The Screening and Morbidity Pattern of Sick Cell Anemia in Chhattisgarh

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Abstract Our objective was to find out prevalence of sickle cell anemia among the population of three districts (Kanker, Dantewada and Raigarh) of Chhattisgarh with clinical and hematological profile of sickle cell disease patients. A cross sectional study was done. A total of 15,701 persons collectively from three districts voluntarily attended the mobile camp and were screened for sickle cell anemia. First solubility test were done and were confirmed by Hb electrophoresis. The prevalence of sickle cell trait (HbAS) was 1,672 (10.6 %), sickle cell disease (HbSS) and inconclusive band was 97 (0.66 %). The HbSS and inconclusive band were subjected to HPLC. Among them 12 (0.076 %) cases were double heterozygous for Hb-S and beta thalassemia minor (SB+), 2 (0.012 %) cases were double heterozygous for Hb-S and Hb-E (S/HBE), 1 (0.006 %) case was double heterozygous for Hb-S and Hb-D Punjab (S/HBD) and 22 (0.14 %) cases had Hb-S with Hb-F level more than 20 % (SSF). Maximum number of HbSS cases were 13 (2.29 %) out of 567 children in the age group 0–5 years and HbAS cases were 124 (15.6 %) out of 794 persons in the age group 21–25 years. On comparison between vaso-occlusive and steady state, homozygous patients showed decrease in Hb, HCT, MCH, RBC in vaso-

occlusive crises ($p < 0.001$) than steady state. Also there was one moderate negative correlation in number of blood transfusion ($r = 0.46$) with fetal hemoglobin (HbF) level. Patients with high HbF can have severe disease. This happens due to uneven distribution of fetal hemoglobin in F-cells with mean HbF remaining constant but in our study, those who had HbF level above 15–20 % were having fewer crises.

Keywords Screening · Morbidity · Sickle cell anemia · Chhattisgarh

Introduction

Sickle cell anemia was first described in south Indian tribal groups [1] and it was also subsequently recognized in Central India [2]. The frequency of sickle cell trait in India is 4.3 % [2]. In Orissa and Chhattisgarh frequency of sickle cell anemia are approximately 9 and 10 % respectively [3, 4]. Chhattisgarh is formed from south eastern parts of Madhya Pradesh in the year 2000 and 32 % of population is tribal origin [4].

As a part of project funded by Department of Biotechnology, Government of India, we screened the population of Raigarh, Kanker and Dantewada districts of Chhattisgarh for Sickle cell anemia and their morbidity pattern. Collectively these three districts have a population of 2,635,950 according to 2001 census [5]. Raigarh have a mixed population of tribal and non-tribal origin where as Kanker and Dantewada area is hilly area with tribal population. We are reporting the 1st phase of screening done in above mention areas of Chhattisgarh with caste wise distribution of the sickle cell anemia and its morbidity.

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Materials and Methods

According to the previous study done in Chhattisgarh, sickle cell trait occurred in 9.30 % and an SS phenotype in 0.21 % [4]. So sample size was calculated by using W.H.O. software of sample size calculation version 2.0 [6]. From previous studies, the prevalence of sickle cell anemia (HbAS + HbSS) is 10 %, with alpha 0.05 and error margin of 5 %, the sample size was estimated to be 15,358. We have screened 15,701 individuals including children above 1 year and reported the study. The study was cleared by ethical committee of Pt.J.N.M. Medical College, Raipur (Chhattisgarh).

The objective of our study was to find out prevalence of sickle cell anemia. To know the clinical and hematological profile of patients suffering from sickle cell disease. Our study was cross sectional, observational and analytical study. The study was done in between March 2009 to July 2011.

For the screening, mobile camps were organized by Dept. of Biochemistry. A doctor, two laboratory technician and a counselor visited the places. A written consent in local language was obtained from the individuals who volunteered themselves for screening. Children above 1 year, whose parents gave a written consent, were included in the study. Team of technicians and other staff take along with them freshly prepared solubility buffer as described in Dacie and Lewis practical hematology (10th edition) [7] with little modification that we add pinch (approx. 0.1 g) of sodium dihyonate to 10 ml buffer solution before testing. We took 10 µl whole blood for 1.0 ml solubility buffer or 20 µl whole blood for 2.0 ml solubility buffer. Since it is screening test finger prick whole blood collection is convenient to patient and is economical to us. In anemic patients we took 20 µl bloods in 1.0 ml buffer. It is an indigenous modification and standardized in our base laboratory against hemoglobin HPLC testing using Bio-Rad system. We run positive and negative controls with each set of test samples. The limit of detection of HbS for this test is 20 %, below which the test may give false negative results that's why it is not suitable for neonatal screening and in post transfusion patients. False negative results are obtained when Hb is low or the reagents are out dated or contaminated. False positive results are obtained in severe leucocytosis, hyperproteinemia and presence of unstable Hb but these cases are sorted out in next step that is Hb electrophoresis. Those patient who tests positive (including false positives) for solubility test we collect 3.0 ml venous blood in EDTA vacutainer from them and send to base laboratory at Medical College Raipur for alkali Hb electrophoresis. Alkali hemoglobin electrophoresis on cellulose acetate gel paper was performed. In electrophoresis we get three types of clear bands that is AS

band, SS band and inconclusive band which may be SSF, SB+, S/HBE, S/HBD, S/HBC and other insoluble Hb variants.

Those samples that had SS band and other inconclusive band were subjected to HPLC Hb variant analysis using beta thal. Short program pack in Bio-Rad variant HPLC machine in the base laboratory at Medical College, Raipur. In homozygous cases, the EDTA sample of blood was preserved; for complete blood count which was done by using BC-300 Plus Auto Hematology analyzer and ICT malaria screening for those who had splenomegaly.

The homozygous and inconclusive band cases which were detected by Hb electrophoresis were checked by the doctor in the next visit. A Performa with detailed clinical history, physical examination, description of previous symptoms, number of transfusion, hospitalizations, severe painful episodes, treatment, complications and finding on physical examinations were filled up. Statistical analysis was done using SPSS software version 10. The statistics like chi-test, *t* test for mean, Pearson correlation coefficient (*r*) in relation to HbF with clinical severity, blood transfusion and hematological parameters were analyzed. For absolute values of *r*, 0–0.19 was regarded as very weak, 0.2–0.39 as weak, 0.40–0.59 as moderate, 0.6–0.79 as strong and 0.8–1 as very strong correlation [8].

Results

15,701 (7,959 were males and 7,742 were females) were screened for sickle cell anemia. 1,769 tested positive for solubility. Their venous blood were collected in EDTA and subjected to alkali Hb electrophoresis. 1,672 (10.6 %) cases were having sickle cell trait (AS) and 97 (0.6 %) cases were having sickle cell disease and other associated bands (Table 1). These 97 cases were subjected to Hb variant analysis by HPLC to find out other associated bands. Among them 12 (0.076 %) cases were double heterozygous for Hb-S and beta thalassemia minor (SB+), 2 (0.012 %) cases were Double heterozygous for Hb-S and Hb-E (S/HBE), 1 (0.006 %) case was double heterozygous for Hb-S and Hb-D Punjab (S/HBD) and 22 (0.14 %) cases had Hb-S with Hb-F level more than 20 % (SSF). We could not do family studies as both parents were not available for screening at a time in all cases. Hardy–Weinberg equilibrium was difficult to apply to our population as the condition of random mating and no migration, mutation or selection was not met. The age wise distribution of cases has been shown in Table 2. Maximum number of sickle cell disease was 13 out of 567 (2.29 %) (95 % CI 1–3.53) in the age group 1–5 years and sickle cell trait was 124 out of 794 (15.6 %) (95 % CI 13–18) persons in the age group 21–25 years. It is notable that as the age increases there is drop in SS cases where as

Table 1 Gender distribution

Phenotype	Male	Female	Total (%)	95 % CI
AS	826	846	1,672 (10.6 %)	10.3–11.2
SS	51	46	97 (0.6 %)	0.49–0.74
Total	877	892*	1,769	

* $P > 0.001$ not significant in gender wise distribution of sickle cell gene

the number of AS cases remain more or less the same in different age groups.

Caste and sub caste wise distribution of sickle cell anemia ($N = 1,769$) (Table 3) shows Teli sub caste of OBCs have highest 232 (13.1 %) prevalence of sickle cell anemia, followed by Panika 97 (5.48 %), Agharia 90 (5.08 %) and Rawat 88 (4.97 %). Among the SCs the Satnami sub caste have highest prevalence 110 (6.21 %) of sickle cell anemia followed by Ganda 101 (5.70 %), Mahar 88 (4.97 %), and Gharsiya 26 (1.46 %). Visibly high prevalence of sickle cell anemia cases were found in Gond sub caste of ST community with 365 (20.63 %) followed by Halbi 184 (10.40 %), Khadiya 89 (5.03 %), Sanwra 67 (3.78 %) and Uranmi 58 (3.27 %).

Out of 97 cases of homozygous and other variants, 47 cases presented with history of different crisis and 50 were in stable state. The most common crisis was vaso-occlusive crisis presenting with musculoskeletal pain 26 (26.8 %), pain abdomen in 22 (22.6 %), Joint swelling in 9 (10.3 %), acute chest syndrome 2 (3 %), dactylitis 1 (2 %), C.N.S crisis 1 (2 %). The second common crisis was hemolytic crisis 13 (14 %), one patients had undergone splenectomy. No case of leg ulcer was found. Large splenomegaly of >9 cm below left costal margin [9, 12, 14] was found in 11 (12 %) and splenomegaly of <9 cm below left costal margin found in 35 (36 %) of cases. Large splenomegaly was found in homozygous cases above the age of 16 years in late adolescence and adults' cases. Four cases in 16–20 years, five cases in 21–25 years and two cases above 25 years. All splenomegaly cases were screened for malaria antigen, which was negative.

Table 2 Age wise distribution

Age (years)	AA (n)	%	AS (n)	%	95 % CI	SS (n)	%	95 % CI	Total (n)
0–5	482	85	72	12.6	9.9–15.3	13	2.29	1–3.53	567
6–10	3,602	91.6	314	7.99	7.1–8.6	13	0.33	0.15–0.51	3,929
11–15	4,973	89.2	580	10.4	9.5–11.2	21	0.37	0.21–0.53	5,574
16–20	2,373	87.4	325	11.9	10.7–13.2	16	0.58	0.30–0.87	2,714
21–25	656	82.6	124	15.6	13–18	14	1.76	0.88–2.63	794
>25	1,849	86.9	257	12.1	10.7–13.4	20	0.94	0.53–1.35	2,123
Total	13,932	88.7	1,672	10.6	10.3–11.2	97	0.61	0.49–0.74	15,701

Complete blood count was done in all the homozygous cases. Most notable finding was microcytic anemia with low hemoglobin (Hb), mean cell volume (MCV), hematocrit (HCT) and mean cell hemoglobin (MCH) values (Table 4). The hematological parameters were compared between vaso-occlusive and steady state homozygous patients. There was statistical significant difference in Hb concentration ($p < 0.0001$), HCT ($p < 0.005$), MCH ($p < 0.0001$), red cell distribution width ($p < 0.0001$) and red blood cell ($p < 0.005$). Other parameters did not show any significant difference (Table 5). The relationship between HbF level as a continuous variable with sickle cell disease severity is shown in (Table 6). There was a moderate negative correlation in number of blood transfusion ($r = 0.46$, 95 CI 0.073–1.08). Other parameters were statistically not significant. Out of 97 cases of sickle cell disease and other variant, 47 cases (48.45 %) were transfused with blood with a median of 3 and range from 1 to 30. Other 50 cases did not required blood transfusion as their fetal hemoglobin was high.

Discussion

In the Present study prevalence of sickle cell anemia is around 11 % (1,769 out of 15,701 persons screened) with HbAS being 1,672 cases (10.4 %), HbSS and other inconclusive band being 97 cases (0.66 %). After doing HPLC of these 97 cases, we found 12 (0.076 %) cases were double heterozygous for Hb-S and beta thalassemia minor (SB+), 2 (0.012 %) cases were Double heterozygous for Hb-S and Hb-E (S/HBE), 1 (0.006 %) case was double heterozygous for Hb-S and Hb-D Punjab (S/HBD) and 22 (0.14 %) cases had Hb-S with Hb-F level more than 20 % (SSF). The percentage have been calculated from the total 15,701 person screened. The 12 cases of double heterozygous for sickle plus beta thalassemia minor were diagnosed solely on basis of elevated A2 levels on HPLC as parental screening was not performed. In absence of parental screening some cases of sickle-beta zero heterozygosity might have been misclassified as SS with iron

Table 3 Caste wise distribution of sickle cell anemia ($N = 1,769$)

Caste	Hob (AS) N (%)	95 % CI	Hob (SS) n (%)	95 % CI	SCA (AS + SS) n (%)	95 % CI
OBC						
Agharia	83 (4.69)	3.70 to 5.67	7 (0.39)	0.10 to 0.68	90 (5.08)	4.06–6.11
Dhobi	7 (0.4)	0.10 to 0.68	1 (0.05)	−0.05 to 0.16	8 (0.45)	0.13–0.76
Kevat	16 (0.9)	0.46 to 1.34	2 (0.11)	−0.04 to 0.26	18 (1.01)	0.54–1.48
Kolta	11 (0.62)	0.25 to 0.98	2 (0.11)	−0.04 to 0.26	13 (0.73)	0.33–1.32
Kumhar	2 (0.11)	−0.04 to 0.26	0 (0)	0	2 (0.11)	0.04–0.26
Kurmi	39 (2.20)	1.52 to 2.88	11 (0.62)	0.25 to 0.98	50 (2.82)	2.05–3.59
Lohar	4 (0.22)	0.004 to 0.44	1 (0.05)	−0.05 to 0.16	5 (0.28)	0.03–0.53
Marar	11 (0.62)	0.25 to 0.98	1 (0.05)	−0.05 to 0.16	12 (0.67)	0.29–1.06
Nayi	13 (0.73)	0.33 to 1.13	1 (0.05)	−0.05 to 0.16	14 (0.79)	0.37–1.2
Panika	95 (5.37)	4.31 to 6.42	2 (0.11)	−0.04 to 0.26	97 (5.48)	4.42–6.54
Ravat	85 (4.80)	3.80 to 5.80	3 (0.16)	−0.02 to 0.36	88 (4.97)	3.96–5.98
Teli	208 (11.75)	10.25 to 13.25	24 (1.35)	0.81 to 1.89	232 (13.11)	11.54–14.68
SC						
Gharsiya	25 (1.41)	0.86 to 1.96	1 (0.05)	−0.05 to 0.16	26 (1.46)	0.90–2.03
Ganda	96 (5.42)	4.37 to 6.48	5 (0.28)	0.03 to 0.53	101 (5.7)	4.62–6.79
Mahar	88 (4.97)	3.96 to 5.98	0 (0)	0	88 (4.97)	3.96–5.98
Satnami	98 (5.53)	4.47 to 6.60	12 (0.67)	0.3 to 1.06	110 (6.21)	5.02–7.34
ST						
Gond	352 (19.9)	18 to 21.7	13 (0.73)	0.33 to 1.13	365 (20.63)	18.74–22.5
Halbi	180 (10.1)	8.7 to 11.8	4 (0.22)	0.004 to 0.44	184 (10.4)	8.97–11.82
Khadiya	86 (4.86)	3.85 to 5.86	3 (0.17)	0.02 to 0.36	89 (5.03)	4.01–6.04
Sanwra	65 (3.67)	2.8 to 4.55	2 (0.11)	−0.04 to 0.26	67 (3.78)	2.89–4.67
Uranwi	56 (3.16)	2.34 to 3.98	2 (0.11)	−0.04 to 0.26	58 (3.27)	2.44–4.10
GEN						
Brahmin	14	0.79	0 (0)	0	14 (0.79)	0.37–1.20
Kshatriya	16	0.90	0 (0)	0	16 (0.90)	0.46–1.34
Muslim	9	0.50	0 (0)	0	9 (0.50)	0.17–0.84
Rajput	13	0.73	0 (0)	0	13 (0.73)	0.33–1.132

The number in parenthesis are percentage

OBC other backward class, *SC* schedule caste, *ST* schedule Tribe, *GEN* general caste, n number of cases, N number of total sickle cell (HbSS + HbAS) from which percentage is calculated, *CI* confidential interval

deficiency. The prevalence of sickle cell anemia was high 16.5 % in children of southern Orissa and 11.5 % in western Orissa [9, 10, 13]. In the spectrum of hemoglobinopathies in Orissa state, the sickle cell trait and sickle cell disease was reported as 29.8 and 7.6 % respectively [11]. In Garasia tribal of Rajasthan, the prevalence of sickle cell gene was 9.2 % [12]. So our study is almost equal to western Orissa study which is adjacent to Chhattisgarh.

In age group distribution max-population came for voluntary screening was in age group 11–15 years, maximum SS were found in 0–5 years and maximum AS was found in 21–25 years of age. 50 % of children in India have 1st manifestation by 5 years of age and another 25 % by 10 years of age [13]. One Clinical study in Orissa have found that 52 % of SS patients are under 15 years of age and maximum frequency was in 5–9 years [10]. In

southern Orissa 31.5 % of sickling positive cases were under 5 years, 60.5 % in 5–9 years and 7.8 % above 10 years of age [9].

In the present study, apart from schedule tribe prevalence 43.0 % and schedule caste prevalence 18.26 %, the prevalence of sickle cell anemia in OBC group is 35.3 % with Teli, Agharia, Kurmi and Panika constituting the major proportion. Sahu et al. [9] have found only SC, ST population having sickle cell anemia. Balgir have found the prevalence of sickle cell disorder 24 and 8 % in SC and ST respectively. In general caste it was 64.5 % in which Khandayat constitute 20.7 %, chasa 19.8 %, Kshatriya 6.8 %, Agharia 6.8 % and Teli 2 %. They have included OBC in General Caste [11].

In Rural area as most of the investigation is not available for detecting sickle cell anemia, the signs and symptoms

Table 4 Hematological data on 97 sickle cell disease patients

Parameters	Mean (SD)	Range	95 % CI
Hb (g %)	8.2 (2.6)	1.2–13.2	7.7–8.7
HCT (%)	28.26 (11.16)	11–63	26–30
Platelet count ($10^3/\mu\text{l}$)	284.18 (171.57)	26–812	250–318
WBC ($10^3/\mu\text{l}$)	14.63 (8.36)	4.9–41.3	12.96–16.29
RBC ($10^6/\mu\text{l}$)	3.74 (2.02)	0.43–15.7	3.3–4.1
MCV (fl)	73.59 (8.92)	51–97	71.8–75.3
MCH (pg)	30.85 (9.24)	11–63.1	29–32.69
MCHC (g/dl)	33.26 (4.39)	23.9–53.9	32.39–34.13
HbF (%)	16.1 (4.89)	4.4–25	15–17

Hb hemoglobin, *HCT* hematocrit, *WBC* white blood cells, *RBC* red blood cells, *MCV* mean cell volume, *MCH* mean cell hemoglobin, *MCHC* mean cell hemoglobin concentration, *HbF* fetal hemoglobin, *CI* confidential interval

Table 5 Comparison of hemogram in VOC and NON-VOC patients

	VOC Mean (SD)	Non-VOC (steady state) Mean (SD)	<i>P</i> value
Hb (g/dl)	6.5 (2.27)	11.39 (3.6)	0.005
MCV (fl)	76 (8.86)	80 (12.28)	0.07
HCT (%)	21.4 (6.79)	34.82 (11.1)	0.005
MCHC (g/dl)	33.3 (3.43)	33.2 (5.16)	0.9
MCH (pg)	26.62 (3.39)	34.82	0.001
RBC ($\times 10^6/\text{l}$)	6.682 (2.15)	11.40 (4.40)	0.005
RDW (%)	19.64 (3.37)	22.74 (20.13)	0.0001
Platelet ($\times 10^9/\text{l}$)	264 (164)	284 (177.67)	0.8
WBC ($\times 10^9/\text{l}$)	15.1 (7.9)	13.86 (8.82)	0.4
Hb F (%)	15.51 (6.16)	20.76 (6.79)	0.004

Hb hemoglobin, *HCT* hematocrit, *WBC* white blood cells, *RBC* red blood cells, *MCV* mean cell volume, *MCH* mean cell hemoglobin, *MCHC* mean cell hemoglobin concentration, *HbF* fetal hemoglobin, *RDW* red cell distribution width. The results of *P*-value in bold letter is significant

like pallor, icterus, musculoskeletal pain, abdominal pain, splenomegaly and hepatomegaly should lead to high suspicious of sickle cell anemia where its prevalence is more. In present study the most common crisis in homozygous cases was vaso occlusive crisis presenting with musculoskeletal pain, abdominal pain, chest pain and hemolytic crisis presenting with jaundice. Splenomegaly was found in 57 % and hepatomegaly 31 %, one patient had undergone splenectomy. Mandot et al. [12] have found musculoskeletal pain 64 %, abdomen pain 35 %, chest pain 7 % and splenomegaly 71 % in homozygous cases. The high incidence may be due to their hospital based study. Sahu et al. [9] have found pain abdomen in 4.7 % cases, musculoskeletal 0.6 and 30 % splenomegaly. Lower incidence may be due to their community based study. Higher prevalence of splenomegaly (67.4 %) has been reported in Indian

Table 6 Correlation between clinical characteristic and Hb F level

	HbF levels Pearson coefficient ' <i>r</i> '	95 % CI
Age of diagnosis of SCA	0.038	−0.1613 to 0.2421
Number of episode of VOC	−0.413	−0.6813 to 0.04727
Age of first B.T	0.029	0.1914 to 0.2478
Number of Hospitalization	−0.211	−0.4780 to 0.08435
Number of blood transfusion	0.46	0.073 to 1.081
Size of spleen	−0.055	−0.279 to 0.1506
White blood count	0.046	−0.1596 to 0.2492
Hemoglobin levels	0.2133	0.008804 to 0.4006
Platelet levels	−0.16	0.3594 to 0.03939

patient by Kar [13, 14]. In our study there were no cases of leg ulcer, priapism, and sequestration crisis.

In comparison of hematological values in sickle cell disease in steady state and during vaso-occlusive crises. The mean Hb, HCT, MCH, MCV and HbF were lower in vaso-occlusive state than stable state where as the red cell distribution width was higher in steady state than in vaso-occlusive state which was statistically significant. Other studies shows MCV and MCHC were higher in VOC than steady state [15]. In our study, we did not observed high MCV and MCHC in VOC state, as in our case the VOC causes mild to moderate degree of hemolysis leading to anemia, decrease in hematocrit level and MCH. The HbF is also low in VOC than in steady state which may contribute to more painful crisis. But when the correlation of HbF level and severity of SCD was compared there was moderate negative correlation in the number of blood transfusion but not in VOC and other crisis state. One study from Uganda have shown statistically negative correlation between HbF level and age of diagnosis, total number of hospitalization, number of severe painful episode and number of blood transfusion [16]. But our study had only one moderate correlation with number of blood transfusion and HbF level. Here the limitation was the sickle cell disease patients and the Parents in case of children could not remember the clinical events and limited medical records availability to know the severity of disease. Patients with high HbF can have severe disease. This happens due to uneven distribution of fetal hemoglobin in F-cells with mean HbF remaining constant. With high HbF, the F-cells might be insufficient to inhibit HbS polymerization. Only if the total HbF concentration near to 30 %, there is possibility of number of protected cells to 70 % [17]. So in our study, those who had HbF level above 15–20 % were having fewer crises. We could not do F cells count and haplotype study of beta globulin gene cluster due to technical limitation. Lack of knowledge about the

disease in the rural population also contributes to negligence of signs and symptoms. So information education and communication about the disease is required in the vulnerable population.

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Conflict of interest None.

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