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# Neonatal Screening in Central India for Sickle Cell Anaemia

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**ABSTRACT**

*Sickle cell disease is an inherited disease of red blood cells. It is characterized by pain episodes, anemia serious infections and damage to vital organs. Symptoms are caused by abnormal hemoglobin.. The sickle gene is prevalent in the tribal populations of India and Carrier frequencies range from 1–40% with the highest prevalence in central India The present study was carried out to estimate the distribution of sickle cell disease in neonates alongwith their various other parameters like caste, age geographic region. A targeted approach was taken and mothers affected with hemoglobinopathies were traced in ANC gynaecology wards to collect cord blood during their delivery. 40 such cases were taken and 10 controls gravida matched were taken.The cord blood samples were tested by HPLC and their complete cell count by Automated cell counter. The data showed that 34% newborns were affected with hemoglobinopathies (15FS & 2FS).Mahar caste showed the maximum prevalence 64.7% positive cases while scheduled castes overall showed 70% positive cases, 3 (17.6%) positive cases were found in scheduled tribes, while 1 (5.8%) in Muslim community.*

**KEYWORDS :**

## INTRODUCTION

Sickle cell disease is an inherited disease of red blood cells. It is characterized by pain episodes, anemia serious infections and damage to vital organs. Symptoms are caused by abnormal hemoglobin. Normally red blood cells are round, flexible and flow easily through blood vessels. But in sickle cell disease, the abnormal hemoglobin causes RBC's to become stiff. Under the microscope, these may look like a C shaped farm tool called a sickle. These stiffer red blood cells can get stuck in tiny blood vessels, cutting off the blood supply to nearby tissues. This is what causes pain (called a sickle cell pain episode/crisis) and sometimes organ damage in sickle cell disease. Sickle shaped red blood cells also die and break down more quickly than normal red blood cells, resulting in anemia.

There are several common forms of sickle cell disease: 1SS (individuals inherit one sickle cell gene from each parent).2SC (child inherits one sickle cell gene and one gene for another abnormal type of hemoglobin called "c") 3.S-beta-thalassemia: (the child inherits one sickle cell gene and one gene for beta thalassemia, another inherited anemia).

The sickle gene is prevalent in the tribal populations of India who are considered the original inhabitants living mainly in rural areas and in some non-tribal population groups like the scheduled castes and other backward classes belonging to a low socio economic status. Carrier frequencies range from 1–40% with the highest prevalence in central India (1).

The present study was carried out to estimate the distribution of sickle cell disease in neonates alongwith their various other parameters like caste, age geographic region.

### Material and Methods

The present study was carried out to study the prevalence of hemoglobinopathies in newborns born to affected mothers and also their caste-wise distribution. The present study was carried out at Regional Hemoglobinopathy Detection and Management Centre, Department of Pathology, Indira Gandhi Government Medical College, Nagpur which serves as a tertiary care institute in Vidarbha region of Maharashtra. Newborns born to the mothers affected with hemoglobinopathies delivered in IGGMC College were Study Subjects **.**Mothers were selected to carry out a targeted study irrespective of their origin, caste and ethnic background. Mothers who had received blood transfusion 3 months prior to delivery.were excluded from the study.

Before carrying out this study prior permission was taken from Obstetrics and Gynaecology HOD and Paediatrics HOD. The present study was duly passed by Ethical Committee. The mothers who were admitted in labour room in department of Obstetrics and Gynaecology for delivery were selected in a targeted manner, as referred above. Blood sample collection was carried out as follows in labour room and gynaecology OT. During labour and lower section caesarian section after separation of newborn, the cord blood was collected from the placental end of umbilical cord after cleaning with sterile swab. The 2 ml cord blood was collected in 4% K2 EDTA anticoagulant bulb. The sample bottles were properly labeled with name, registration number and date.

Relevant information regarding parents like name, address, caste and educational status, SCD status was noted. Detailed clinical history and h/o previous blood transfusion within 3 months prior to delivery was noted. Birth weight and sex of newborns were also noted.

The cord blood samples collected were subjected to various hematological tests in the hematology laboratory in Pathology department. Hematological parameter was studied using system KX-21 an automated multiparameter blood cell counter which analyses 18 parameters of blood and automated high performance liquid chromatography, which separates and determines the relative percentage of normal and abnormal hemoglobin. Maternal contamination of cord blood can occur at any stage of collection from cord, which can alter the hematological values.

The mothers affected with hemoglobinopathies were traced in the ANC Gynecology wards for counseling. They were told about the probability of their passing on the disease to their offspring, its complication and hence the need for collection of cord blood samples at delivery for early detection. Sometimes deliveries of the affected mothers could not be traced due to deliveries at home.

The newborns detected for haemoglobinopathies were asked for follow-up at 3 months and six months of age. The parents of the affected newborns were counseled about the disease and its clinical manifestations that indicate impending emergency such as increasing abdominal girth secondary to splenomegaly and increasing respiratory rate. The infants who were diagnosed with sickle cell disease were advised penicillin prophylaxis and active immunization by pneumococcal vaccine to achieve the objective of reducing morbidity and mortality.

## OBSERVATIONS & RESULTS

50 mothers were taken as subjects of which 10 were taken as controls and 40 as cases.

**TABLE 1**
**Hb Pattern-wise distribution of Mothers**

| S.No. | Hemoglobin Type | Total Cases | Percentage Distribution |
|---|---|---|---|
| 1) | AA | 10 | 20% |
| 2) | AS | 35 | 70% |
| 3) | AD | 1 | 2% |
| 4) | SS | 4 | 8% |

**TABLE - 2**
**Distribution of Cord Blood Samples for Hemoglobinopathies**

| Cord Blood Sample | Normal Hb pattern (FA) | Abnormal Hb (FS/FAS) | Total Cases |
|---|---|---|---|
| Number | 33 | 17 | 50 |
| Percentage | 66% | 34% | 100% |

**TABLE - 3**
**Hb Pattern-wise distribution of Cord Blood Samples**

| S.No. | Hemoglobin Type | Total Cases | Percentage Distribution |
|---|---|---|---|
| 1) | FA | 33 | 66% |
| 2) | FAS | 15 | 30% |
| 3) | FS | 2 | 4% |
| | Total | 50 | 100% |

**TABLE - 4**
**Caste-wise Distributions of Cord Blood Samples**

| Caste | Sub-Caste | Cases | Total | Percentage |
|---|---|---|---|---|
| Scheduled Caste | Mahar<br>Bouddha<br>Chambhar | 27<br>6<br>1 | 34 | 68 |
| Scheduled Tribes | Gauli<br>Halba<br>Gond<br>Katiya | 1<br>1<br>3<br>1 | 6 | 12 |
| O.B.C.* | Kunbi | 1 | 1 | 2 |
| Others | Muslim<br>Maratha<br>Thakur<br>Brahmin | 6<br>1<br>1<br>1 | 6<br>3 | 12<br>6 |
| TOTAL | | | 50 | 100 |

*OBC (Other Backward Classes)

**TABLE - 5**
**Caste-wise and Pattern-wise distribution of collected Cord Blood Samples**

| | Sub caste | Positive | Normal | Pattern-wise Distribution | | |
|---|---|---|---|---|---|---|
| | | | | FAS | FS | FA |
| Scheduled Caste | Mahar<br>Bouddha<br>Chambhar | 11<br>1 | 16<br>5<br>1 | 10 (37.03%)<br>1 | 1 (3.7%) | 16<br>5<br>1 |
| Scheduled Tribes | Gauli<br>Halba<br>Gond<br>Katiya | 1<br><br>1<br>1 | <br>1<br>2 | 1<br><br>1<br>1 | | <br>1<br>2 |
| O.B.C. | Kunbi | | 1 | | | 1 |
| Others | Muslim<br>Maratha<br>Thakur<br>Brahmin | <br>1<br>1 | 5<br><br>1<br>1 | <br>1 | 1 | 5<br><br>1<br>1 |
| | TOTAL | 17 | 32 | 15 | 2 | 33 |

**TABLE - 6**
**Hematological Profile of Normal Cord Blood Samples**

| S. No. | Hematological Parameter | Range | Mean | Standard Deviation | **Mean ± 2SD** |
|---|---|---|---|---|---|
| 1) | RBC in mil/ cu.mm. | 1.24 - 7.4 | 3.66 | 1.367 | (3.66 ± 2.73) |
| 2) | Hb gm% | 4.4 - 15.2 | 11.44 | 2.67 | (11.44 ± 5.34) |
| 3) | HCT % | 12.9-52.2 | 37.46 | 9.9 | (37.46 ± 19.8) |
| 4) | MCV (fl) | 94-132.1 | 107.15 | 8.45 | (107.15 ±16.9) |
| 5) | MCH (pg) | 27.5-39.7 | 32.48 | 2.84 | (32.48 ± 5.68) |
| 6) | MCHC (gm/dl) | 20.2-37.1 | 29.83 | 2.93 | (29.83 ± 5.86) |
| Hemoglobin fraction | | | | | |
| 1) | HbF% | 65 - 92.4 | 76.38 | 7.45 | (76.38 ± 14.9) |
| 2) | $HbA_0$ % | 5.8 - 27.8 | 16.87 | 5.71 | (16.87 ± 11.42) |
| 3) | $HbA_2$ % | 0- 1.5 | 0.07 | 0.29 | (0.07 ± 0.58) |

**TABLE - 7**
**Hematological Profile of FAS Cord Blood Samples**

| Sr. No. | Hematological Parameter | Range | Mean | Standard Deviation | **Mean ± 2SD** |
|---|---|---|---|---|---|
| 1) | RBC in mil/ cu.mm. | 2.4-4.91 | 3.6 | 0.76 | (3.6 ± 1.52) |
| 2) | Hb gm% | 7.3-15.1 | 11.23 | 2.66 | (11.23 ± 5.32) |
| 3) | HCT % | 21.4-53.6 | 36.72 | 9.65 | (36.72 ± 19.3) |
| 4) | MCV (fl) | 79.4-114.7 | 102.8 | 8.96 | (102.8 ± 17.92) |
| 5) | MCH (pg) | 22.8-40.3 | 32.1 | 4.03 | (32.1 ± 8.06) |
| 6) | MCHC (gm/dl) | 27.9-40.4 | 31.18 | 3.01 | (31.18 ± 6.02) |
| Hemoglobin fraction | | | | | |
| 1) | HbF% | 66.1-84.2 | 76.96 | 5.76 | (76.96 ± 11.52) |
| 2) | $HbA_0$ % | 3.1-18 | 8.36 | 4.26 | (8.36 ± 8.52) |
| 3) | $HbA_2$ % | 0-1 | 0.08 | 0.25 | (0.08 ± 0.5) |
| 4) | HbS % | 3.8-9.5 | 6.70 | 1.74 | (6.70 ± 3.48) |

**TABLE - 8**
**Hematological Profile of FS Cord Blood Samples**

| | RBC in mil/ cu.mm | Hb gm% | HCT % | MCV (fl) | MCH (pg) | MCHC (gm/ dl) | HbF % | $HbA_0$ % | HbS % |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 3.065 | 10.55 | 32.75 | 107.2 | 34.2 | 31.9 | 74.65 | 0.8 | 15.75 |

**DISCUSSION**

50 cord blood samples of newborns were screened and their various hematological parameters and hemoglobin fractions were studied. Out of these, 40 were from affected mothers with hemoglobinopathy and 10 were controls with normal Hb pattern.

**Table – 1 :** Shows pattern wise distribution of mothers. Out of 50, 35 cases were AS, 4 cases were SS, 10 cases were AA and 1 case was AD, Sickle Cell trait was the most prevalent hemoglobinopathy in traced mothers with 70% cases of AS and 8% cases of SS.

**Table - 2 :** Shows distribution of cord blood samples for hemoglobino-pathies, Out 50, 33 cases had normal Hb pattern while 17 cases had abnormal Hb pattern. Thus 34% showed hemoglobinopathy. Thus out of 78% cases of affected mothers, cord blood showed hemoglobinopathy in 34% cases. Thus gene of sickling passed with a frequency of 43.5%.

**Table - 3 :** Shows pattern wise distribution of cord blood samples. Out of 50 cases, 33 (66%) were FA, 15 (30%) were FAS & 2 (4%) were FS.

**Table - 4 :** Shows the caste wise distribution of cord blood samples. The highest no of samples were from Mahar Community – 27 (54%) out of 50.Scheduled caste showed 68% of total cases, their no. was 34. In scheduled caste Mahar were 27 (79%), Bouddha were 6 (17.6%) and Chambhar were 1 (2.9%).Scheduled tribes had 6 (12%) cases with Gauli - 1, Halba-1, Gond - 3 & Katiya -1 case.Other Backward class had 1 (2%) case of Kunbi, Muslim had 6 (12%) Cases while Maratha, Thakur, Brahmin had 3(6%) cases with 1 case each.

Pearson[2] et al (1974) screened 756 cases from black and Peutorican newborn at Yale New Heavan's Hospital and found 657 (86.97%) normal FA, Sickle Cell trait 61 (8.1%) Sickle Cell anemia 6 (2.1%).Griffith[3] et al (1982) screened 43, 500 newborns in Birmingham of which 10.3 were Negros and 22.6% Asians, rest were white population. They found 534 AS patient, 205 AC patients and 7 SS patients. The cases reported mostly were from black population.

Gulbise[4] et at (1999) screened 23, 126 cord blood samples in Brussels where 45% of newborn were from high risk group i.e. mothers from Afro- Carribean, Mediterranean and Indians, sickle cell disease was diagnosed in 11 neonates 0.048%, 350 (1.57%) AS and 672 (2.9%) were Hb Barts.

**Table - 5 :** Shows caste wise and pattern wise distribution of collected cord blood samples. In scheduled caste 11 (32.3%) were FAS, 22 (64.7%) were FA & 1 (2.9%) was FS, Mahar showed 11 positive and 16 normal cases while Buddha had 1 positive and 5 normal cases. Thus 40.7% Mahar Samples were positive and 64.7% positive cases in all the cord blood cases were from Mahars.In scheduled tribes Gawli, Gond & Katiya had 16.6% incidence of positive cases.Kunbi cord blood sample was negative. In Muslims 1 (16.6.%) out of 6 cases was positive.In others Maratha had 1 positive case of FS thus 33% cases in others were positive. Mahars had 66.67% incidence of positive Sickle Cell trait.

Lehman H et at reported first case in India and 30% prevalence of sickle cell trait in rural tribe of South India.Shukla[5] et al from a study in Nagpur showed the highest incidence of sickle cell trait in Mahar (22.3%), Teli (11.3%) and Kunbi (9.7%).Mohanty[6] et al (1988) reported gene frequency ranging from 22.5% to 44.4% for sickle cell disorder in Central India.

Abhyankar[7] et al (2000) reported that Central India is a focus of sickle cell disorder and prevalence in general population is 12%.Kate et al (2000) reported 20% prevalence of sickle cell disorder among SC/ST population from 2194 people screened in Aheri Taluka of Gadchiroli districts. Otkar tribe show highest 35% and Gond Tribe show 20.8% prevalence for sickle cell trait. In Muslim community sickle cell gene and HbAE was found due to the practice of endogamous marriage. It was seen in 9 cases.

Henthorn[8] et al (1984) screened consecutive cord blood samples in a hospital at Brent, out of all, 59% births were from European mothers with 22% belonging to Afrocarribean origin. 2.8% patients of AS pattern were diagnosed. HbAC shows 0.91% prevalence and 0.15% belong to SS.

**Table - 6 :** Shows the hemotological profile of 33 normal cord blood samples. The mean RBC count was found to be 3.66 $\pm$ 2.73 mil / cu.mm., mean Hb 11.44 $\pm$ 5.34 gm% mean HCT 37.46 $\pm$ 19.8%, mean MCV 107.15 $\pm$ 16.9 fl, mean MCH 32.48 $\pm$ 5.68, mean MCHC 29.83 $\pm$ 5.86 mean HbF 76.38 $\pm$ 14.9% mean $HbA_0$ 16.87 $\pm$ 11.42% mean $HbA_2$ 0.07 $\pm$ 0.58%.Heygi[9] et al (1977) reported 80-90% HbF, 10 to 20% HbA.Mason et al (1982) reported mean HbF 69.6 $\pm$ 8.650 in 266 normal cord bloods from Jamaica.Fucharoen[10] et al (1998) reported mean HbF 74.1 $\pm$ 6.43% $HbA_0$ 17.8 $\pm$ 6.325% & $HbA_2$ 0.6 $\pm$ 0.43% in 326 normal cord blood samples.Eastman[11] et al (1999) reported median HbF 61.6%, Median $HbA_0$ 10.3% and Median $HbA_2$ < 0.27% in 4 million newborn-screened specimens in dried blood spots.

**Table showing Hematological Profile in other studies**

| Hematological Parameter | Wintrobe Hematology | Natthans & Oski's Hematology | Fuchareon et al |
|---|---|---|---|
| RBC Count Mil/Cm | | 4.64 $\pm$ 0.68 | - |
| Hbgm% | 16.8 $\pm$ 1.65 | 15.9 $\pm$ 1.86 | 15.4 $\pm$ 1.65 |
| HCT% | 53 $\pm$ 5 | 50.2 $\pm$ 6.9 | - |
| MCV fl | 106.4 $\pm$ 5.7 | 110 $\pm$ 5.05 | 105 $\pm$ 6.2 |
| MCH pg | 34.0 | 34.6 $\pm$ 1.5 | 35 $\pm$ 22 |
| MCHC gm% | 31.7 | 31.9 $\pm$ 1.13 | 33 $\pm$ 1 |

**Table – 7 :** Showing hematological parameters and hemoglobin fractions in 15 cord blood FAS samples. Mean value of RBC 3.6 $\pm$ 1.52 mil/cu.mm, mean Hb 11.23 $\pm$ 5.32 gm% mean HCT 36.72 $\pm$ 19.3%, mean MCV 102.8 $\pm$ 17.92 fl mean MCH 32.1 $\pm$ 8.06 pg, mean MCHC 31.18 $\pm$ 6.02 gm/dl, mean HbF 76.96 $\pm$ 11.52% mean $HbA_0$ 8.36 $\pm$ 8.52% mean $HbA_2$ 0.08 $\pm$ 0.5%, mean HbS 6.70 $\pm$ 3.48%.

Wilson[12] et al (1986) the quantity of HbA & HbS in AS newborn was reported to be mean 8.56 $\pm$ 3.17% and mean 6.82 $\pm$ 2.15 SD % respectively in 67 patients by fast HPLC in Georgia.Eastman[11] et al (1999) reported the distribution of F, A and D quantity in 4 million newborn screening specimens.

| Pattern | Fo% | $A_0$% | Variants S, C or D% | E% |
|---|---|---|---|---|
| FA | | 10.3 | | |
| FAC<br>FAS<br>FAD | 61.6 | 5.3 | > 4.0 | |
| FAE | | 5.3 | - | > 2.0 |
| FS, FC, FD | 61.6 | - | 6.8 | |

**Table – 8 :** Shows hematological profile of 2FS cord blood samples. Mean RBC 3.065 mil cu.mm, mean Hb 10.55 gm% an HCT 32.75% mean MCV 107.2 fl, mean MCH 34.2 pg mean MCHC 31.9 gm/dl mean HbF 74.65% mean $HbA_0$ 0.8% mean HbS 15.75%.Wilson[12] et al (1986) reported mean HbS level of 13.10 $\pm$ 3.2% in FS patients in absence of HbA.Eastman[11] et al (1999) reported cases of FS, FC, FD with median HbF 61.6 & variant median 6.8% in absence of HbA.

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