Prevalence of Splenomegaly in Sickle cell Anemia patients in relation to Hemoglobin F

Parmar D, Likhar KS

ABSTRACT

Background: The sickle cell disease (SCD) is a very common single gene disorder; 50% of world's population affected by SCD resides in India. The average frequency of SCD gene ranges between 22-44%. High prevalence of sickle gene has been demonstrated in various tribal communities of Gujarat, India. Hematological profile of SCD is extremely variable.

Aim: To determine fetal Hb value in sickle cell disease patients and the percentage of other major Hb fractions cellulose acetate electrophoresis as well as assessing the significance of high fetal hemoglobin level on persistence of splenomegaly

Methods: Patients with sickle cell disease showing positive sickling test attending V.S.S Medical College Hospital, Burla, during Nov'2003 - 2005, were included in study. Detail history was obtained along with laboratory investigations in a pre-made proforma. Hemoglobin electrophoresis was done for the detection of most common clinically important hemoglobin variants. Foetal hemoglobin was estimated by Betke's method.

Results: Out of 95 cases splenomegaly was found in 50 cases (52.63%) of which 42 cases (44.21%) were males while only 8 cases (8.42%) were females. In cases with splenomegaly the mean level of fetal hemoglobin in males was 23.1% and in females was 21.9%. In cases without splenomegaly the mean level was 21.5% in males and 20.6% in females. The mean Hb F level was between 1 – 20% in 35 cases (36.84%) out of which 26 were males and 9 were females. 60 cases (63.16%) had Hb F level greater than 20% with male prepondence.

Conclusion: High fetal hemoglobin level suggests Hb F as one of the important etiological cause of persistence splenomegaly.

Key words: sickle cell disease, splenomegaly, hemoglobin F

INTRODUCTION

The sickle haemoglobinopathies are heterozygous (sickle cell trait), homozygous (SS disease) states for Haemoglobin S and conditions in which HbS is combined with other hemoglobin structural variants or thalassaemia. The sickle cell disease (SCD) is a very common single gene disorder; 50% of world population affected by it resides in India. The average frequency of SCD gene ranges between 22-44%. High prevalence of sickle gene has been demonstrated in various tribal communities of Gujarat, India.[1]

The level of haemoglobin varies considerably in the various forms of sickle cell disorders. In sickle cell anaemia, Hbs ranges from 75-95% and Hb F ranges from 1-20%, and in sickle cell thalassaemia the amount of Hbs is above 60% and HbF ranges from 2-30%. Similarly in sickle cell trait HbS 38-45% and HbF is normal. Homozygous sickle cell disease in parts of Saudi Arabia and India is associated with elevated HbF and is typically a very mild disorder.[2]

Sickle haemoglobin in deoxygenated state is responsible for a wide spectrum of disorders that vary with respect to degree of anaemia, frequency of crisis, extent of organ injury and duration survival. The clinical features can be divided into acute and episodic (crisis) and those that are chronic and unremitting.[3] The cardinal clinical manifestations of sickle cell disease are chronic hemolytic anemia, recurrent painful episodes, chronic organ

damage, particularly of the spleen, bones, brain, kidney, lungs, skin and heart.[4]

In the present study, Hb F and Hb S concentration has been estimated in different types of sickle cell disorders and attempt has been made to find out the pattern of quantitative distribution of HbS and correlate the HbF concentration to the severity of clinical symptoms.

MATERIALS AND METHODS

Ninety five cases attending V.S.S Medical College Hospital, Burla, during November'2003-2005, showing positive sickling test were selected for the study. Their parents and other siblings were also studied when available. Detail history, clinical findings along with laboratory investigations were recorded in the pre-made proforma.

Sickling Test was performed with 2% sodium meta-bisulphite. Hemoglobin electrophoresis at Alkaline pH was done using cellulose acetate membrane. Foetal hemoglobin was estimated by Betke's method (Dacie & Lewis, 2001).

Test: 0.25 ml of haemolysate (10 gm%) was added to 4.75 ml of Drabkin's solution. 0.2 ml of 1.2 NaOH is added to 5.0 ml of resultant HiCN solution and the mixture was gently agitated foe 2 minutes. 2 ml of saturated Ammonium Sulphate was added and after shaking, the mixture is allowed to stand for at least 5 minutes. It was then filtered through a double layer of Whatman no.1 filter paper.

Standard: As a standard, 0.4 ml of HiCN solution, 13.9 ml of water was mixed together.The absorbance of both the test and standard are read using 420 nm filter against water blank. The percentage of HbF was calculated as follows:

$$\%HbF = \frac{\text{Test (Abs) x 100}}{\text{Std.(Abs) x 20}}$$

RESULT

The value of hemoglobin above 13 and 11.5 gm% was considered normal in male and females respectively. The hemoglobin level was more than 10 gm% in 11 cases (11.57%), between 6 -10 gm% in 59 cases (62.10%), below 6 gm% in 25 cases (26. 33%).

The highest incidence of Sickle cell disease (45.26%) was seen in 21 – 31 yrs of age, followed by 26.31% in 11 – 20 yrs and lowest 1.05% was found in 0 – 10yrs and 61 – 70 yrs decade of life.

Splenomegaly was found in 50 cases (52.63%) of which 42 cases (44.21%) were males while only 8 cases (8.42%) were females. In cases with splenomegaly the mean level of fetal haemoglobin in males was 23.1% and 21.9% in females. In cases without splenomegaly the mean level was 21.5% in males and 20.6% in females.

Table.1. HbF in Patients with Sickle cell disease with and without Splenomegaly

	Cases with Splenomegaly		Cases without Splenomegaly	
	50 (52.63%)		45 (47.37%)	
	Males	Females	Males	Females
Incidence of cases SCD	42 (44.21%)	8(8.42%)	30 (31.58%)	15 (15.79%)
HbF	23.1%	21.9%	21.5%	20.6%

In patients with crisis, the mean fetal Hb level in males was 22.01% and in females it was 22.47%, whereas in patients without crisis the mean fetal Hb level was 23.82% in males and 23.21% in females. The mean Hb S level in patients with crisis was 74.28% in males and 75.5% in females, where as in patients without crisis the

mean Hb S level was 70.35% in males and 73.88% in females.

In patients with crisis the total hemoglobin level in males was 7.28 gm% and in females was 6.53 gm%, where as in patients without crisis the total haemoglobin level was 7.92 gm% in males & 7.05 gm% in females.

Table.2. Haematological Parameters in SCD patients with crisis and without crisis

	Subject Observed with Crisis 60 (63.15%)		Subject Observed without Crisis 35 (36.85%)	
	M - 49	F – 11	M - 23	F - 12
HbF	22.01	22.47	23.82	23.21
HbS	74.28	75.50	70.35	73.88
Hb (gm%)	7.28	6.53	7.92	7.05
TLC	12,660	9072	9591	8159
Neutrophils in Diff. Count	73.92	70.75	70.91	65.81

The mean Hb S level is found to be less than 75% in 53 cases (55.78%) out of which 41 were males and 12 were females. The mean Hb S level was between 75 – 95% in 42 cases (44.22%) out of which 31 were males and 11 were females.

Table.3. Variability of Major fractions of Hemoglobin

	% of Hb fractions	Male	Female	Total	% of Incidence
Hb S	< 75%	41	12	53	55.78
	75 – 95 %	31	11	42	44.22
	Total	72	23	95	100
Hb F	1 – 20	26	9	35	36.84
	> 20	46	14	60	63.16
	Total	72	23	95	100
Hb A2	< 2	21	6	27	28.42
	2 – 5	50	16	66	69.47
	> 5	1	1	2	2.11
	Total	72	23	95	100

The mean Hb F level was between 1 – 20% in 35 cases (36.84%) out of which 26 were males and 9 were females 60 cases (63.16%) had Hb F level greater than 20% out of which 46 were males and 14 were females. The mean Hb A2 level is found to be less than 2% in 27 cases (28.42%), between 2 – 5% in 66 cases (69.47%) and only 2 cases (2.11%) had HbA2 greater than 5% .

The most common symptom was fever in 42 cases followed by pain including limb, joint, chest, abdomen and body in 42cases, jaundice in 18 cases, general weakness in 12 cases and 37 cases were associated with diseases like tuberculosis, malaria and pneumonia etc.

Table.4. Features of Sickle Cell Disease

Signs & Symptoms associated with Disease	No. of cases presented
Fever	42
Jaundice	18
Pain including limb,joint,chest, abdominal & Body	42
Generalized weakness	12
Others	37

DISCUSSION

Higher levels of HBF has its effect on persistence of splenomagaly has been demonstrated in a study carried out on 340 patients by an author.[5] Results reflected in our study relates with the findings in patients with higher mean HbF in patients with splenomegaly.

A work on 81 'SS' cases of age range 6 months to 64 yrs in central India with an average age of 14.55 yrs in males and 18.13 yrs in females.[6] Average hemoglobin in males was 7.11 ± 2.13 gms/dl and in females 6.75 ± 1.85 gms/dl. Hb F in males was 19.58 + 5.86% and in females is 20.99+ 4.9%. In present study including 95 patients of age group 2 to 65 yrs , the mean Hb level in patients with crisis was 7.28gm% in males and 6.5% l in females. The mean Hb level in patients without crisis was slightly higher (Males 7.92gm% and Females 7.05gm %).

A study carried out in Rajasthan found found the homozygous state SS in 14 patients. M:F ratio was 3.6 and the common presentations were anemia, pain, recurrent infection and splenomegaly.[7] The male female ratio in our study was 3.13 and most common symptom was fever (42cases) followed by pain.

A study in South Gujarat on 33 sickle cell disease patients of age range 5 to 15 years,with M:F

ratio of 2.6; Hb in g/dl was 7.73±1.86 (male 7.86±1.87 female 7.41±1.92), mean HbF was 12.3% (male 12.56±7.60% female 11.97±6.68%). Mean HbF level was high and no sex related difference in HbF values was observed in their study (P>0.05).[8] In our study difference in mean HbF level was 23.1% in males and 21.9% in females in patients with splenomegaly while in cases without splenomegaly had mean HbF level 21.5% in males and 20.6% in females.

Yet another study in Uganda 216 children with sickle cell disease aged 1 to 18 yrs found the mean HbF level 9.0% (SD 5.58) and the median was 7.9% . High levels of Hb F ≥10% were found in 80 (37.0%) out of which 24 (30%) had palpable spleen. Low levels of Hb F<10% was found in 136 (63 %) out of which 20 (20.6%) had palpable spleen.[9] Our study had 60 cases with HbF >20%,35 cases had HbF between 1-20%. Thus the mean HbF level in most of our cases was significantly higher thus relating with the higher mean HbF found in homozygous SCD patients of Saudi Arabia and India who have typically a milder form of this disorder.

CONCLUSION

High fetal hemoglobin level found in sickle cell disease suggests Hb F as one of the important etiological cause of persistent splenomegaly. Also the high Hb F level has an ameliorating effect on the clinical severity of the disease, mainly the occurrence of sickle cell crisis and late onset of symptoms.

AUTHOR NOTE

Deepankar Parmar (**Corresponding Author**),
Email: dr.komallikhar@yahoo.com
Komal Singh Likhar
Department of Pathology; PCMS & RC, Bhopal

REFERENCES

1. B Vasava, R Chudasama, N Godara, R Srivastava. Prevalence of sickle cell disease in tribal adolescents of the South Gujarat region, India. *The Internet Journal of Tropical Medicine*. 2008 ; (6)1.
2. De Gruchy, 1989. Clinical hematology in medical practice. 5th edition.Disorders of hemoglobin structure and synthesis, p.142-46. Blackwell publisher.
3. Wintrobe. 2009. Clinical hematology.12th edn.Vol. 1. Sickle cell anaemia and other sickling sydromes. Winfred C Wang. P.1038-44. Lippincott Williams and Wilkins.
4. Hoffman. 2009. Hematology Basic Principles and Practice. 5th edn. Pathobiology of sickle cell disease. P.579. Churchill Livingstone.
5. Al-Salem AH, Al-Aithan S, Bhamidipati P, Al-Jam'a A, Al Dabbous I. Sonographic assessment of spleen size in Saudi patients with sickle cell disease. *Ann Saudi Med*. 1998 May-Jun;18(3):217-20.
6. Shrikhande AV, Dani AA, Tijare JR, Agrawal AK. Hematological profile of sickle cell disease in central India. *Indian J Hematol Blood Transfus*. 2007Dec;23(3-4):92-8.
7. Mandot S, Khurana VL, Sonesh JK. Sickle cell anemia in Garasia tribals of Rajasthan. *Indian Pediatr*. 2009 Mar;46(3):239-40.
8. Mandot S, Khurana VL, Sonesh JK. Sickle cell anemia in Garasia tribals of Rajasthan. *Indian Pediatr*. 2009 Mar;46(3):239-40.
9. Mpalampa L, Ndugwa CM, Ddungu H, Idro R. Foetal haemoglobin and disease severity in sickle cell anaemia patients in Kampala, Uganda. *BMC Blood Disord*.2012 Sep 7;12:11.