 YRS		>31 YRS	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Hb A	0.97±0.62	0-3.5	0	0	0	0	0.97±0.59	0~3.3
Hb F	21.55±1.80	14.2-30.5	23.6±3.39	11.4-37.4	22.63±3.75	16-29	21.6±1.79	10.7-28.7
Hb S	75.1±1.45	68-77.6	74.14±3.15	61.1-85.8	75.03±3.37	69.9-81.2	74.92±1.25	69.6-80.3
Hb A_2	2.67±0.47	1.4-2.8	2.21±0.33	1.2-3.4	1.63±0.61	0.7-2.8	2.74±0.60	0.3-5.5

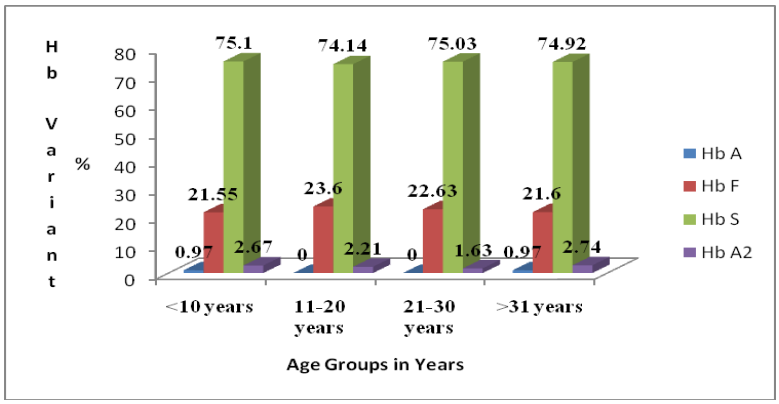

Fig: Showing the HbA%, HbF%, HbS% and HbA2% in SS patients of different age groups.

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Discussion

The level of Hb F was found to be highest in SS individuals (22.15±1.162) followed by AS individuals (1.01±0.32). In three different studies conducted at Nigeria a mean fetal haemoglobin level of (5.16±4.04) (Olaniyi *et al.,* 2010), (6.4±4.0) (Enosolease *et al.,* 2005) and (7.4±3.6) (Kotila *et al.,* 2000) was reported in SS individuals. A similar study performed in Calabar, Nigeria, reported that the mean HbF value in HbSS subjects was higher (3.05±1.61%) than in HbA (0.20±0.25%) and HbAS (1.07±0.98%,) subjects (Uko *et al.,* 1997). The variations in the HbF levels in HbSS patients and others from different localities could be due to common single-nucleotide polymorphisms (SNPs) at the BCL11A and HBS1L-MYB loci, which have been implicated previously in HbF level variation in non-anemic European populations (Uda *et al.,* 2008). 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).

The HbF level in SS females (22.85±1.62) was recorded higher as compared to SS males (20.76±1.24) and the difference was statistically significant ($p<0.001$). However, another study showed statistically higher value of HbF in males than in females (Kotila *et al.,* 2000). The mean HbF level was higher in females than in males, with female HbSS and HbSC subjects having the highest mean HbF level. This is in agreement with a study showing that, after the age of 10, HbF levels were consistently higher in females than in males, and this was statistically significant (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).

When the level of HbF was compared among different age groups, highest value was observed in the age group of 11-20 year (23.6±3.39) followed by 21-30 year (22.63±3.75) and then >30 years(21.6±1.79). When age is considered, the 1-10-year age group had the lowest mean HbF level (21.55±1.80) among all hemoglobin genotypes and the relationship was statistically significant ($P<0.05$). The mean HbF level appears to be declining as age advances (Olaniyi *et al.,* 2010). This increased HbF level is a compensatory mechanism for sickling in SS subjects because HbF reduces the tendency of HbS to polymerize within the red cell (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 betaglobin 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

Within the study population, the HbF level was found to be highest in HbSS and very low in HbAS and HbAA. In SS subjects the HbF level is higher compared to other hemoglobin variants. When the HbF status was compared between SS males and females, it was found that the level of HbF was higher in HbSS females than their male counterparts. However, the highest level was recorded for the age group 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.

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