IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 10, 2022

"Unravelling Haemoglobin Variations in Sickle Cell Disease: Patterns and Implications"

[1*]Sandeep M. Chede, [2]Alka M. Vikhar
[1*]Assistant Professor, G.S.G. Mahavidyalaya, Umarkhed
Dist. Yavatmal (M.S., India)
[2]Assistant Professor, Vidyabharati Mahavidyalaya, Camp Amaravati.
Dist. Amaravati (M.S., India)

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 individuals.

Keywords: Fetal Haemoglobin-HbF, Sickle cell disease-SCD, Erythrocytes-RBC, hemoglobinopathy

Introduction

Hemoglobinopathies are inherited single-gene (β-globin gene) disorders; in most cases, they are inherited as autosomal co-dominant traits[1]. Most common hemoglobinopathy like sickle cell disease (SCD) causes life threatening medical emergencies, chronic disability to families and major drain on health resources[2]. However, the SCD patients with high HbF levels not only have less severe clinical courses, but also have mild clinical complications because an increase in haemoglobin F inhibits polymerization of sickle haemoglobin[3]. HbF (α2 γ2), the main haemoglobin component in the foetus, is present at levels of 65 to 90% at birth and usually drops to less than 2% by 6 to 12 months of age[4]. After birth, the HbF-gama-gene is switched down and the HbA beta-gene is switched on so that adults mainly produce HbA (α2β2). After this developmental switch, low levels of HbF are still produced, and this is distributed heterogeneously with some red cells (F cells) expressing more HbF than others[5]. Hemoglobinopathies can be diagnosed by detecting and quantifying various haemoglobin fractions like HbF (α2γ2), HbA (α2β2) and HbA2 (α2δ2). In healthy adults, Hb is composed of Hb A (~95%) and Hb A2 (~3.5%), with only trace amounts of Hb F. While HbF levels may also be elevated as a result of genetic abnormalities of haemoglobin production or because of haemopoietic stress.

This β-globin chain structure disorder comprises a heterogeneous group of conditions, in which HbF production persists through adult life in the absence of hematological abnormalities called hereditary persistence of fetal hemoglobin (HPFH) [6]. 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) [7]. The varying levels of foetal haemoglobin in RBCs account for a larger part of clinical heterogeneity observed in patients with sickle cell anaemia[8]. It is also a major prognostic factor for several clinical complications[9,10,11].

Hb F and S are heterogeneously distributed within the RBC population of patients with Hb SS disease, and their transfusion studies indicated that those RBCs with higher proportions of Hb F had longer life spans[12]. In SCD patients the RBC changes quantitatively and qualitatively. Intravascular haemolysis of RBC results from the lysis of complement-sensitive red cells [13] and haemoglobin lost during sickling-induced membrane damage[14,15]. The extracellular haemolysis occurs by phagocytosis of red cells that have undergone sickling [16,17] and physical entrapment of rheologically compromised red cells[18]. As the RBCs are being affected by SCD the other red cell parameters like Cell volume (MCV) and cell hemoglobin content (MCH) and even the white blood cells and platelets are also affected by the mutation. So the aims of this study were to determine the HbF levels of normal (AA), sickle cell trait (AS), SCD (SS) and the haematological parameters of studied individuals.

Materials and Methods

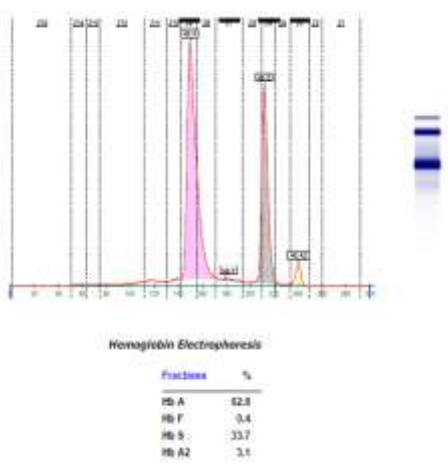
A total of 100 individuals belonging to 5 different tribal caste were screened for SCD in some tribal villages from Umarkhed region of Yavatmal district. Blood samples were collected from both patients and controls into Ethylene Diamine Tetraacetic acid (EDTA) anticoagulant bottles with prior consent of all individuals. Among those 59 were found to be sickling positive (AS)/(SS) and 41 were found to be sickling negative (AA). Capillarys Electrophoresis (CE) is the recent most advanced technology which provides walkway convenience for electrophoresis. CE is the approved method offers quantitation and detection of normal and abnormal haemoglobins, as an aid in the diagnosis of hemoglobinopathies. Capillrys Hemoglobin also provides very enhanced resolution and foculisation in the separation of HbA2, F, A and S especially useful in Sickle Cell anemia diagnosis[19,20,21]. Sebia Capillary Electrophoresis was used for detecting the levels of Hb A, Hb A_2, Hb S and Hb F of all the individuals studied. For complete blood count (CBC) analysis was done by

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 10, 2022

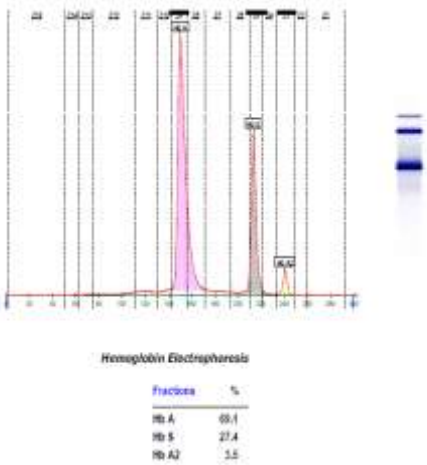
using Beckman Coulter [22,23] in the laboratory of Anthropological survey of India, Nagpur regional centre.

Results and Discussion

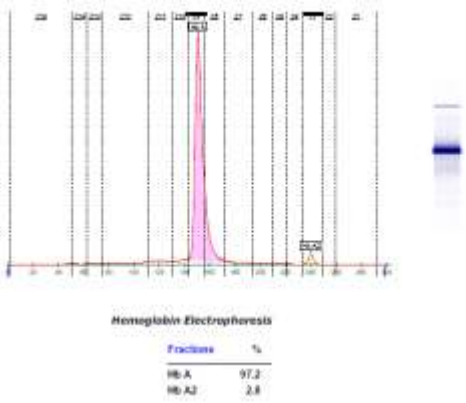
Fig :1 Showing CE electrophoresis Hb profile of Hb AA, AS and SS individuals.



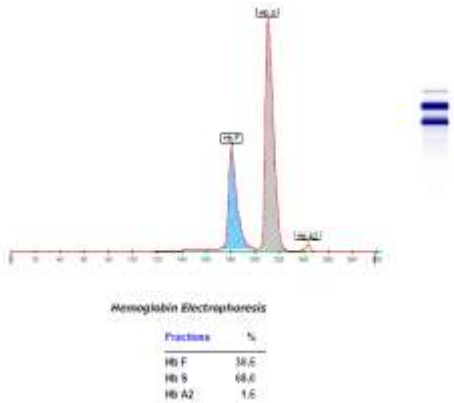
1: Heterozygous (AS) with traces of Hb F.



2: Heterozygous (AS) without Hb F.



3: Homozygous (AA) without Hb S and Hb F.



4 : Homozygous (SS) with Hb S and Hb F.

(Where, HbA- Hemoglobin A, HbA_2- Hemoglobin A_2 , HbS-Hemoglobin S, and HbF-Hemoglobin F.)

Table :1 Data showing the values of four haemoglobin variants and some haematological parameters of the Sickle cell Positive Tribal Population as compared with Sickle cell gene carriers and normal individuals from Amravati district.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 10, 2022

Parameters	Sickle cell patient (SS) n=21		Sickle cell gene carriers (AS) n=38		Normal (AA) n=41	
	Mean±S.E.	Range	Mean±S.E.	Range	Mean±S.E.	Range
Hb A	0.89±0.54	0.3-4,2	61.67±1.52	55.1-68	97.58±0.15	96.9-98.8
Hb F	21.76±2.4	9.4-28.6	1.02±0.27	1-2.7	-	-
Hb S	74.85±2.30	71.9-87.8	34.57±1.5	27.1-40.9	-	-
Hb A_2	2.49±0.35	1.4-4.8	2.74±0.3	2.5-3.4	2.37±0.13	1.2-2.7

(Value are expressed in mean± SE (Slandered error), n= 10, *P<0.05, **P<0.01, ***P< 0.001, ns= non-significant) (Hb- Haemoglobin Count)

The level of HbA was found to be in negligible amounts and HbA_2 was found only 2-4% however HbS was found 71-88% and HbF was recorded 10-30% in SS individuals.(Table:1, Fig:1) This shows the persistence of fetal hemoglobin in SS positive tribal adults. However, in AA individuals HbS and HbF were not observed at all.

Sebia Capillarys Electrophoresis was used for detecting the levels of HbA, HbA_2, HbS and HbF of all the individuals studied. The HbF level was found to be highest in HbSS and lowest in HbAS individuals. HbF is the best known genetic modulator of sickle cell anaemia and its concentration varies in the blood of these patients and it results in milder forms of SCD in Indian SS individuals.

Conclusion

The values of HbF were found to be higher in SS, lower in AS and negligible in AA individuals. In SS individuals the level of HbF was found to be more as compared to SS individuals of other regions of the world, showing that the disease is less severe in the Indian population.

References

1. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ. 2001;79(8):704-712.

2. . Weatherall DJ. Genetic disorders of haemoglobin. In: Hoffbrand AV, Lewis SM, Tuddenham EG, editors. Postgraduate haematology. 4th ed. Oxford, UK: Butterworth-Heinemann; 1999. pp. 91–119.

3. Wood WG. Increased HbF in adult life. Baillieres Clin Haematol. 1993;6:177–213.

4. Haewon C. Kim, Elias Schwartz. Foetal Haemoglobin. In Williams J. Williams et al; Haematology; 4th Edition 1990. pg 1720-1721.

5. Boyer SH, Belding TK, Margolet L, Noyes AN: Fetal hemoglobin restriction to a few erythrocytes (F cells) in normal human adults. Science.(1975; 188:361–363.

6. . Kan YW, Holland JP, Dozy AM, Charache S, Kazazian HH. Deletion of the beta-globin structure gene in hereditary persistence of foetal haemoglobin. Nature. 1975;258:162–163

7. Kotila TR, Fawole OI, Shokunbi WA. Haemoglobin F and clinical severity of sickle cell anaemia among Nigerian adults. Afr J Med Med Sci. 2000;29:229–231.

8. Bailey K, Morris J, Sergent GR. Foetal Haemoglobin and early manifestation homozygous sickle cell diseases. Arch Dis Child 1972.

9. Molineaux L, Fleming AF, Cornille-Brogger R, Kagan L. Abnormal haemoglobin in the suddan savannah of Nigeria III. Ann Trop Med Parasitol 1979; 73: 301-310

10. Bordin JO, Kerbauy J, Lourenco DM and Sesso R. Level of foetal haemoglobin as an indicator of clinical complications in sickle cell anaemia. Braz J Med Biol Res 1989; 22(11): 1347–1353.

11. Olatunji PO. Sickle cell disease in developing countries: magnitude and challenges 1. Postgrad Doc Afr 2002; 25(3): 61–64.

12. Singer, K., and B. Fisher. 1952. Studies on abnormal hemoglobins. V. The distribution of type S (sickle cell) hemoglobin and type F (alkali resistant) haemoglobin within the red cell population in sickle cell anemia. Blood. 7: 1216.

13. Test ST, Kleman K, Lubin B: Characterisation of the complement sensitivity of density-fractionated sickle cells. Blood 1991, 78(Suppl):202a.

14. Allan D, Lumbrick AR, Thomas P, Westerman MP: Release of Spectrin-free Spicules on re- oxygenation of sickled erythrocytes. Nature 1982, 295(5850):612–613.

15. Platt OS: Exercise–induced haemolysis in Sickle cell anaemia: Shear sensitivity and erythrocyte dehydration. Blood 1982, 59(5):1055–1060.

16. Galili U, Clark MR, Shohat SB: Excessive binding of natural anti-alpha galactosyl immunoglobulin G to sickle erythrocytes may contribute to extravascular cell destruction. J Clin Invest 1986, 77(1):27–33.

17. Green GA, Kalra VK: Sickling-induced binding of immunoglobulin to sickle erythrocyte. Blood 1988, 71(3):636–639.

18. Kaul DK, Fabry ME, Nagel RL: Vaso-Occlusion by Sickle cells, evidence from selective trapping of dense red cells. Blood 1986, 68(5):1162–1166.

19. Chen FT et al (1991) Capillary electrophoresis—a new clinical tool. Clin Chem 37:14–19

20. Ishioka N et al (1992) Detection of abnormal haemoglobin by capillary electrophoresis and structural identification. Biomed Chromatogr 6:224–226

21. Gulbis B et al (2003) The place of capillary electrophoresis techniques in screening for haemoglobinopathies. Ann Clin Biochem 40:659–662.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 10, 2022

22. Bourner G, Dhaliwal J, Sumner J. Performance evaluation of the latest fully automated hematology analyzers in a large, commercial laboratory setting: a 4-way, side-by-side study. Laboratory Hematology. 2005;11:285–297.

23. Fernandez T, Domack LB, Montes D, Pineiro R, Landrum E, Vital E. Performance evaluation of the coulter LH 750 hematology analyzer. Laboratory Hematology. 2001;7:217–228.