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Paediatrics

A STUDY TO SCREEN NEWBORNS FOR HEMOGLOBINOPATHIES

Bhavya Verma	Senior resident, Dept of Paediatrics, RNT Medical College, Udaipur.
Ashok Gupta	Senior Professor, Dept of Paediatrics, SMS Medical College, Jaipur.
Nitin Gupta	Senior resident, Dept of Paediatrics, RNT Medical College, Udaipur.
Shivani Sharma*	Resident, Dept of Paediatrics, RNT Medical College, Udaipur. *Corresponding Author

ABSTRACT **Background:** The main objective of this study was to diagnose hemoglobinopathies in newborns as early as possible to minimize morbidity and mortality and to detect the proportion of various types of hemoglobinopathies among the screened neonates.

Methods: A hospital based observational study was done in department of pediatrics, SMS hospital over a period from june 2019 to june 2020. Institute Ethics Committee approval was obtained before the start of the study. Total 1175 newborns were screened in the study. 2 ml of cord blood samples of the newborn were analyzed through high performance liquid chromatography to detect the abnormal hemoglobin.

Results: Out of the 1175 newborns screened, 20 (1.7%) had variant hemoglobin and 1155 (98.3%) had normal hemoglobin. The mean concentration of HbA was (16.2 ± 3.14) mg/dl and range of HbA was (7.9 - 24.7) mg/dl. The concentration of HbA2 was (0.38 ± 0.21) mg/dl and the range of HbA2 was (0.1 - 0.9) mg/dl.

Conclusion: Newborn screening with high Performance Liquid Chromatography (HPLC) achieves good separation and quantitation of HbF and HbA2 in addition to screening for variant hemoglobins along with thalassemia.

KEYWORDS : hemoglobinopathies, newborns, HPLC

INTRODUCTION

Hemoglobinopathies are a group of inherited single gene disorders found in the Mediterranean region, Middle East, Indian subcontinent and Southeast Asia. The hemoglobinopathies pose a major health burden and are more confined at variable frequencies to certain states and caste populations.[1] It is estimated that, worldwide, approximately 7.0% of the population are carriers of hemoglobinopathies including the thalassemias, Hb E and Hb S. Annually, 300,000–500,000 children are born with a severe hemoglobin (Hb) disorder. In India, Hb E is the most prevalent hemoglobinopathy in the eastern and northeastern states. Indian Council of Medical Research study showed that the HbE is mainly seen in Assam (23.9%) and Kolkata, West Bengal (3.92%). In India, the cumulative gene frequency of hemoglobinopathies is around 4.2%.[1,2]

Abnormalities of Hb involve thalassemias and Hb variants which are due to abnormal globin chain synthesis. Qualitative changes due to change in the amino acid sequence of the globin result in sickle cell disease.[2,3] Quantitative changes due to decreased or imbalanced production of structurally normal globins result in the thalassemia syndromes.[2,3] The prevalence of -thalassemia trait and sickle cell in various regions of India is around 3%–17% and 1%–44%, respectively, because of consanguinity, caste, and area endogamy. Every year, around ten thousand children with -thalassemia major are born in India, which constitutes about 10% of the total global load of -thalassemia.[3]

Newborn screening for sickle cell disease as one element of prevention programs for hemoglobinopathies has been in place in several regions in the world for more than 40 years now. Newborn screening is a public health program with the purpose of identifying pre-symptomatic newborn infants with treatable condition associated with significant morbidity and mortality.[4]

High Performance Liquid Chromatography (HPLC) achieves good separation and quantitation of HbF and HbA2 in addition to screening for variant hemoglobins along with thalassemia. HPLC involves the separation of haemoglobin based on their ionic interaction with the cartridge. The separated fractions pass through a flow cell, where absorbance is measured over time producing a chromatogram. Each hemoglobin has its own characteristic retention time and is measured from the time of sample injection into the HPLC to the maximum point of each peak.[4]

The main objective of this study was to diagnose hemoglobinopathies in newborns as early as possible to minimize morbidity and mortality and to detect the proportion of various types of hemoglobinopathies among the screened neonates.

METHODS

The study was a hospital based observational study, conducted at department of Paediatric medicine, SMS hospital and attached group of hospitals, Jaipur over a period from June 2019 to June 2020. Sample size was calculated at 95% confidence level with an alpha error of 0.05 assuming 13.6% hemoglobin abnormalities identified in the newborns screened as per the reference study. At an absolute error of 2%, total 1175 newborns were included in the study.

All the newborns delivered in the SMS Medical College and attached hospitals Jaipur, Rajasthan were included in the study. Institute Ethics Committee approval was obtained before the start of the study. Total 1175 newborns were screened in the study. The parents not giving consent were excluded from the study. All the steps of study were explained to the parents and informed written consent was taken.

The newborns included in the study were subjected to a detailed history, clinical examination and laboratory evaluation. 2 ml of cord blood sample of each newborn was collected in Ethylene Diamine Tetra Acetic Acid (EDTA) vial at the time of delivery. Hemoglobin analysis was done on the collected cord blood sample through High Performance Liquid Chromatography (HPLC).

Appropriate documentation was completed, including the patient's name, date of birth and time of the sample collection. The entire data was collected in a pretested proforma. Nominal / categorical variables were summarized using frequency and percentage and analysed using Chi square test. Continuous variables were summarized as mean and standard deviation and were analysed using independent sample t test. A P value <0.05 was taken as statistically significant.

RESULTS

In our study, the mean concentration of HbA was (16.2 ± 3.14) mg/dl and range of HbA was (7.9 - 24.7) mg/dl. The concentration of HbA2 was (0.38 ± 0.21) mg/dl and the range of HbA2 was (0.1 – 0.9) mg/dl. The concentration of HbF was (80.12 ± 3.14) mg/dl and the range of HbF (71.3 - 89.7) mg/dl. [Table 1, Figure 1].

In our study, 667 (56.8 %) were males and 508 (43.2 %) were females out of the 1175 newborns screened. Out of the 667 males, 659 (97.78 %) had normal haemoglobin and 15 (2.2 %) had variant haemoglobin. Out of the 508 females, 496 (5%) had normal haemoglobin and 5 (1%) had variant haemoglobin. The sex difference in both the groups is statistically insignificant. [Table 2, Figure 2].

In our study, out of the 1175 newborns screened, 1155 (98.3 %) newborns had normal hemoglobin while 20 (1.7%) newborns had Variant hemoglobin. [Table 3, Figure 3]

Table 1: Different type of Hemoglobin concentration among the newborns screened

Hb Concentration	Mean ± SD	Range
HbA	16.2± 3.14	7.9 – 24.7
HbA2	0.38± 0.21	0.1 – 0.9
HbF	80.12± 3.14	71.3- 89.7

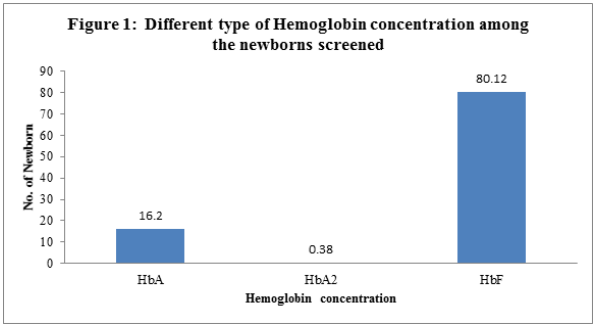

Table 2: Variant Hemoglobin in relation to gender

Gender	Normal Hb		Variant Hb		Total
	Number	%	Number	%	N
Female	496	99	5	1	501
Male	659	97.78	15	2.22	674
Total	1155	98.3	20	1.7	1175
Chi-square = 1.906 with 1 degree of freedom; P = 0.167 (NS)					

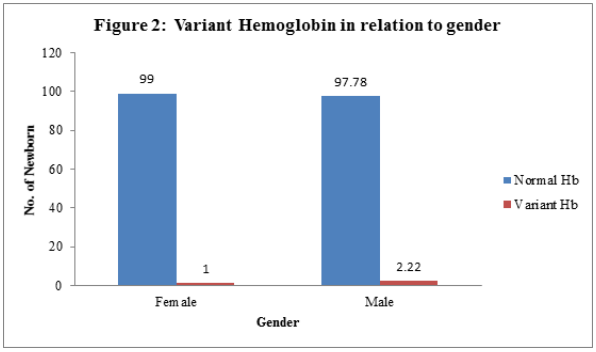

Table 3: Distribution of the newborns according to the Variant Hemoglobin

Hemoglobin	Number	Percentage (%)
Normal	1155	98.3%
Variant Hb	20	1.7%
Total	1175	100

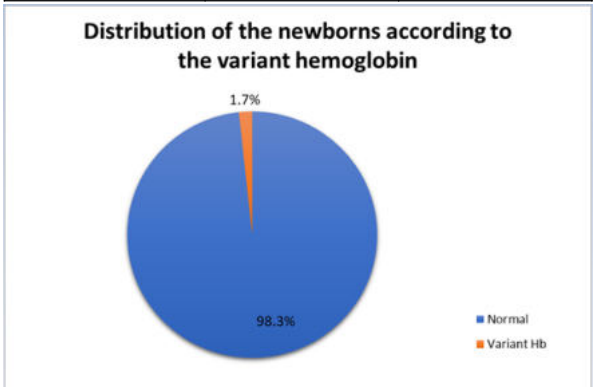

Figure 3:

DISCUSSION

Newborn screening is mainly recommended for sickle cell disorders among those tribal and nontribal populations where the prevalence of Hb S is high.[1] Ideally, universal screening should be done where all newborn babies in these high-risk groups are screened, as this would allow identification of other clinically significant disorders such as homozygous β-thalassemia and all cases of Hb S–β thalassemia.[3,4] The normal separation pattern of the haemoglobin done through HPLC consists of two fractions with some derivatives. After 36 weeks of gestation the main fraction is HbF with an average expression of 80%. The second fraction is HbA with an average expression of 20%.

In case of sickle cell disease or β-thalassemia major, no HbA will be present. In sickle cell disease, HbA is substituted by the HbS fraction only or by the common combinations HbS/HbC, HbS/HbE or HbS/HbD. When the HbA level is below 5% and the neonate is not significantly premature, probable β-thalassemia major or intermedia must be considered and investigated further.[5,6] At birth, the HbA2 is usually below detection levels. When HbA_2 is visible, HbF is lower and HbA is higher than expected, then a blood transfusion has probably been given to the baby, either intrauterine or shortly after birth. These cases should be investigated to exclude large deletion defects or other intrauterine haemoglobinopathies.[5,6]

Normal newborn samples mainly contain HbF (α2/γ2) and a smaller amount of HbA (α2/β2). HbA levels range from 6 to 40% with an average of 19%, showing inter-individual variations. HbA levels in newborns are dependent on gestational age and date of sampling, reflecting the stage of hemoglobin switch. Today, there are more than 1000 variants of hemoglobin known.[7]

The α-globin gene mutations are already expressed during embryonic, foetal and postnatal life. In all the newborns with α-thalassemia the non-functional abnormal fraction Hb Bart's will always be detected. The carriers of β-thalassemia cannot be diagnosed by measuring their elevated HbA_2 expression at birth because HbA_2 is not sufficiently expressed until one year of age. Newborn carriers of β-thalassemia do not express one of their β-globin genes and are born with a reduced HbA level. They can be recognized with reasonable sensitivity by measuring the expression of HbA at birth according to the weeks of gestation.[8,9]

As it is established that the level of normal or abnormal fractions at birth in full term newborn must be 80% HbF and 20% HbA, the absence of HbA suggests β-thalassemia major. Samples with 3% or even up to 5% HbA should be considered as possible β-thalassemia intermedia or β-thalassemia carriers and should be investigated further. The absence of HbA and the presence of about 20% HbS indicates homozygous HbS or the compound heterozygous genotype of HbS/β-thalassemia. The measurement of HbA and a cut-off <15% may be indicative for the β-thalassemia trait in newborns. β-thalassemia major can be expected in samples with a hemoglobin pattern of HbF only, or HbF and HbA below a certain cut-off from 0–5%.[10]

In our study, out of the 1175 newborns screened, 1155 (98.3%) had normal haemoglobin and 20 (1.7%) had variant haemoglobin. In a study done by Dipti Upadhye[1] in Tripura state, out of the 2400 newborns screened, 327 (13.6%) had variant haemoglobin. In our study, the mean concentration of HbA was (16.2 ± 3.14) mg/dl and range of HbA was 7.9 - 24.7 mg/dl. In a study done by Salam Alkindi (2010)[11] 7837 newborns were screened in Oman, out of which 67.5% had variant haemoglobin. In a study done by Roshan B Colah (2018)[12] in India, out of the 18,003 newborns screened, 3244 (18%) had variant haemoglobin. In a study done by Clarisse Lopes (2013)[13] out of the 1,217,833 newborns screened in Brazil over 10 years, 4.87% of the newborns were heterozygous for a haemoglobin variant, 0.08% were homozygous of doubly heterozygous for variant hemoglobin and 95.02% had normal haemoglobin. In a study done by SS Rwezaula (2015)[14] in Muhimbili, Tanzania in 2009, out of the 2053 newborns screened, the prevalence of variant haemoglobin was 18.2%. In a study done by R.D. Hanmante (2009)[15] out of the 500 newborns screened, 8% showed the variant haemoglobin.

The concentration of HbA2 was (0.38 ± 0.21) mg/dl and the range of HbA2 was 0.1 - 0.9 mg/dl. The concentration of HbF was (80.12 ± 3.14) mg/dl and the range of HbF was 71.3 - 89.7 mg/dl. In a study done by Suzane Dal[16] for determining the reference interval of haemoglobin fractions in the cord blood, was 19.9% (10.5-36.7%) for HbA, 80.1% (62.7-89.4%) for HbF and 0.1% (0.0-0.6%) for HbA2. Primary screening with HPLC can thus help to streamline the follow-up tests needed for the identification of a hemoglobin disorder in the majority of cases.

Limitations of HPLC include its inability to distinguish β-thalassemia from benign conditions (Hb E/b-thalassemia and Hb EE show an FE pattern by HPLC) or detection of unusual Hb variants.[3,4] Thus, a

confirmatory test with DNA analysis is essential to detect the variant hemoglobins. The major limitation of the study was the small sample size. Also, the confirmatory diagnosis of the variant hemoglobin by DNA analysis could not be done.

ABBREVIATIONS

Hb: Hemoglobin
HbA: Adult Hemoglobin
HbF: Fetal Hemoglobin
HbS: Sickle Hemoglobin
HbC: Hemoglobin C
HbD: Hemoglobin D
HbE: Hemoglobin E

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