Newborn Screening for Hemoglobinopathies in Tertiary Care Centre in Central India

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ABSTRACT
Inherited abnormalities of hemoglobin synthesis include structurally abnormal hemoglobin & decreased rate of synthesis of one or more of normal polypeptide chain of hemoglobin. These are collectively called as hemoglobinopathies. These hemoglobinopathies are further classified as heterozygous (AS pattern) or homozygous (SS pattern) or double heterozygous form (SC pattern) and others with quantitative defect in globin chain synthesis. Some homozygous or double heterozygous form of hemoglobinopathy can lead to significant morbidity and mortality. Early detection of hemoglobinopathy in these patients can reduce morbidity & mortality. The main aim of this study is to identify affected infants at birth, initiate prophylactic therapy as early as possible and educate parents against disease.

Material And Methods
Around 300 cord blood samples of newborn were screened using High Performance liquid chromatography, Biorad variant system by using Beta thal short program, whose mothers were referred for delivery in tertiary care Centre. The patients were followed up at 3 and 6 months.

Results
Hemoglobinopathy in newborn is commonly seen in SC, ST communities followed by Muslim and OBC. Sickle cell disorder (SCD) is the most prevalent hemoglobinopathy followed by Hb E and HbD. SCD is prevalent in Scheduled Caste, HbE in Muslim and Teli community

Conclusions
Follow up of SS infants as per protocol is important to prevent morbidity and mortality due to infection in infancy and early childhood. Family awareness, parental education and genetic counseling would help to lower the birth of SS child

Keywords: Hemoglobinopathies, Cord blood sampling, sickle cell disease

INTRODUCTION
An erythrocyte has more than 95% of cytoplasmic protein as hemoglobin. It is a tetramer consisting of 2 pair of similar polypeptide chain called globin that exhibits a diad axis of symmetry. To each of the four chains is attached a prosthetic group “heme” a complex of iron and protoporphyrin. The two pair of polypeptide chain from six polypeptide chain i.e. α, β, γ, δ, ε, ζ form a hemoglobin molecule from embryonic, fetal, neonatal to adult life (1). Inherited abnormalities of hemoglobin synthesis are characterized by structurally abnormal hemoglobin variants called hemoglobinopathies & others in which one or more of normal polypeptide chain of hemoglobin are synthesized at a reduced rate called thalassemia (1). These conditions forms a spectrum of diseases known collectively as the hemoglobinopathies (2). These hemoglobinopathies are inherited in form of heterozygous (AS pattern), homozygous (SS pattern) or double heterozygous form (e.g. SC pattern) and others with quantitative defect in globin chain synthesis with structural variants like S/β0 thal. Most are functionally normal and hence clinically silent others form polymer (HbS) or crystal (HbC) giving rise to hemolytic disease,
while other are unstable forming intermittent or chronic hemolysis.

Some homozygous or double heterozygous form of hemoglobinopathies when inherited produce significant morbidity and mortality in newborns, infants, children and in later ages (3).

The newborn screening for hemoglobinopathies primarily started for sickle syndrome. In a hospital set up during newborn period, newborns are easily available for screening. Early detection of the hemoglobinopathies in homozygous or in double heterozygous form gives time to educate the parents against disease, offer genetic counseling, initiating prophylactic penicillin therapy, pneumococcal vaccine, providing ongoing care by knowledgeable health professionals and allow the sufferer to lead an effective human life(4,5).

The first statewide newborn hemoglobinopathies started in New York in 1975 but the major thrust for newborn screening came from the demonstration that early diagnosis and comprehensive care could reduce morbidity and mortality in infants with sickle cell anemia, particularly through the prevention of pneumococcal sepsis with penicillin prophylaxis(4).

Pearson, O’Brien Aspnes et al in 1972 carried out routine screening of umbilical cord for sickle cell disease. They screen 756 patient and found sickle cell trait 61 FAS (8.1%), FS 6(2.1 %), sickle/thal 1 (6).

Henthom Aniwu, M.Brozoric et al 1984 screened 3165 consecutive cord blood with 6.9% of cord blood showing abnormal hemoglobin(7).

Vichinsky et al in 1988 demonstrated that cord screening was both practical and effective. It can effectively reduce the mortality of SCD, when linked to comprehensive clinical program with a strong family education component (5).

Gulbis et al in 1999 started newborn screening program in Brussels and diagnose 12 patients with significant hemoglobinopathies and 350 traits (8).

D Mohanty et al in 1998 stated that India lies in a rich zone involving HbS, HbD, HbE and Thalassemia. In India the prevalence of HbS varies from 0 – 44% the frequency varies between:0 – 18.5% in North East Zone, 0 – 33.5% in Western Zone, 22.5 – 44.4% in Central India and 1 – 40% in Southern Zone(9).

Abhyankar et al in 2000 concluded that Central India is a focus of sickle cell disorder and prevalence in general population is 12%(10). The average gene frequency of sickle cell is 4.3% & that of HbD is 0.86% & for HbE is 10.9% in North Eastern region of India. The cumulative gene frequency for hemoglobinopathies is 5.3%(11). Hence the present study has been carried out in Central India at a tertiary health care center to know the prevalence of hemoglobinopathies in newborns ultimately to reduce the morbidity and mortality in infancy and childhood due to Sickle Cell Disease.

**MATERIAL AND METHODS**

The present study is hospital base cross sectional study was carried out at Regional Hemoglobinopathy Detection and Management Centre, at a tertiary care institute in Vidarbha region for a period of two year. Mothers were randomly selected irrespective of their origin, caste and ethnic background. All mothers delivered in the tertiary health care center were included in the study. Mothers who had received blood transfusion 3 months prior to the delivery were excluded from the study. The present study was duly passed by Ethical committee. The cord blood was collected during labor & lower section caeserian section (LSCS) from the mothers who were admitted in labor room in the department of Obstetrics and Gynecology for delivery randomly. Blood sample was collected in labor room and gynecology Operation Theater. After separation of newborn, the cord blood was collected from the placental end of umbilical cord after cleaning with sterile swab. The 2ml cord blood was collected in K2-Ethylene diamine tetraonic acid (EDTA) anticoagulant bulb. The sample bottles were properly labeled with name, registration number and date.

After labour and LSCS the patients were followed in the wards and detail name, address, caste, educational status of the parents were noted. Detail clinical history and h/o previous blood transfusion within 3 month prior to delivery was noted. Birth weight, sex and Apgar score of newborn was also noted. An automated Complete Blood Count(CBC) using sKX-21 an automated multiparameter blood cell counter which analyses 18 parameter of blood and automated high performance liquid chromatography (HPLC).
which separate and determine the relative percentage of normal and abnormal hemoglobin were carried out.

The newborn detected for hemoglobin were followed after 3 month and 6month. The parents of the affected newborn were counseled and their hemoglobinopathy status was studied by testing their blood for sickling, solubility, hemoglobin electrophoresis/HPLC. Those parents whose blood was not tested previously were tested during follow up. The parents were counseled about the disease during follow up. Those parents where were trait (both), they were offered genetic counseling. A reminder in the form of postcard letter was sent to the parents as well telephonic reminder was given. During follow up infants were examined by pediatrics in Sickle Cell OPD and blood sample were taken to study hematological profile and hemoglobin fraction for confirmation. The infants who were diagnosed as sickle cell disease were advice penicillin prophylaxis after 6 months and active immunization by pneumococcal vaccine after 2 years to reduce morbidity and mortality.

**OBSERVATIONS**

Under newborn screening for hemoglobinopathies 300 cord blood samples of the new born were screened and their various hematological parameters and hemoglobin fraction were studied. Follow up of the patients were carried out at 3 month and 6 month for confirmation of diagnosis. Parents of the newborn who had abnormal hemoglobin were also screened and their hemoglobinopathy status was studied.

Table 1 shows distribution of 300 study subject showing hemoglobin pattern

<table>
<thead>
<tr>
<th>Study Subjects</th>
<th>Normal Hb Pattern</th>
<th>Abnormal Hb Pattern</th>
<th>Total Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>256</td>
<td>44</td>
<td>300</td>
</tr>
<tr>
<td>Percentage</td>
<td>85.34</td>
<td>14.66</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2 showing Caste wise and Pattern wise distribution of positive cases

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Group</th>
<th>Ethnic group</th>
<th>Abnormal hemoglobin</th>
<th>Total positive</th>
<th>%age in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S.C. Group</td>
<td>96 (cases)</td>
<td>FS, FAS, FAE, FAD</td>
<td>20</td>
<td>20.81</td>
</tr>
<tr>
<td></td>
<td>S.C. Group</td>
<td>Mahar + Bouddha</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S.C. Group</td>
<td>Khatik</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S.C. Group</td>
<td>Burrud</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S.C. Group</td>
<td>Teli</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S.C. Group</td>
<td>Lodhi</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>ST</td>
<td>Gond</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>04</td>
<td>16.67</td>
</tr>
</tbody>
</table>
Later diagnosed after 6 month of follow up

32 AS newborn showed 2 SF, 20 AS, 10 AA patterns in mothers. 2 AS pattern and 6 AA pattern in father were seen and 24 lost to follow up. 3 SS newborn show sickle cell trait in both parents. All 6 AE newborn parents were lost to follow up. 2 AD newborn show mothers having HbE as AD pattern. 

\[ \beta \text{ thal minor newborn have mother known case of S/} \]

Table 3: Hematological profile of 32 AS patients

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Hematological parameter</th>
<th>Range</th>
<th>Mean</th>
<th>Standard Deviation (SD)</th>
<th>Mean ± 2 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>RBC COUNT mil/cumm</td>
<td>2.86-4.9</td>
<td>3.77</td>
<td>0.42</td>
<td>3.77±0.84</td>
</tr>
<tr>
<td>2.</td>
<td>Hb gm %</td>
<td>9.5-15.2</td>
<td>12.28</td>
<td>1.27</td>
<td>12.28±2.54</td>
</tr>
<tr>
<td>3.</td>
<td>HCT %</td>
<td>31.6-49.5</td>
<td>40.6</td>
<td>4.88</td>
<td>40.6±9.76</td>
</tr>
<tr>
<td>4.</td>
<td>MCV (fl)</td>
<td>82.9-123.3</td>
<td>108.06</td>
<td>9.5</td>
<td>108.06±19</td>
</tr>
<tr>
<td>5.</td>
<td>MCH (pg)</td>
<td>24-36.6</td>
<td>32.66</td>
<td>2.48</td>
<td>32.66±4.96</td>
</tr>
<tr>
<td>6.</td>
<td>MCHC (gm/dl)</td>
<td>27.5-35.1</td>
<td>30.53</td>
<td>1.53</td>
<td>30.53±3.06</td>
</tr>
<tr>
<td></td>
<td><strong>Hemoglobin Fraction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>HbF%</td>
<td>59.6-82.2</td>
<td>74.53</td>
<td>5.46</td>
<td>74.53±10.92</td>
</tr>
<tr>
<td>2.</td>
<td>HbA0%</td>
<td>5-20</td>
<td>10.27</td>
<td>3.50</td>
<td>10.27±7</td>
</tr>
<tr>
<td>3.</td>
<td>HbA2%</td>
<td>0.2-0.7</td>
<td>0.35</td>
<td>0.17</td>
<td>0.35±34</td>
</tr>
</tbody>
</table>
4. HbS% 4.2-13.1 6.53 2.03 6.53±4.06

Table 4: Hematological profile of 3SS patients

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Hematological parameter</th>
<th>Range</th>
<th>Mean</th>
<th>Standard Deviation (SD)</th>
<th>Mean ± 2 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>RBC COUNT mil/cumm</td>
<td>3.01-4.22</td>
<td>3.51</td>
<td>0.62</td>
<td>3.51±1.24</td>
</tr>
<tr>
<td>2.</td>
<td>Hb gm %</td>
<td>9.8-11.3</td>
<td>10.46</td>
<td>0.76</td>
<td>10.46±1.52</td>
</tr>
<tr>
<td>3.</td>
<td>HCT %</td>
<td>32.5-34.2</td>
<td>33.63</td>
<td>0.98</td>
<td>33.63±1.96</td>
</tr>
<tr>
<td>4.</td>
<td>MCV (fl)</td>
<td>84.2-108</td>
<td>98.4</td>
<td>12.54</td>
<td>98.4±25</td>
</tr>
<tr>
<td>5.</td>
<td>MCH (pg)</td>
<td>25-34</td>
<td>30.53</td>
<td>4.84</td>
<td>30.53±9.68</td>
</tr>
<tr>
<td>6.</td>
<td>MCHC (gm/dl)</td>
<td>29-33</td>
<td>30.73</td>
<td>2.05</td>
<td>30.93±4.1</td>
</tr>
<tr>
<td>1.</td>
<td>HbF%</td>
<td>73.1-77</td>
<td>75.03</td>
<td>1.95</td>
<td>75.03±3.9</td>
</tr>
<tr>
<td>2.</td>
<td>HbA0%</td>
<td>0.4-0.7</td>
<td>0.56</td>
<td>0.15</td>
<td>0.56±0.3</td>
</tr>
<tr>
<td>3.</td>
<td>HbA2%</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>HbS%</td>
<td>15-19.5</td>
<td>17.13</td>
<td>2.25</td>
<td>17.13±4.5</td>
</tr>
</tbody>
</table>

Table showing range, mean, standard deviation (SD) and mean + 2SD of hematological parameters i.e. RBC count, Hb, HCT, MCV, MCH and MCHC and hemoglobin fractions i.e. HbF, HbA, HbA2 of 3 SS newborns cord blood samples.

DISCUSSION

Table 1 &2 show the 300 cord blood sample that were screened during study period out of which 256 (85.34%) show normal hemoglobin pattern & 44 (14.66 %) cases showed abnormal hemoglobin pattern for new born. These 44 new born were followed at 3 and 6 month for confirmation of diagnosis. Sickle cell disorder was the most prevalent hemoglobinopathy in the study showing 32 (10.66%) cases of AS and 3(1%) SS, followed by 6 (2%) cases of AE and 2 (0.67%) of AD,1 (0.33%) case of β thal. Serjeant et al 1986 screened 100000 cord blood in Kingston Jamaica and found 10049 (10%) cases of sickle cell trait. 315 (.3%) cases of SS, and 3511(3%) Hb AC (12). The present study shows Sickle cell hemoglobinopathy as most prevalent hemoglobinopathy in central India.

The highest patients screened were from schedule caste 96 (32 %) out of which maximum were from Mahar and Baudhha caste (79 cases). Followed by this OBC group constitute 93 cases (31%) out of which Kunbi 32 cases and Teli 26 cases. 70 cases (23.33%) belong to Muslim community as they were having large population residing in the vicinity of the center.

8% patients belonged to ST tribe while 5.67% belong to other population comprising Brahmin 6 and Thakur 6 and 1 each from Bengali, Jaiswal, Sindhi, Punjabi and Naidu.

The highest prevalence was seen in schedule caste group i.e. 20.81% followed by scheduled tribes (16.67%) Muslim (12.85%) and OBC (9.67%). 2 cases showing 2, 1 from Sindhi and other from
Thakur caste were seen. The overall prevalence was 14.66%.

Shukla et al 1985 from a study in same geographical region showed the highest incidence of sickle cell trait in Mahar (22.3%), Teli (11.3%) and Kunbi (9.4%) (13). Almost similar findings are seen in our study.

Mohanty et al 1998 reported gene frequency ranging from 22.5 to 44.4 for sickle cell disorder in central India (14).

Abhyankar et al 2000 reported that central India is a focus of Sickle Cell Disorder and prevalence in general population is 12%(10).

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 (15).

HbE is prevalent in South East Asian country. Chatterjea et al reported 525 cases of E thal out of which 48 were from Bengali Muslim. Although HbE had assumed importance due to transmigration of peoples carrying these genes(16) The average gene frequency has been found to be 10.9% in North Eastern state(16)

FAE( long form) show variable frequency ranging from 0.1 to 0.24%in different region probably due to transmigrations of people from high prevalent area into these areas. The present study show prevalence of 6 cases (2%) of hemoglobin E

Hemoglobin D is also prevalent worldwide. 2% prevalence among Sikhs of Punjab and 1% in Gujarat. 1% prevalence in Iran also reported (3). The reported gene frequency for HbD in India is 0.86%. Maharashtra show 42 (0.6%) cases of HbD detected from a survey of 5082 people screened (17). Increased number of cases was also seen from family migration from Pakistan. Prevalence of HbD is seen in Punjab (3.6%) Jammu and Kashmir (3.3%), Uttar Pradesh (2.3%)(11).The present study show 2 (0.66) cases of AD, one from Sindhi and other from Thakur community.

Reported frequency for β Thal varies between 3.5 to 14.9 %. Dash S et al 2004 reported 1-17% of prevalence of β Thal in different population of India (18). Higher prevalence is seen in north, west and east India. Cases from Punjab, Gujarat, Maharashtra, Uttar Pradesh, Goa, Bengal, Orissa, Assam, Karnataka, and Tamil Nadu & Andhra Pradesh have been reported. The present study show 1 (0.33%) case of β Thal minor of the total.

The present study shows Sickle cell hemoglobinopathy as most prevalent hemoglobinopathy in central India. It also show high population of SC, ST and OBC where hemoglobinopathy is prevalent. Amongst all caste, Scheduled caste show high prevalence for sickle cell disorder. Muslim community show high prevalence for HbS and HbE both.

12 patients were follow up during 3rd & 6th months. At 3rd months the diagnosis of 8 newborns was confirmed on HPLC as AS. In 3 newborn of FS pattern, the follow up at 3rd month confirmed cases as SS pattern.

Due to high level of HbF(α2γ2) at birth hemoglobinopathies involving β chain synthesis, such as sickle cell disease & β thalassemia do not present in neonatal period(19). In one case of β thal minor the mother was K/c of Sβ+ thal i.e. SFAA2 pattern. HPLC of newborn show normal FA pattern at birth, but during follow up at 6th month, HPLC of infant show HbA2= 5.1 (β thal minor have HBA2 ranging between (4-7%)(20).

Only 12 cases turned up for follow up at 3 & 6 months and rest were lost to follow up. The patients which were lost to follow up may be due to ignorance, low level of education, social stigma & relatively asymptomatic coarse of the disease in early childhood.

Hb electrophoresis of parent of 44 newborn with hemoglobinopathy was done. There were 32 AS newborn having mothers whose hemoglobin electrophoresis pattern showed 2 SF pattern, 20 AS pattern & 10 AA pattern. Fathers of AS newborn showed 2 AS pattern, 6 AA pattern & 24 were lost to follow up. All three SS newborn had sickle cell trait parents. All 6 AE patient and parents lost to follow up. The Hb electrophoresis status of the mother & father could not be assessed.
The 2 AD newborn have mother whose electrophoresis pattern was AD.

β thal minor patients have mother with K/c of S/B+ thal. The father shows Hb Ep as AA pattern.

The parents of 5 newborns, 3 from SS & 2 from AS group, showed sickle cell trait parents. All couples were offered genetic counseling. Information about prenatal diagnosis was given to them. The parents of 3 SS newborns were informed about development of signs & symptoms in infancy & when to seek medical help.

They were taught the importance of palpation of spleen, avoidance of cold and maintenance of hydration.

The importance of giving penicillin prophylaxis & pneumococcal vaccines to these infants to prevent pneumococcal sepsis & pneumococcal pneumonias was explained to them. This active intervention would subsequently lead to reduction in morbidity & mortality in future (5,20).

The present study showed mean HbF 76.41 + 5.12SD%, mean HbA 14.39+3.68 SD% & mean HbA2 0.24 + 0.12SD% in control group.

HbF was reported to be in range of 50 – 85%, HbA15-40% & HbA2 <0.3% in cord blood (21).Heygi et al 1977 reported 80-90% HbF, 10to 20% HbA(22).

Mason et al 1982 reported mean HbF 69.6 + 8.650 in 266 normal cord blood from Jamaica(3).

Fucharoen et al 1998 reported mean F 74.1 + 6.43 SD, HbA0 17.8 + 6.32SD & Hb A2 0.6 + 0.43SD in 326 normal cord blood (23).

Eastman et al 1999 reported median HbF 61.6%, Median HbA0 10.3% & Median HbA2 < 0.27% in 4 million newborn screened specimen in dried blood spots (24). The study showed 15 % less for HbF & 22-40% less value for all other hemoglobin. The difference was attributed to HPLC methodology & chemical degradation of Hb during formation of dried blood spots.

In present study the value of mean HbF is 76.41% which is higher the Jamaican cord blood data & dried blood spot from California.

The value of HbA and HbA2 was found to be within range of published data.

The hemoglobin fractions in 32 AS patient in current study as follows-the mean HbF is 74.53 + 5.46 SD comparable to the control group. The mean value of HbA was 10.27+ 3.5, HbS 6.53 + 2.03 & A2 is 0.35 + 0.34.

Wilson et al 1986 the quantity of HbA & HbS in AS newborn was reported to be mean 8.56 + 3.17% & mean 6.82+ 2.15 SD% respectively in 67 patients by Fast HPLC in Georgia The present study show mean HbA 10.27 + 3.5% and mean Hb is 6.57 + 2.03% comparable to above studies(25).

CONCLUSION:-Hemoglobinopathy is highly prevalent in Central India. It is seen in newborn in SC, ST communities followed by Muslim and OBC. Sickle cell disorder is the most prevalent hemoglobinopathy followed by Hb E and HbD. Sickle cell disorder is prevalent in Scheduled Caste, HbE in Muslim and Teli community. Follow up of SS infants as per protocol is important to prevent morbidity and mortality due to infection in infancy and early childhood. Family awareness, parental education and genetic counseling would help to lower the birth of SS child.

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