Hematological profile of sickle cell disease in central India

A. V. Shrikhande · A. A. Dani · J. R. Tijare · A. K. Agrawal

Abstract Hematological profile of homozygous sickle cell disease patients attending RHDMC from Central India is presented. Central India has a huge population of sickle cell disease patients. Though predicted SS in the region is 22–44 %, 81 homozygous of sickle cell patients reported during study period of Jan 2003–Dec 2005. The clinical course of these patients is characterized in most of the cases by relatively long period without any symptoms punctuated by acute clinical events. Hematological profile of these 81 patients with age ranging from 6 month to 64 years is presented. There are 44 males and 37 females with an average age of 14.55yrs in males and 18.13 yrs females. Males outnumber females in pediatric age group where as females with SCD are attending hospital more in reproductive age group. Very few patients are reported after the age of 30 yrs. Average hemoglobin in males is 7.11 ± 2.13 gms/dl and in females 6.75 ± 1.85 gms/dl with parallel low RBC count. Hemoglobin rise is seen after 14 years of age in males and females. Age related rise in MCV is more noted in females after the age of 5 as compared to males. No age or sex related difference was seen in MCHC values. Hb A₂

levels for males is $2.13 \pm 0.95\%$ and for females $2.04 \pm 0.91\%$. Hb F in males is $19.58 \pm 5.86\%$ and in females is $20.99 \pm 4.9\%$. There is no age and sex related difference in Hb F levels. Moderate to severe anemia with high Hb F dominate Central Indian sickle cell disease patient's hematological profile. The hematological profile in Central India is similar to the profile in other parts of India and Saudi Arabia but different from Jamaica and Africa.

Keywords Hematological profile · HPLC · Sickle cell disease · Central India

Abbreviations

ANC – Ante natal clinic
F - female
Hb - Hemoglobin
HbA₂ – Hemoglobin A₂
Hb Ep – Hemoglobin electrophoresis
HbF – Fetal hemoglobin
HbS – Sickle hemoglobin
HPLC – High performance liquid chromatography
IGGMC – Indira Gandhi Government Medical College
M - male
MCHC - Mean corpuscular hemoglobin concentration
MCV – Mean corpuscular volume
OPD – Out patient department
RBC – Red blood cell
RHDMC – Regional hemoglobinopathy detection and management centre
SCD – Sickle cell disease

A. V. Shrikhande¹ (✉) · A. A. Dani² · J. R. Tijare² · A. K. Agrawal³

¹Prof. & Head

²Sr. Lecturer

³Lecturer

Dept. of Pathology,
Indira Gandhi Government Medical,
Nagpur, India

E-mail: dravshrikhanderrhdmc@yahoo.com

Introduction

The SCD is a commonest single gene disorder; 50% of world population of SCD resides in India. The average frequency of SCD gene ranges between 22 to 44 % [1] Solanki and Shukla reported the first sickle cell case from Central India in the year 1958 [2]. The disease is found predominantly amongst certain high-risk communities belonging to schedule caste, schedule tribe and other backward classes. Approximately 3.2 million population belongs to high-risk communities in Central India. Anemia dominates the hematological profile so this study was undertaken to document and compare the hematological finding with other studies.

Material and method

Inclusion Criteria – Patients included in the study were in their steady state with relatively long period without any symptoms pertaining to SCD, and without any chronic conditions like renal failure, which can affect the hematological findings; those transfused within last 3 months were excluded from the study. These cases were attending the speciality clinic of RHDMC and reported from Central India and other neighboring states. Detail history was elicited to obtain information on all SCD related symptoms. Most of the blood samples are collected during OPD visit. Venous blood of all patients was collected in EDTA and hematological indices were measured on KX 21 Sysmex auto analyzer. Electrophoresis was done on hemolysate prepared from EDTA sample on cellulose acetate medium at pH 8.6. Quantitation of hemoglobin was done on Bio-Rad variant system using Beta-thal short program. Hematological profile was compared with other studies.

Observation

Total 81 ‘SS’ cases registered with RHDMC, IGGMC are studied for their hematological parameters. There are 44 males and 37 females (Table 1). Age ranges from 6 months to 64 years. Maximum numbers i.e. 27(61.36%) of males reported up-to 14 years of age, whereas maximum no. 23 (62.16%) of females reported in reproductive age group (Table 1). Males outnumbered females in pediatric age group whereas females outnumbered males in reproductive age group. Number of patients reporting after the age of 30 years is less in both sexes.

Average Hb observed in males was 7.11 ± 2.13 gms/dl while that in females was 6.75 ± 1.85 gms/dl (Table 2A).

No significant difference was observed in total hemoglobin of both the sexes (P value 0.42). Total hemoglobin is low in SCD patients more so in females. Total hemoglobin is significantly low till 14 years of age in both the sexes (P value for males < 0.0001 and for females 0.005) then, there is rise in total hemoglobin, till 29 years of age in males and till 39 years of age in females, and again there is fall in total hemoglobin in both the sexes after these ages (Table 2B). Fall is more in males as compared to females.

Average R.B.C count in Males is -2.69 ± 0.76 millions/cu mm while that in Females is -2.45 ± 0.72 millions/cu mm (Table 3). RBCs count is low in our study and significant positive correlation was found between total hemoglobin and total RBCs count (Figs. 1, 2). Average MCV in males is 85 ± 13.1 fl as against 89.8 ± 13.4 fl. in females (Table 4). Difference in MCV in males and females is not statistically significant (P value 0.19). Age related rise in MCV is seen in both the sexes after the age of 5 yrs, however this rise is not statistically significant (P value 0.25 for males and 0.41 for females). Rise in MCV in females is more marked in 10 – 39 yrs of age. There is no significant correlation between MCV and total hemoglobin in both the sexes (Figs 3 and 4). (P value for males 0.02 and for females 0.99). Average MCHC in males is $31 \pm 64\%$, while that in females is $31.02 \pm 1.76\%$ (Table 5). No age or sex related difference was seen in MCHC values.

Average Hb A₂ values in males and females were $2.13 \pm 0.95\%$ and $2.04 \pm 0.91\%$ respectively (Table 6A). There is fall in Hb A₂ % above 19 yrs of age and this is statistically significant in females (P value 0.021) but not in males (P value 0.11) (Table 6B). It is also observed that in males there

Fig. 1 Showing correlation of RBC count with total Hb in males.

Fig. 2 Showing correlation of RBC count with total Hb in females

Fig. 3 Showing correlation between MCV and total hemoglobin in males. (P value 0.02).

Fig. 4 Showing correlation between MCV and total hemoglobin in females. (P value 0.99).

Table 1 Age and sex wise distribution of sickle cell disease cases

S.No.	Age Grp.	Male No-44 (%)	Female No-37 (%)
1	0-4 yrs	8 (18.18)	3 (8.1)
2	5-9 yrs	12 (27.27)	7 (18.91)
3	10-14 yrs	7 (15.9)	3 (8.1)
4	15-19 yrs	2 (4.54)	8 (21.62)
5	20-24 yrs	5 (11.36)	11 (29.72)
6	25-29 yrs	7 (15.9)	-
7	30-39 yrs	1 (2.27)	4 (10.81)
8	40-49 yrs	1 (2.27)	-
9	50-59 yrs	1 (2.27)	-
10	60-70 yrs	-	1 (2.7)

Maximum numbers i.e. 27(61.36%) of males reported up-to 14 years of age, whereas maximum no. 23 (62.16%) of females reported in reproductive age group.

Table 2A Age and Sex wise distribution of Total Hemoglobin

S. No	Age Grp.	Male Hb gms/dl M±SD	Female Hb gms/dl M±SD
1	0-4 yrs	6.1 ±1.89	5.43±2.19
2	5-9 yrs	6.37± 2.01	5.3 ±1.97
3	10-14 yrs	5.85 ±0.88	6.33 ±1.0
4	15-19 yrs	7.7± 1.27	7.28± 2.23
5	20-24 yrs	10.1 ±1.25	7.37± 1.49
6	25-29 yrs	8.67 ± 2.03	-
7	30-39 yrs	6.5	7.55 ±0.61
8	40-49 yrs	6.8	-
9	50-59 yrs	6.9	-
10	60-70 yrs	-	7.9

Average Hb observed in males was 7.11 ± 2.13 gms/dl while that in females was 6.75 ± 1.85 gms/dl. No significant difference was observed in total hemoglobin of both the sexes (P value 0.42).

Table 2B Age and Sex wise distribution of Total Hemoglobin below and above 14 yrs of age

Age Grp.	Hemoglobin in Males	Hemoglobin in Females
Below 14 yrs	6.16±1.71	5.57±1.76
Above 14 yrs	8.64±1.88	7.4±1.6
P value	< 0.0001	0.005

Total hemoglobin is significantly low till 14 years of age in both the sexes (P value for males < 0.0001 and for females 0.005) then, there is rise in total hemoglobin.

Fig. 5 Showing inverse correlation between MCV and Hb A₂ in males (P 0.05).

Fig. 6 Showing correlation between MCV & Hb A₂ (P 0.09) in females.

Fig. 7 Showing correlation between MCV & HbF in males (P 0.74).

Table 3 Age and sex wise distribution of RBC count

S. No.	Age Grp.	Male RBCs millions/ cumm	Female RBCs millions/ cumm
1	0-4 yrs	2.29 ± 0.89	2.21 ± 0.96
2	5-9 yrs	2.37 ± 0.64	2.14 ± 1
3	10-14 yrs	2.36 ± 0.4	2.29 ± 0.44
4	15-19 yrs	2.96 ± 0.18	2.7 ± 0.88
5	20-24 yrs	3.58 ± 0.33	2.51 ± 0.57
6	25-29 yrs	2.9 ± 0.8	-
7	30-39 yrs	2.51	2.63 ± 0.22
8	40-49 yrs	2.23	-
9	50-59 yrs	4.32	-
10	60-70 yrs		2.63

Table 4 MCV analyzed according to age & sex

.		Male	Female
S.No	Age Grp.	MCV fl M±SD	MCV fl M±SD
1	0-4 yrs	88.22±8.22	82.66±12.34
2	5-9 yrs	81.65±13.74	85.88±15.68
3	10-14 yrs	80.04±7.12	90.76±10.4
4	15-19 yrs	83.85±12.79	90.61±18.69
5	20-24 yrs	89.86±12.45	93.6±12.41
6	25-29 yrs	94.3±17.58	-
7	30-39 yrs	82.5	88.7± 4.9
8	40-49 yrs	93.7	-
9	50-59 yrs	57.6	-
10	60-70 yrs	-	94.7

Difference in MCV in males (85 ± 13.1) & females (89.8 ± 13.4 fl) is not statistically significant (P value 0.19). Age related rise in MCV is seen in both the sexes after the age of 5 yrs, however this rise is not statistically significant (P value 0.25 for males & 0.41 for females). Rise in MCV in females is more marked in 10–39 yrs of age

is inverse correlation between MCV and Hb A₂ (P 0.05, Fig. 5), however no significant correlation is seen in females (P value 0.09, Fig. 6).

Average Hb F values in males and females were 19.58 ± 5.86 % and 20.98 ± 4.79 % respectively (Table 8). Significant negative correlation between HbA₂ and HbF is seen in males (p value 0.002 Fig. 9) as well as in females (P value 0.02 Fig. 10). Age related difference in HbF levels is not seen. There is no significant correlation between MCV and HbF in males (P 0.74, Fig. 7) as well as in females (P value 0.09 Fig. 8).

Fig. 8 showing correlation between MCV & HbF in females (P value 0.09) .

Fig. 9 showing correlation between HbA₂ & HbF in males (p value 0.002).

Fig. 10 showing correlation between HbA₂ & HbF in females (P value 0.02).

Table 5. Age and Sex wise distribution of MCHC

S. No.	Age Grp.	Male	Females
		MCHC % M ±SD	MCHC % M ±SD
1	0-4 yrs	30 ±1.08	30.1± 1.03
2	5-9 yrs	30.98 ±1.47	29.8 ±1.72
3	10-14 yrs	31.64 ±2.03	30.73± 1.88
4	15-19 yrs	31.05± 2.33	30.85 ±1.82
5	20-24 yrs	31.62± 1.31	31.6 ±1.78
6	25-29 yrs	31.37 ±1.65	-
7	30-39 yrs	31.4	32.65±0.22
8	40-49 yrs	32.5	-
9	50-59 yrs	27.3	-
10	60-70 yrs	-	31.7

Average MCHC in males is 31± 64%, while that in females is 31.02 ± 1.76 %.No age or sex related difference was seen in MCHC values.

Table 6A Age and Sex wise distribution of Hb A₂

S. No.	Age Grp.	Males	Females
		Hb A ₂ % M±SD	Hb A ₂ % M±SD
1	0-4 yrs	2.25± 1.1	2.33±1.01
2	5-9 yrs	2.49±1.02	2.9±0.85
3	10-14 yrs	2.18±0.77	2.33±1.15
4	15-19 yrs	1.6±0.98	2.02±0.43
5	20-24 yrs	1.68±0.91	1.63±0.81
6	25-29 yrs	1.74±0.57	-
7	30-39 yrs	1.5	1.62± 1
8	40-49 yrs	1.1	-
9	50-59 yrs	3.5	-
10	60-70 yrs	-	0.8

Average Hb A₂ values in males and females were 2.13 ± 0.95 % and 2.04 ± .91 % respectively.

Table 6B Age and Sexwise distribution of Hb A₂

Age Grp.	Hb A ₂ % in Males	Hb A ₂ % in Females
Below 19 yrs	2.21±1.05	2.29±0.96
Above 19 yrs	1.70±0.96	1.58±0.83
P value	0.11	0.021

There is fall in Hb A₂ % above 19 yrs of age and this is statistically significant in females (P value 0.021) but not in males (P value 0.11).

Discussion

Hematological profile of 81 sickle cell disease patients from Central India is reported. Males outnumber females in pediatric age group whereas females outnumber males in repro-

ductive age group. This may be on account of the repeated crisis during this period and the attention that the male child gets as compared to female child. Where as females get more attention in reproductive age group, as additional burden of pregnancy predispose these patients to frequent crisis and awareness created about SCD in last 5 years has prompted them to register early in ANC clinic. Number of elderly patients attending SCD OPD is less either on account of death or apathy towards elderly because of economic constraints. Total hemoglobin is low in SCD patients more so in females as compared to males (Table 8B), although this difference is not statistically significant(P value 0.42). It may be due to chronic hemolytic process, blood loss due to haematuria [7], burden of multiple pregnancies, nutritional deficiencies because of low socio-economic status. Total hemoglobin is low till 14 years of age in both the sexes (P value 0.0001 in males and 0.005 in females) on account of repeated infections in childhood, nutritional deficiencies and increase requirement in the growing age in addition to chronic hemolytic process and frequent crisis in this period. There is significant rise in total hemoglobin, from 14 yrs till

29 years of age in males and till 39 years of age in females (P value 0.0001 in males and 0.005 in females) . Rise in hemoglobin after 14 years of age maybe due to hormonal changes at puberty in males [8] and in females may be due to additional medical and dietary attention during pregnancies. Fall in hemoglobin level after this age may be due to sub clinical renal insufficiency which progresses with the age [9] or possibly some marrow failure of unexplained cause [6]. More or less similar age related pattern of hemoglobin level was observed by RJ Hayes et al 1985 [6]. RBCs count is low in our study as compared to other studies (Table 8) and significant positive correlation was found between total hemoglobin and total RBCs count (Figs. 1, 2). Age related rise in MCV is seen in both the sexes after the age of 5 yrs (Table 6). This is not statistically significant (p value for males 0.25 and for females 0.41). Rise in MCV in females between 10–39 yrs of age is more marked but not significant. Normally sickle cell disease patients are at a critically balanced state of Vit B-12 and folic acid so slight increase in demand due to increase need for erythropoiesis because of chronic hemolysis or haematuria and pregnancy can precipitate deficiency state, leading to macrocytosis. High values of MCV in females and rise in MCV values between 5-29 yrs of age was also observed by RJ Hayes et al 1985 [6]. No significant correlation was found between MCV and total hemoglobin (Figs. 3, 4). But Kar et al found significant negative correlation between MCV and total Hb only in males and not in females [3]. No significant correlation was found between MCV and Hb F in our study (Figs 7 and 8). Kar et al [3] noted similar finding. Significant negative correlation was found between MCV and Hb A₂ levels in our study (Figs. 5, 6). Kar et al [3] note similar findings. No age or sex related difference was seen in MCHC values (Table 5).

Average HbA₂ levels in our studies are comparable with other studies (Table 8) . Average Hb F is 19.62% in our study this is significantly higher as compared to other studies. Central Indian patients have high HbF and low hemoglobin be high in this region. No sex related difference in Hb F values was observed in present study

Table 7 Age and sexwise distribution of Hb F %

S. No.	Age Grp.	Males	Females
		Hb F % M ±SD	Hb F % M ±SD
1	0-4 yrs	23.87±3.64	25.66±4.48
2	5-9 yrs	17.85±6.29	18.92±3.42
3	10-14 yrs	20.61±3.9	18.8±4.01
4	15-19 yrs	19.6±1.55	18.85±6.15
5	20-24 yrs	23.06±4.79	22.62±4.32
6	25-29 yrs	15.77±4.58	-
7	30-39 yrs	26.6	21.15±3.32
8	40-49 yrs	15.3	-
9	50-59 yrs	5	-
10	60-70 yrs	-	25.9

Average Hb F values in males and females were 19.58 ± 5.86 % and 20.98 ± 4.79 % respectively (Table VII).

Table 8 Comparison of Haematological finding of present study with Other studies from Orissa and Jamaica

Haematological parameter	Present study N-82	Kar et al ³ 1986 N - 130	Roy et al ⁴ 1996 N - 21	Kaur et al ⁵ 1997 N - 34	Hayes et al ⁶ 1985, Jamaica N - 1071
Hb gms/dl	6.95±2.01	8.73±1.69	8.1±1.4	8.6±2.1	8.04±1.12
RBCmillions/cumm	2.58±0.75	3.35±0.79	3±0.6	2.7±0.8	2.77±0.54
MCV fl	87.63±13.29	83.6±9.4	78.6±9.0	98.3±10.5	87.3±8.1
MCHC pg	31.01±1.68	31.5 ±2.7	33.1±5.8	30±2	29.7±3.4
Hb A ₂ %	2.09±0.93	1.89±0.52	-	-	2.96±0.45
Hb F %	20.21±5.41	16.64±5.42	-	-	6.11±4.21

(Table 7). Similar findings are noted by Rucknagel et al 1979 [12]. Significant negative correlation was found between HbF and Hb A₂ levels (Figs. 9, 10). Kar et al [3] noted similar finding. There is inverse correlation between Hb F and Hb A₂ [13, 14]. Hb A₂ values are low in Indians as compared to Jamaican patient [3]. No significant correlation was found between Hb F and total hemoglobin. This is evident from our study that both sexes are having more or less similar and higher Hb F levels but total haemoglobin is low in females as compared to males. Moderate to severe anaemia and high HbF dominate the hematological profile of central Indian Sick cell Disease.

References

1. Mohanty D and Pathare AV (1998) Sick cell Anemia – the Indian scenario. *Indian J. Hematol Blood Transfus* 16(1–1): 1–2
2. Solanki BR, Shukla RN (1958) Sick cell trait in Central India *Lancet* 1:297
3. Kar BC, Satapathy RK, Kulozik M, Sirm S, Serjeant BE, Serjeant GR. (1986) Sick cell disease in Orissa state, India. *Lancet* ii, 1198–1201
4. Roy B, Dey B, Balgir RS, Dash BP, Chakraborty M, Bhattacharya SK, Banerjee S, Roy M, Majumder PP. (1996) Identification of sickle cell homozygotes using haematological parameters. *J Indian Anthropol Soc* 31:191–199
5. Kaur M, Das GP, Verma IC. (1997) Sick cell trait and disease among tribal communities in Orissa, Madhya Pradesh and Kerala. *Indian J Med Res* 105:111–116
6. Hayes RJ, Beckford M, Grandison Y, Mason K, Serjeant BE, Serjeant GR. (1985) The haematology of steady state homozygous sickle cell disease: frequency distributions, variation with age and sex, longitudinal observations. 59:369–382
7. Wong YW, Elliott-Mills D, Powars D. (1996) Renal failure in sickle cell anaemia. *Haematol Oncol Clinics of N Am* 10: 1321–1331
8. Lundh B, Gadner FH. (1970) The haematologic response to androgens in sickle cell anaemia. *Scandinavian J Hematol* 7:389–397
9. Sklar AH, Campbell H, Ccaruana RJ, Ligjtfoot BO, Gaier JG, Milner P. (1990) A population study of renal function in sickle cell anaemia. *Internatl J Artific Organs* 13: 231–236
10. De Ceulaer K, Higgs DR, Weatherall DJ, Hayes RJ, Serjeant BE, Serjeant GR. (1983) A – thalassaemia reduces the haemolytic rate in homozygous sickle cell disease. *N Engl J Med* 309:189–190
11. Serjeant GR, Serjeant BE (1983) sickle cell disease. 3rd ed. Jamaica: Oxford university press year: 430–431
12. Rucknagel DL, Hanash SH, Sing CF, Winter WP, Whitten CF, Prasad AS. (1979) Age and sex effect on haemoglobin F in sickle cell anemia In Stamatouannopoulos G, and Nienhuis AW. cellular and molecular regulation of haemoglobin switching; Grune and Stratton, New York pp 107–118
13. Serjeant GR, (1975) Fetal haemoglobin in homozygous sickle cell disease. *Clinics in Haematol* 4:109–122
14. Mason KP, Grandison Y, Hayes RJ, Serjeant BE, Serjeant GR, Vaidya S, Wood WG. (1982) Postnatal decline of fetal haemoglobin in homozygous sickle cell disease: relationship to parental HbF levels. *British J Haematol* 52:455–463