

# Neonatal Screening for Sickle Cell Disorders

R. D. Hanmante<sup>1\*</sup>, S. W. Chopade<sup>2</sup>, K. S. Dhumure<sup>2</sup>, S. Shere<sup>3</sup>, R. S. Bindu<sup>4</sup>

<sup>1</sup>Assistant Professor, Dept. of Pathology, Dr Shankarrao Chavan Govt. Medical College, Nanded (MS) INDIA.

<sup>2</sup>Resident, Dept. of Pathology, Govt. Medical College, Aurangabad (MS) INDIA.

<sup>3</sup>Assistant Professor, Dept. of Pathology, Govt. Medical College, Aurangabad (MS) INDIA.

<sup>4</sup>Professor & Head, Dept. of Pathology, Govt. Medical College, Aurangabad (MS) INDIA.

Corresponding addresses:

[\\*drrajharshhanmante@gmail.com](mailto:drrajharshhanmante@gmail.com)

## Research Article

**Abstract:** 500 newborns (age group 0 – 1 month) were screened using the sickle cell short programme kit by HPLC. First the blood samples of newborns were subjected for CBC. The neonates having low haemoglobin content [i.e. Hb < 14 gm %] were subjected for High Performance Liquid Chromatography (HPLC) study. Out of 500 newborns, 8 (1.6%) cases were sickle cell trait with male 6 (75%) preponderance with the more common clinical presentation of fever. Sickle cell trait was more prevalent in Bouddha 3 (37.5%) community followed by Muslim 2 (25%) and Matang 2 (25%). Hb S gene is also found in Banjara community 1 (12.5%).

**Key words:** Neonate, Sickle cell disease, HPLC, Community, Chromatograph.

## Introduction:

Sickle cell disorders (SCD) are genetic blood disorders that affect the Haemoglobin in red blood cells.<sup>1</sup> In SCD, there is abnormal haemoglobin i.e. **Hb-S** which results from the replacement of Glutamic acid by Valine at the 6th position on  $\beta$  chain from N terminal. Sickle cell anaemia is an autosomal recessive condition, so inheritance of an affected gene from both parents results in a disorder whilst inheritance of one abnormal gene results in a healthy carrier.<sup>1</sup> Sickle cell disorder is a life long condition that can be diagnosed from a simple blood test, before the baby becomes ill.

The incidence of this condition is most common in people of African – Caribbean origin and from sub-Saharan region but is also found in those with Arab, Mediterranean and Indian origin.<sup>1</sup>

In India, Hb S is predominantly found in central India i.e. Vidarbha in Maharashtra, Madhya Pradesh, Orissa, Andhra Pradesh, Gujarat and to

a lesser extent in Tamil Nadu, Karnataka, Kerala and Uttar Pradesh. Sickle cell gene is widely spread in all district of Eastern Maharashtra i.e. Vidarbha, North Maharashtra i.e. Satpuda ranges and some parts of Marathwada region.<sup>2</sup> Prevalence of sickle cell disorder (carrier) amongst scheduled caste population in Aurangabad region is 07%<sup>2</sup> and In Nanded region, the prevalence of sickle cell disorder (carrier) amongst Banjara communities is 05 %.<sup>2</sup> The causes of mortality among sickle cell disease patients were septicemia, acute splenic sequestration, severe anaemia and haemolytic crisis. Improvement in life style of these patients helps to control morbidity and mortality. Extensive health education programme and early detection of SCD is extremely useful for improving the quality of life. Therefore, neonatal screening for SCD is recommended which helps to reduce the morbidity and mortality in SCD. The main aim of the neonatal screening is the early detection of babies with sickle cell disorders, so that penicillin and other forms of treatment, as well as parent education can be started as soon as possible, preferably by the time the baby is two months old. Early treatment improves the health of babies with sickle cell disorder and can prevent death.<sup>1,3</sup>

## Material & Methods:

This study was carried out in the department of pathology, Government Medical College Aurangabad during the period from January 2009 to November 2010. This is the observational Study.

During this study, the newborns who were admitted in the paediatric ward for various reasons like for under observation after routine delivery cases or for any significant clinical history like suspected haemolytic anaemia, Fever etc. were included in the study.

The samples of the newborns were first subjected for CBC and the neonates having low haemoglobin content [i.e. Hb < 14 gm %], and MCV < 100 fl, and MCH < 32 pg, were further subjected for HPLC study using the sickle cell short programme kit.

**HPLC [High Performance Liquid Chromatography – variant™ BIO-RAD]: Equipment / Reagent System:**

It consist of 1) 30 mm column (cartridge) is packed with a nonporous 4.6 mm internal diameter polymeric cation exchange material

(silica gel). Each column can be used for as many as 500 injections.

2) Two sodium phosphate buffers were used as eluents, run as a gradient from 4 g / L (**Buffer 1**) to 14 g / L (**Buffer 2**) at pH 6.5 (Buffer 1) and at pH 6.6 (Buffer 2).<sup>4</sup>

3) The reagent kit include whole blood primer , wash solution, three linearity calibrators containing Hb F and A and two lyophilized control, one containing Hb - F , A, E and S and another one is Hb – F, A ,D and C. and ROM card.<sup>4,5</sup>

**Principle of High Performance Liquid Chromatography:**

HPLC is a fully automated cation exchange based instrument. It depends on the interchange of charged groups on the ion exchange material with charged groups on the haemoglobin molecule.

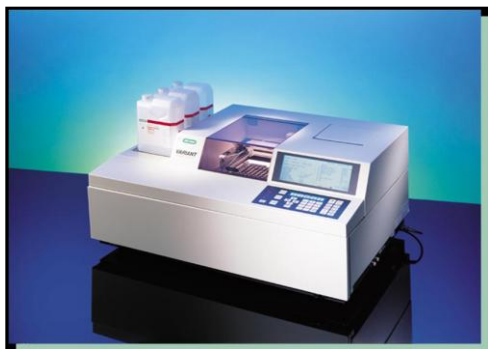


Fig [1]: HPLC machine - variant™ [Bio – Rad]



Fig [2]: ROM Card



Fig [3]: Cartridge

**Specimen collection and preparation:**

Patient’s blood samples were collected from heel sticks and absorbed into S and S 903 specimen collection paper (DBS filter paper).<sup>4</sup> A

disc 0.31 cm (1/8 inch) in diameter was punched from the DBS specimen and the blood is eluted into 0.5ml (half) of deionized water for 30 min with periodic shaking.<sup>4</sup>



Fig [4] collection of Blood from heel prick filter paper



Fig [5] Punch Machine

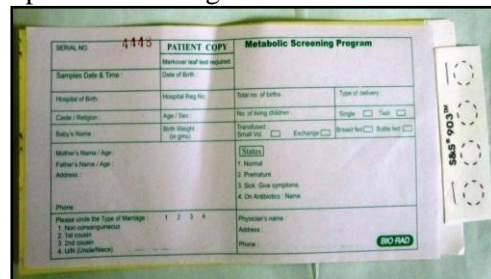


Fig [6] The patient History Form along with filter on Paper

**Observations & Results:**

During this study, 500 newborns were screened for sickle cell disorders using sickle cell short

programme kit by HPLC. Age group ranges from 0 to 1 month.

316 (63.2%) male and 184 (36.8%) female newborns were screened. Male to Female ratio is 1.72:1.

Out of 500 newborns screened, the data according to their caste is as follows.

Muslim 131 (26.2%), OBC 106 (21.2%), Bouddha 101 (20.2%), Matang 42 (8.4%), Maratha 38 (7.6%), Chambhar 35 (7%), Banjara 24 (4.8%) and Brahmin 23 (4.6%).

Majority i.e. 402 (80.4%) newborns were asymptomatic at the time of screening. Remaining 98 (19.6%) newborns were presented with one or combination with the clinical features of Fever, Irritability, Paleness of body, Swelling of abdomen, Not accepting feeds, convulsions and Breathlessness.

Most common presentation was Fever (72 cases), followed by paleness of body (22 cases),

convulsions (18 cases), not accepting feeds (16 cases), irritability (12 cases), swelling of abdomen (08 cases) and breathlessness (04 cases).

During this study, out of 500 neonates screened, 23 (4.6 %) neonates have their parental history of consanguineous marriage while 477 (95.4 %) neonates with no history of consanguinity.

The Haematological parameters of screened newborns were studied. The findings are as follows. Most of the newborns are with Hb < 12 gm%, PCV < 34 %, MCV < 95 fl, MCH < 31 pg and MCHC < 30 gm%.

Out of 500 newborns screened, 8 (1.6 %) newborns were diagnosed as sickle cell trait.

No case of sickle cell disease, Hb SC disease, Hb E trait or Hb S β thalassaemia was found.

Of these 8 cases, 6 (75%) were male and 2 (25%) were female newborns. Male to Female ratio is 3:1.

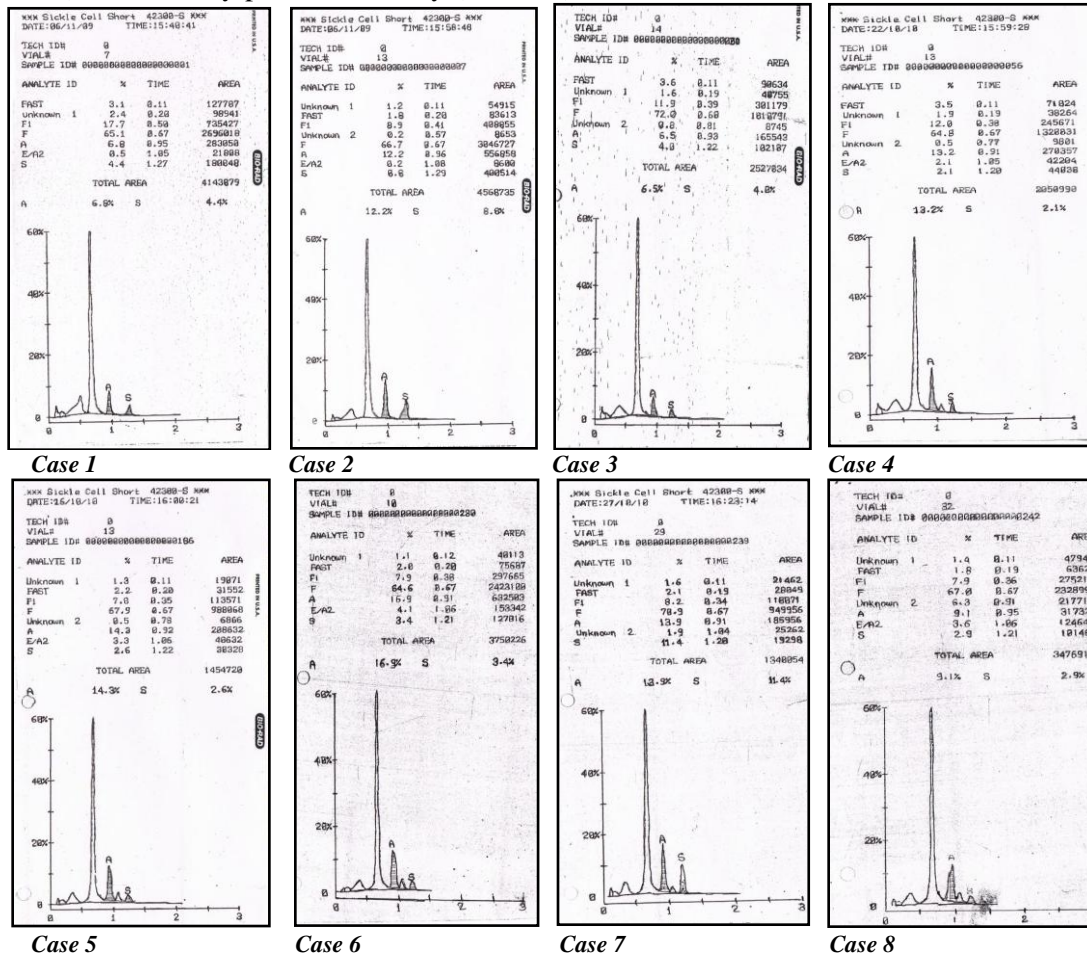


Fig [7] Chromatographs of sickle cell trait cases

Table 1. Chromatographic values according to cases.

Case	Hb A %	Hb S %
1	6.8%	4.4%
2	12.2%	8.8%
3	6.5%	4%
4	13.2%	2.1%
5	14.3%	2.6%
6	16.9%	3.4%
7	13.9%	11.9%
8	9.1%	2.9%

Of these 8 sickle cell trait cases, the majority cases belong to the Bouddha community followed by Muslim and Matang and finally Banjara community. The incidence of cases according to their caste and community are as, Bouddha = 3 cases (37.5 %), Muslim = 2 cases (25 %), Matang = 2 cases (25 %) and Banjara = 1 case (12.5 %).

During the study history of consanguineous marriage was noted. Among these 8 sickle cell trait cases, it was found that 7 (87.5%) couples were with the history of consanguineous marriage and 1 (12.5%) couple was not having the history of consanguineous marriage.

It is found that prevalence of sickle cell gene is more common in couples with consanguineous marriage.

Family study was also noted. It was found that, out of these 8 cases, 4 cases had family history of sickle cell trait. Of these 4 cases, the mothers were K/C/O Sickle cell trait in 3 cases and in 4 th case the father of newborn was K/C/O Sickle cell trait. Family study was not completed in the remaining 4 cases because the cases were lost to follow up.

Out of 8 sickle cell trait cases, 7 newborns were presenting with one or combination of clinical features such as Fever, Paleness of body, Breathlessness, Not accepting feeds, Convulsions.

Remaining one case had no any presenting clinical features.

Most common presenting feature was Fever (7 cases) followed by Paleness of body (3 cases), Breathlessness (2 cases), Not accepting feeds 1 (case), and Convulsions (1 case).

The Haematological parameters of the sickle cell trait cases were studied. The findings are as follows.

The haemoglobin level of most of the newborns with sickle cell trait was < 10 gm%.

Similarly other haematological parameters like PCV less than 28 % and MCV less than 86 fl, MCH less than 28 pg and MCHC less than 28 gm%. Usually, the newborns do not manifest the features of anaemia but from the above findings it is found that the haematological parameters in the sickle cell cases are below the normal level.

The Comparison of haematological parameters among the screened normal newborns and sickle cell trait cases is done using Student's T test.

It is found that the difference of low Haemoglobin and all other haematological parameters among diagnosed sickle cell trait cases and normal screened newborns is statistically very significant.

Hence, the criteria like low haematological parameters like Hb, MCV, MCH and MCHC in newborns can be used for screening to rule out sickle cell trait / disease.

## Discussion:

At present, screening of neonates for SCD is carried out in a number of areas throughout the world, but the evidence used for establishing and appraising screening programmes has come mainly from observational studies.<sup>3</sup>

In India, Sickle haemoglobin was first detected by Lehman and Cutbush in 1952 among the tribals from Nilgiris. During the last 58 years, several groups of investigators conducted hospital based or epidemiological surveys in

various ethnic groups.<sup>6</sup> Based on these surveys, prevalence of sickle cell gene found to be 0 – 18% in North eastern India, 0 – 33.5% in western India, 22.5 – 44.4% in central India and 1- 40% in Southern India.

**Kamble M et al (2000) [Maharashtra]**<sup>7</sup> carried out the prospective descriptive hospital based study on 1753 patients admitted in paediatric ward at Mahatma Gandhi Institute of Medical Sciences, Sewagram Wardha. Of these 1753 patients, 61 (61.6%) had Hb S homozygous state whereas 38 (38.4%) had heterozygous state. Of these, only 6 homozygous cases and 8 heterozygous cases were below the age of 12 months. SCD was more common in males. The male: female ratio being 1.65: 1 in Hb SS and 1.71: 1 in Hb AS cases.<sup>7</sup>

**R Ducrocq et al (1995) [France]**<sup>8</sup> carried out the neonatal screening for sickle cell disease over the period of 5 year since 1995 in Northern part of the Paris. During this study 115480 newborn were screened for sickle cell disease using HPLC. Of these identified 250 newborns with SCD. Of these, 175 SS, 52 SC, 1 SE and 22 S  $\beta$  thalassaemia were identified.<sup>8</sup>

**Soad et al (2001-2002)**<sup>9</sup> carried out study on neonatal screening for haemoglobinopathies using HPLC in Saudi Arabia. During this study, out of 834 neonates screened, 37 (4.44%) newborns were diagnosed as sickle cell trait and M:F ratio was approximately 1:1.<sup>9</sup>

In the present study, out of 500 newborns screened, 8 (1.6%) cases were identified as sickle cell trait. Of these 8 cases, 6 were males and 2 were female. Male to Female ratio is 3:1.

According to the study by Kamble M et al (2000), the prevalence of SCD was 5.7% of which Hb SS was 3.51% and that of Hb AS was 2.1%. 81 Studies from Orissa report the incidence of SCD in Hospitalized paediatric patients to be 6.42%.<sup>10</sup>

In present study, the prevalence of sickle cell trait was 1.6% in hospitalized paediatric patients. No case of Hb SS was found. Preponderance of males over female was found in the study by Kamble et al and also in the present study.

**S.L.Kate (2000)** (Emeritus Medical Scientist, Department of Paediatric, B J Medical college Pune) carried out the study on Health problems of Tribal Population Groups from the state of

Maharashtra. The epidemiological data of this study suggest that<sup>11</sup>

1. Prevalence of sickle cell disorder is very high among tribal population groups, Bhil and Pawara from Nandurbar district and amongst the Madia, Pardhan, Oktar population from Gadchiroli district i.e. 20%.

2. The highest recorded prevalence is amongst the Oktar group i.e. 35%.

3. The overall prevalence among tribal population is about 10% for the carrier state and 0.5% for sufferers.

Most of the studies from Maharashtra show that the prevalence of Hb S is more common in Schedule Caste followed by Scheduled Tribes population. Scheduled Caste mainly consists of Mahar [now termed as Bouddha], Matang and Chambhar. Scheduled tribes consist of Bhil, Pardhan, Andha, Mahadeo koli, Pawara etc.<sup>11</sup>

According to the studies by Lele et al (1962), Shukla R.N. et al (1957), Kamble et al (2000), Hb S prevalence was more common in Mahar communities.

The prevalence of Hb S in present study is more common in Bouddha (37.5%) followed by Muslim (25%) and Matang (25 %) and Banjara (12.5%) among the screened population.

No appropriate data was found regarding the clinical presentation of neonates in relation to sickle cell disease. Data is available regarding the clinical presentation of sickle cell disease in higher age groups.

**Kamble et al (2000) [Maharashtra]** found that Vaso-occlusive crisis (23.3 %) was the commonest crisis encountered followed by hyper-haemolytic crisis (16.3 %).

**Topley et al (1981) [Jamaica]** found that 20 % incidence of sepsis reported in infants under the age of one year and reported mortality rate up to 35 %.<sup>12</sup> Topley et al also state that the incidence of splenic sequestration episodes is reported in 24.1% of cases.<sup>3, 13</sup>

**Powers et al (1981) [California]** identified that out of 182 sickle positive newborns 23 cases presents with pneumococcal sepsis and of these 23 cases 8 (34.8 %) were died due to non comprehensive care and no early intervention.<sup>13</sup>

Similarly, **Vichinsky et al (1988) [California]** found that out of 64 sickle positive infants, 11 were presents with pneumococcal sepsis and of



these 11 cases, 4 (36.4 %) died due to non comprehensive care and no early intervention.<sup>13</sup>

The high level of Hb F at birth prevents the newborn's red cells from sickling. Over the ensuing months, however, the concentration of Hb F declines, usually reaching a level that is too low to offer protection by the age of 6 months. It should be emphasized, however, that like most paediatric milestones, there is no magic age; rather, it is a continuum, and the infant must be considered at risk for sickle cell related complications by the age of 2 months.<sup>13</sup>

In the present study, among 8 sickle cell trait cases, the newborns are presented with one or combination of clinical features. Most common presentation was Fever (7 cases), followed by Paleness of body (3 cases), Breathlessness (2 cases), Not accepting feeding (1 case) and Convulsion (1 case).

### Conclusion:

To conclude from the present study, that HPLC is a sensitive as well as specific test for the neonatal screening for sickle cell disorders. HPLC is a successful approach in detecting neonates who are carriers.

The screening programme should be supplemented by screening modalities like the extended family studies and couples at risk particularly where the Consanguineous marriage is common, giving a high yield of information on carriers. As SCD is autosomal recessive disease, it is best to restrict the consanguineous marriages and also to restrict the marriages between the known sickle cell trait persons.

***“Prevention is always better than Cure”.***

Neonatal screening for sickle cell disorders using HPLC is a comprehensive approach to reduce SCD morbidity and mortality in children. The Sickle cell trait / Disease should be suspected in the newborns with low haematological parameters with or without clinical features like Fever, Paleness of body etc. The low haematological parameters in the newborns give us a clue for the neonatal screening to rule out Sickle cell trait / Disease. Anaemic newborns with signs of sepsis or haemolytic crisis should be treated as medical emergencies. Early detection of cases makes

opportunities for newborns to get early medical treatment to improve the quality of Life.

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