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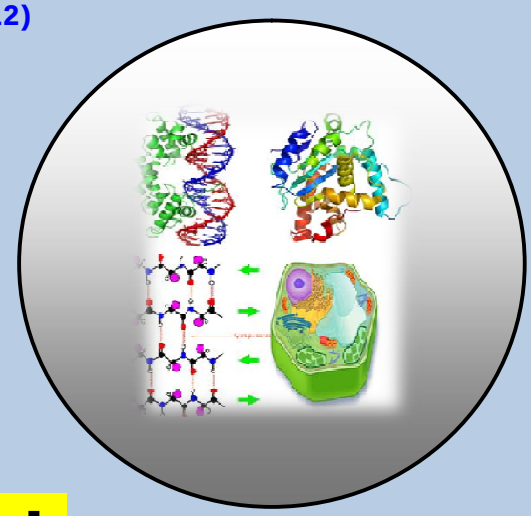

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Hematological Parameters in the Tribals with Sickle Cell Disease of Melghat Region, Amravati District (Maharashtra), India

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ABSTRACT

Sickle cell disease (SCD) is the most common hemoglobinopathy and a genetic abnormality involving HbF. Although, it is primarily a red cell disorder, the WBC's and the platelets are also affected by the mutation. However, HbF is the best known genetic modulator of sickle cell anaemia and its concentration varies in the blood of these patients. So the aim of this study was to determine the HbF levels of normal (AA), sickle cell carriers (AS) and SCD (SS) tribal individuals and to study the various haematological parameters. Homozygous sickle cell disease patients have lower values of red cell parameters but higher values of white cells and platelet counts as compared to haemoglobin phenotypes AS and AA. Within the study population, MCV was found highly significant at p<0.001 and its value in SS cases is more as compared to AS and AA individuals. The HbF level was found to be highest in HbSS and lowest in HbAS individuals.

KEY WORDS: Sickle Cell Disease (SCD), Fetal haemoglobin HbF, Hematological indices, Hemoglobinopathy and Tribals.

INTRODUCTION

Hemoglobinopathies are inherited single-gene (β-globin gene) disorders; in most cases, they are inherited as autosomal co-dominant traits (Weatherall & Clegg 2001). Most common hemoglobinopathy like sickle cell disease (SCD) causes life threatening medical emergencies, chronic disability to families and major drain on health resources (Weatherall 1999). However the SCD 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 (Wood 1993). 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 (Haewon *et al.,* 1990). After birth, the HbF- γ -gene is switched down and the HbA- β -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 (Boyer *et al.,* 1975). In healthy adults, Hb is composed of HbA (~95%) and HbA2 (~3.5%), with only trace amounts of HbF. Hemoglobinopathies can be diagnosed by detecting and quantifying various haemoglobin fractions like HbF (α2γ2), HbA (α2β2) and HbA2 (α2δ2).
Persistent production of variable levels of HbF into childhood and adult life is a characteristic finding in sickle cell anaemia 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). The varying levels of foetal haemoglobin in RBCs account for a larger part of clinical heterogeneity observed in patients with sickle cell anaemia (Bailey, 1992). It is also a major prognostic factor for several clinical complications (Molineaux *et al.,* 1979; Bordin *et al.,* 1989 and Olatunji 2002). HbF 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 HbF had longer life spans (Singer and Fisher 1952). In SCD patients the RBC changes quantatively and qualitatively. Intravascular haemolysis of RBC results from the lysis of complement-sensitive red cells (Test *et al.,* 1991) and haemoglobin lost during sickling-induced membrane damaged (Allan *et al.,* 1982 and Platt 1982). The extracellular haemolysis occurs by phagocytosis of red cells that have undergone sickling (Galili *et al.,* 1986 and Green & Kalra 1988) and physical entrapment of rheologically compromised red cells (Kaul *et al.,* 1986). 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 aim of this study was to determine the HbF levels of normal (AA), sickle cell trait (AS), sickle cell anaemic tribal individuals (SS) and study their various haematological parameters.

MATERIAL AND METHODS

A total of 100 individuals belonging to 5 different tribal castes were screened for SCD in some tribal villages from Melghat forest region of Amravati district. Blood samples were collected from both patients and controls into Ethylene Diamene Tetraacetic Acid (EDTA) anticoagulant bottle with prior consent of all individuals. Sebia Capillary Electrophoresis (CE) is the approved method offering 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 Hb A2, F, A and S especially useful in Sickle Cell anaemia diagnosis (Chen *et al.,* 1991; Ishioka *et al.,* 1992 and Gulbis *et al.,* 2003). Capillarys Electrophoresis was used for detecting the levels of Hb A, Hb A_2, Hb S and Hb F of all the individuals studied. Complete blood count (CBC) analysis was done by using Beckman Coulter AcT Diff II Hematology Analyzer (Bourner *et al.,* 2005 and Fernandez *et al.,* 2001) in the laboratory of Anthropological survey of India, Nagpur Central Regional Centre, Nagpur.

RESULTS

Highest level of Hb F and Hb S was recorded in SS individuals, negligible in AS and not seen in AA individuals. However, highest level of Hb A was observed in AA individuals, moderate in AS and lowest in SS individuals (Table 1.).

Table 1. Data showing the values of four haemoglobin variants of the Sickle cell Positive Tribal Population as Compared with Sickle cell gene carriers and Normal individuals from Amravati district.

Parameters	Sickle cell patient (SS)		Sickle cell gene carriers (AS)		Normal (AA)	
	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	0	0
Hb S	74.85±2.30	71.9-87.8	34.57±1.5	27.1-40.9	0	0
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

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

Table 2. Data showing the values of some haematological parameters of the Sickle cell Positive Tribal Population as Compared with Sickle cell gene carriers and Normal individuals from Amravati district.

Parameters	Sickle cell patient (SS)		Sickle cell gene carriers (AS)		Normal (AA)	
	Mean±S.E.	Range	Mean±S.E.	Range	Mean±S.E.	Range
RBC	3.40 ± 0.21^{ns}	2.58-4.16	$4.94\pm0.28^{**}$	4.19-5.88	3.807±0.61	2.2-4.36
Hgb	$8.6\pm0.72^{***}$	6.2-13.2	12.85 ± 0.7^{ns}	10.4-13.2	12.77±1.32	11.2-18.9
Hct	$26.52\pm1.3^{*}$	22.4-35.1	$38.51\pm1.5^{***}$	33.4-50.2	20.95±3.97	19.4-36.2
MCV	$79.27\pm3.64^{***}$	57.4-90.8	$77.63\pm2.57^{***}$	70-88.8	54.45±4.00	44.4-69.4
MCH	$24.99\pm1.25^{*}$	18.8-31.1	$25.29\pm1.05^{*}$	21.6-30.8	42.11±7.05	36-76.2
MCHC	$30.14\pm0.88^{***}$	26.8-34.7	$33.04\pm0.72^{***}$	30.3-38.0	67.7±7.61	53.5-99.9
Plt	346 ± 52.35^{ns}	243-457	$273\pm32.7^{***}$	170-538	316.4±32.12	247-538
WBC	11.67 ± 1.59^{ns}	6-17.6	$8.01\pm0.81^{***}$	4.2-11.2	8.08±0.8	5.8-10.6

Value are expressed in Mean ± SE (Standard error), n= 10, *P<0.05, **P<0.01, ***P< 0.001, ns= non-significant.

Lower levels of RBC, Hgb and Hct were recorded in SS individuals as compared to AS and AA individuals. Similarly, lower levels of MCH and MCHC were observed in SS as compared to AS and AA individuals. However, highest level of MCV was recorded in SS individuals, moderate in AS and lowest in AA individuals (Table 2.)

Levels of Hb F, Hb S, Hb A and Hb A_2 were recorded by Sebia Capillary electrophoresis. AS individuals consists of Hb A, Hb A_2 and Hb S but may or may not consist of Hb F (Fig 1. and 2.). Whereas, AA individuals consist of Hb A and Hb A_2 only and not Hb S and Hb F (Fig 3.). However, SS individual consists of Hb S, Hb F and Hb A_2 and not Hb A (fig 4.).

(RBC-Red blood Cell Count, Hb- Haemoglobin Count, HCT- Hematocrit Count, MCV- Mean Corpuscular volume, MCH-Mean Corpuscular Hemoglobin, MCHC-Mean Corpuscular Hemoglobin Concentration, MPV-Mean Platelet Volume, PLT-Platelet Count, WBC-White Blood Cell Count)

Figure: Showing CE Haemoglobin profile of AA, AS and SS individuals.

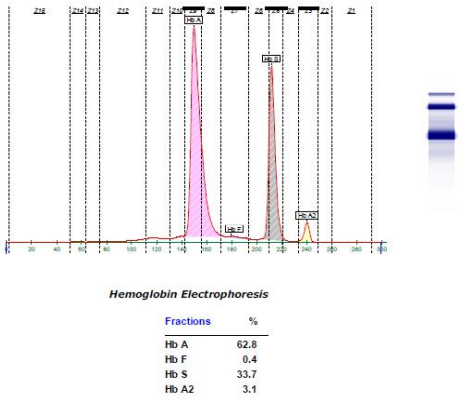

Fig 1. Heterozygous (AS) with traces of Hb F.

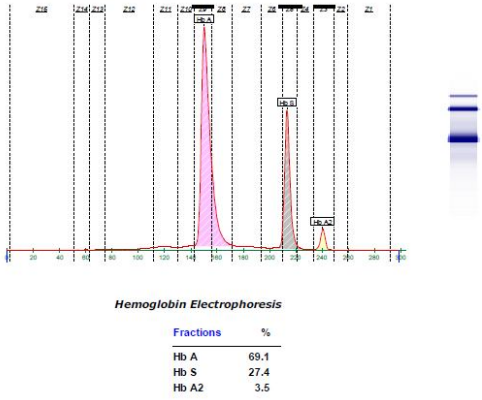

Fig 2. Heterozygous (AS) without Hb F.

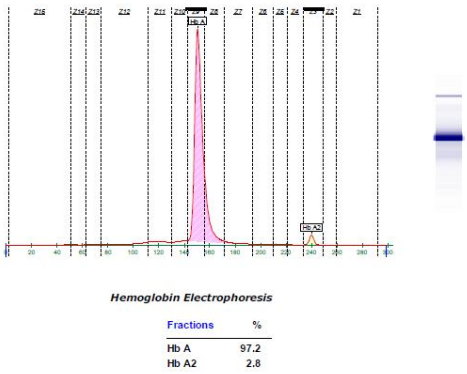

Fig 3. Homozygous (AA) without Hb S and Hb F.

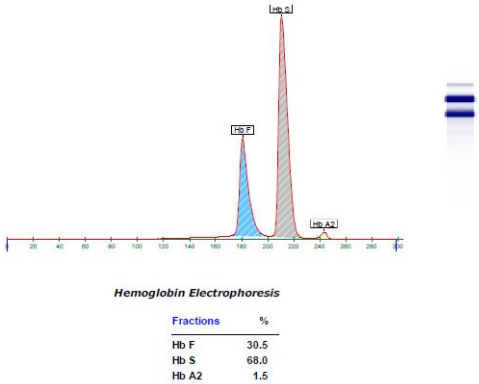

Fig 4. Homozygous (SS) with Hb S and Hb F.

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

DISCUSSION

For SS tribal patients, the overall mean haemoglobin concentration was 8.6±0.72 g/dl which was lower as compared to carriers (AS) 12.85±0.7 g/dl and normal individuals (AA) 12.77±1.32 g/d (Akinsegun *et al.,* 2012). However, mean cell volume (MCV) of SS was recorded as 79.27±3.64 fl which was higher as compared to carriers (AS) (77.63±2.57 fl) and normal (AA) individuals (54.45±4.00 fl). Higher values of MCV were noted earlier in sickle cell patients (SS) as compared to carriers (AS) and normal (AA) individuals (Odenheimer *et al.,* 1983). The hematocrit (Hct) (26.52±1.3) of SS was found to be higher than the Hct (20.95±3.97) of normal (AA) individuals. A study indicated an increased hematocrit (Hct) in subjects with sickle cell trait, suggesting a possible impairment in oxygen delivery in these individuals (Bowers *et al.,* 2011).

The level of four haemoglobin variants HbA, HbS, HbF and HbA_2 were also assessed for SS patients. 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. This shows the persistence of foetal haemoglobin in SS positive tribal adults. Whereas for sickle gene carriers (AS), the HbA level was found to be in higher amount (55-70%) and HbA_2 was found only 2-4%, however HbS was found in moderate amount (27-40%) and HbF in negligible amounts. This indicates that the carriers consist of both heamolgobin variants HbS and HbA. Whereas, the HbF was found only in trace amounts. Within the study population, the HbF level was found to be highest in HbSS and lowest in HbAS. This increased HbF level is a compensatory mechanism for sickling in SS subjects (Bhagat *et al.,* 2013).Whereas, it was found that the normal individuals AA, do not possess HbS and HbF at all. These people only possessed HbA (96-99%) and HbA_2 (1-2%). In a similar study done in Chhattisgarh, high HbF values in sickle cell patients (SS) (22.71±5.23) as compared to heterozygous (AS) (20.85±3.02) and normal (AA) individuals were recorded (Wood 1993).

When the HbF levels were estimated in Calabar, Nigeria, it was reported that the mean HbF value in HbSS subjects was higher than in carriers (HbAS) and normal (HbA) subjects (Uko *et al.,* 1997 and Falusi & Esan 1989). In a study comparing the hematological indices in homozygous sickle cell patients, it was discovered that beyond age 10, there is no consistent age-related trend in HbF levels (Maude *et al.,* 1987). The unusually elevated levels of Hb F are not due to an associated high frequency of a gene for hetero-cellular hereditary persistence of fetal haemoglobin in the Oasis population, but rather from a genetically determined absolute increase in Hb F production related in some way to the SS genotype (Pembrey *et al.,* 1978).

CONCLUSION

Within the study population, the HbF level was found to be highest in SS and lowest in AS tribal individuals. Amongst the red cell parameter the level of Hgb was the least in sickle cell aneamic tribal individuals as compared to the carriers and the normal individuals. MCV was found highly significant at $p<0.001$ showing that its value for SS tribal individuals is more as compared to AS and AA individuals.

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REFERENCES

Akinsegun, A., Dosunmu, A., Adediran, A., Oshinaike, O., Adebola P. and Arogundade. O. 2012. Haematological values in homozygous sickle cell disease in steady state and haemoglobin phenotypes AA controls in Lagos, Nigeria. *BMC Research Notes* 5:396.

Allan, D., Lumbrick, A.R., Thomas, P. and Westerman, M.P. 1982. Release of Spectrin-free Spicules on re-oxygenation of sickled erythrocytes. *Nature*, 295(5850):612–613.

Bailey, K., Morris, J., Thomas, P., Sergent, G.R. 1992. Foetal Haemoglobin and early manifestation homozygous sickle cell diseases. *Arch. Dis. Child.* 67: 517-520.

Bhagat, S., Patra, P.K. and Thakur, A.S. 2013. Fetal Haemoglobin and β-globin Gene Cluster Haplotypes among Sickle Cell Patients in Chhattisgarh. *Heamatology Section*. 7(2): 269-272.

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

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

Bowers, A.S., Pepple, D.J. and Reid, H.L. 2011. Optimal haematocrit in subjects with normal haemoglobin genotype (HbAA), sickle cell trait (HbAS), and homozygous sickle cell disease (HbSS). *Clin Hemorheol Microcirc*. 47(4):253-60.

Boyer, S.H, Belding, T.K., Margolet, L. and Noyes, A.N. 1975 Fetal hemoglobin restriction to a few erythrocytes (F cells) in normal human adults. Science. 188:361–363.

Chen, F.T., Liu, C.M., Hsieh, Y.Z. and Sternberg, J.C. 1991. Capillary electrophoresis—a new clinical tool. *Clin Chem*. 37:14–19

Falusi, A.G. and Esan, G.J. 1989. Foetal haemoglobin levels in sickle cell anaemia in Nigerians. *Afr J Med Med Sci*. 18:145–149.

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

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

Green, G.A. and Kalra, V.K. 1988. Sickling-induced binding of immunoglobulin to sickle erythrocyte. *Blood*, 71(3):636–639.

Gulbis, B., Fontaine, B., Vertongen, F. and Cotton, F. 2003. The place of capillary electrophoresis techniques in screening for haemoglobinopathies. *Ann Clin Biochem* 40:659–662.

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

Ishioka, N., Iyori, N., Noji, J. and Kurioka, S. 1992. Detection of abnormal haemoglobin by capillary electrophoresis and structural identification. Biomed Chromatogr. 6:224–226

Kaul, D.K., Fabry, M.E. and Nagel, R.L. 1986. Vaso-Occlusion by Sickle cells, evidence from selective trapping of dense red cells. *Blood*. 68(5):1162–1166.

Kotila, T.R., Fawole, O.I. and Shokunbi, W.A. 2000. Haemoglobin F and clinical severity of sickle cell anaemia among Nigerian adults. Afr J Med Med Sci. 29:229–231.

Maude, G.H., Hayes, R.J. and Serjeant, G. 1987. The haematology of steady state homozygous sickle cell disease: interrelationships between haematological indices. *Br J Haematol*. 66:549–558.

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

Odenheimer, D.J., Whitten, C., Rucknagel, D., Sarnaik, S.A. and Sing. F.C. 1983. Heterogeneity of Sickle-Cell Anemia Based on a Profile of Hematological Variables. *Am J Hum Genet* 35:1224-1240.

Olatunji, P.O. 2002. Sickle cell disease in developing countries: magnitude and challenges 1. *Postgrad Doc Afr*. 25(3): 61–64.

Pembrey, M.E., Wood, W.G., Weatherall, D.J. and Perrine, R.P. 1978. Fetal haemoglobin production and the sickle gene in the oases of Eastern Saudi Arabia. *Br J Haematol*. 40(3):415-29.

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

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.

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

Uko, E.K., Useh, M.F. and Gwanmesia, F.N. 1997. Frequency of foetal haemoglobin and haemoglobin values in various haemoglobin genotypes in Calabar, Nigeria. *East Afr Med J.* 74:809–811.

Weatherall, D.J. 1999. Genetic disorders of haemoglobin. In: Hoffbrand AV, Lewis SM, Tuddenham EG, editors. Postgrauate haematology. 4th ed. Oxford, UK: *Butterworth-Heinemann*. pp 91–119.

Weatherall, D.J. and Clegg, J.B. 2001. Inherited haemoglobin disorders: an increasing global health problem. *Bull World Health Organ*. 79(8):704-712.

Wood, W.G. 1993. Increased HbF in adult life. Baillieres Clin Haematol. 6:177–213.

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