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Hemoglobin fractions in Indian pediatric population – Do we need to look westward?

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Abstract:

BACKGROUND: Hemoglobin (Hb) is a tetramer of two alpha and two beta globin polypeptide chains, The fractions of HbF, HbA2, and HbA vary gradually in pediatric population (age 0–12 years) and show a dynamic change in the 1st year of life.

OBJECTIVE: The objective of the study is to establish the levels of normal hemoglobin (Hb) fractions by using high-performance liquid chromatography (HPLC) in Indian pediatric population.

MATERIALS AND METHODS: A total of 169 children of 0–12 years of age were recruited from a pediatric outpatient clinic. Eleven cord blood samples from normal deliveries were collected, and 2 ml peripheral blood was drawn from each subject in EDTA vial for CBC and Hb HPLC. CBC was performed by hematology analyzer XT 2000i, and the proportion of HbA, HbA_2, and HbF was obtained from HPLC using the β thalassemia short program.

RESULTS: The fractions of HbF, HbA_2, and HbA gradually changed with increasing age. HbF levels decreased rapidly from 80.9% ± 0.48% (mean ± standard deviation) in the cord blood to 3.6% ± 1.04% at 6 months of age. HbA_2 was 0% in cord blood, was 0.04 ± 0.12 in newborn, reached to a level of 2.52% ± 0.3% at 6–12 months of age, and thereafter marginally increased to 2.65 ± 0.89% at 2–12 years of age. HbA was 20.41% ± 5.14% in the cord blood and increased substantially to 84.7% ± 1.83% at 6–12 months of age.

CONCLUSION: HbF levels show a more dynamic change in the first 6 months of life than later half. It reaches adult levels by the age of 2 years. HbA_2 levels reach a plateau at 6 months of age. HbA levels rise substantially until the 6th month and sustain thereafter. These data can serve as a quick practical reference guide for the analysis of Hb fractions in the Indian pediatric population.

Keywords:

Hemoglobin, high-performance liquid chromatography, newborn screening, pediatrics

Introduction

Hemoglobin (Hb) is a tetramer of two alpha and two beta globin polypeptide chains.[1] There are three genes within the alpha gene cluster – zeta (ζ), alpha 1 (α1) and alpha 2 (α2) – and five genes within the beta gene cluster – epsilon, delta (δ), beta (β), and 2 gamma (γ) genes.[2] The early embryonic Hbs include Gower 1 (ζ2ε2), Gower 2 (α2ε2), and Portland (ζ2γ2), found in 4–13 weeks of gestation. Beyond 13 weeks of fetal life, the major Hb is HbF (α2γ2) which functions as a principle oxygen-carrying protein. The adult Hb, i.e., HbA (α2β2), appears at ~1 month of fetal life but does not become dominant until after birth.[1]

The term "hemoglobinopathy" is restricted to disorders with structurally abnormal Hb, and "thalassemia" is used for those that have a reduced synthesis of a globin chain.[3] Although many clinicians consider thalassemia as a subtype of hemoglobinopathies, they have different

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causative factors.[4] Point mutations or single nucleotide polymorphisms in the alpha or beta gene cluster can result in hemoglobinopathies, while quantitative changes such as amino acid insertions, deletions, or mutations in the noncoding sequences (introns) lead to thalassemias.[3]

Hemoglobinopathies are the most common recessive inherited disorders globally, and approximately 7.0% of the world's population are carriers.[5] It is estimated that over 50,000 new patients are born worldwide each year with severe forms of thalassemia, out of which nearly 80% of these births occur in developing countries.[6] Screening of at-risk couples and prenatal diagnosis for affected fetuses is the practical and effective approach to reduce the incidence of hemoglobinopathies. Various techniques to detect Hb disorders include high-performance liquid chromatography (HPLC), immunoelectrophoresis, cellulose acetate electrophoresis, citrate agar electrophoresis, alkaline globin chain electrophoresis, capillary zone electrophoresis, and advanced molecular methods.[3]

The fractions of HbF, HbA_2, and HbA vary gradually in pediatric population (age 0–12 years) and show a dynamic change in the 1st year of life.[7] Diagnosis of thalassemia and other hemoglobinopathies in the pediatric population requires identifying the reference values for each Hb fraction in our population. Cutoff values for adults cannot be used for diagnosis in pediatric population. Only western studies and limited literature on Indian pediatric population are available to date. The aim of this study was to establish the levels of normal Hb fractions by using HPLC in Indian pediatric population.

Materials and Methods

This was a prospective study conducted in the departments of pathology, pediatrics, and obstetrics and gynecology in a tertiary care center between July 2018 and July 2019. A total of 169 children of 0–12 years of age were recruited from the pediatric outpatient clinic and thereafter categorized into a series based upon their ages. Eleven cord blood samples from normal deliveries were collected, and 2 ml peripheral blood was drawn from each subject in EDTA vial for CBC and Hb HPLC. CBC was performed using hematology analyzer XT 2000i, and the proportion of HbA, HbA2, and HbF was obtained from HPLC using the β thalassemia short program (BIO-RAD VARIANT II). Patients with Hb levels less than 10 g/dl and samples with detected hemoglobinopathies were excluded from the study.

Ethical clearance was obtained from the institutional ethical committee. Statistical analysis was performed using SPSS Software Version 24 IBM Corp. Released 2016. (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp). $P \leq 0.5$ was considered statistically significant.

Results

After exclusion of 44 specimens, 125 samples were used for evaluation. Of these, 85 samples were from males and 40 were from females (M:F ratio of 2.1:1). The distribution of cases among various age groups is summarized in Table 1.

The fractions of HbF, HbA2, and HbA gradually altered with increasing age [Table 1 and Figure 1]. HbF levels decreased rapidly from 80.9% ± 0.48% (mean ± standard deviation) in the cord blood to 3.6% ± 1.04% at 6th month. Percentage of HbF in a newborn was almost similar to that of cord blood (80.8% ± 5.4%). The levels of HbF came down to 49.56% ± 9.7% at 15 days to 1 month of age and have further been reduced to 17.68% ± 6.8% at 2–3 months of age. HbA2 was 0% in the cord blood and reached to a level of 2.52% ± 0.3% at 6–12 months and thereafter marginally increased to 2.65% ± 0.89% at 2–12 years. HbA was 20.41% ± 5.14% in the cord blood and increased substantially to 84.7% ± 1.83% at 6–12 months of age. HbF

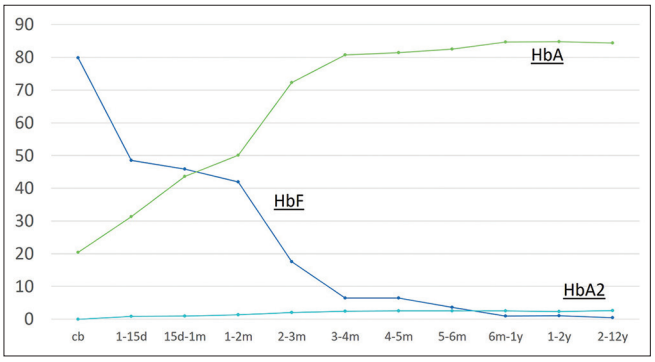

Figure 1: Percentage of fetal hemoglobin, hemoglobin A_2, and hemoglobin A in children less than 12 years of age

Table 1: Percentage of fetal hemoglobin, hemoglobin A_2, and hemoglobin A in children <12 years of age

Age	Number (*n*)	HbF (%)	HbA_2 (%)	HbA (%)
Cord blood	11	80.9±0.48	0	20.41±5.14
Newborn	11	80.8±5.4	0.04±0.12	17.24±5.28
1-15 days	10	65.3±20.5	0.83±0.57	31.27±18.58
15 days-1 month	6	49.56±9.7	0.96 ±0.26	43.58±8.65
1-2 months	8	43±17.29	1.32±0.36	50.12±15.98
2-3 months	5	17.68±6.8	2.04±0.41	72.28±6.9
3-4 months	4	6.37±1	2.45±0.46	80.67±2.88
4-5 months	3	6.4±2.08	2.56±0.15	81.4±0.95
5-6 months	5	3.6±1.04	2.52±0.22	82.54±1.45
6 months-1 year	13	0.9±0.39	2.52±0.3	84.7±1.83
1-2 years	10	1.01±0.53	2.28±0.26	84.74±1.24
2-12 years	50	0.44±0.23	2.65±0.29	84.36±1.23

Hb: Hemoglobin, HbF: Fetal hemoglobin

normalized to adult levels at 2 years of age, while HbA2 and HbA showed normalization at 6 months of age.

Out of 44 blood samples that were excluded from this study, 33 samples had Hb less than 10 and hemoglobinopathies were detected in 11 children. Of these, eight samples were that of β thalassemia trait and one sample each with HbE trait, HbJ, and hereditary persistence of fetal Hb (HPFH) [Figure 2]. The prevalence of hemoglobinopathies detected was 6.5%.

Discussion

Estimation of Hb fractions in children can assist in diagnosis of various Hb disorders if the normal reference range of each Hb type is defined in our own pediatric population.

Our findings of HbF levels in the pediatric population were similar to the study in Thailand,[7] in contrast to western literature,[8] where the levels of HbF in newborn were significantly different from this study [Table 2]. This difference may be possibly explained due to ethnic variation. HbF is clinically useful in the detection of many hemoglobinopathies. It is raised in hereditary disorders such as β thalassemia, HPFH, δβ thalassemia, sickle cell anemia, other hemoglobinopathies (HbC, HbE, some unstable Hbs), and acquired conditions such as pernicious anemia, paroxysmal nocturnal hemoglobinuria (PNH), refractory normoblastic anemia, sideroblastic anemia, pure red cell aplasia, aplastic anemia, pregnancy, hyperthyroidism, and juvenile myelomonocytic leukemia.[9]

HbA2 levels in the cord blood were zero in the current study. HbA_2% increased with age till 6 months, then remained stable. The levels of HbA2 in newborns in our study (0.04% ± 0.12%) were significantly low in comparison to the studies conducted by Wong *et al*. (0.32% ± 0.19%) and Mosca *et al*. (0.4% ± 0.2%).[7,8]

Table 2: Comparison of fetal hemoglobin between our study and study from Thailand and Italy

Current study (Hb HPLC)		Thailand, Wong *et al*.[7] (Hb HPLC)		Italy, Mosca *et al*.[8] (Hb HPLC)	
Age	**HbF (%)**	**Age**	**HbF (%)**	**Age**	**HbF (%)**
Cord blood	80.9±0.48		-	-	-
New born	80.8±5.4	At birth/cord blood	78.39±7.59 (*P*=0.40)	At birth	65.1±7.5 (*P*=0.001)
1-15 days	65.3±20.5		-		-
15 days-1 month	49.56±9.7	1st month±2 weeks	71.49±16.03 (*P*=0.01)	-	-
1-2 months	43±17.29	2nd month±2 weeks	42.79±15.36	-	-
2-3 months	17.68±6.8	3rd month±2 weeks	20.04±11.32	3 months	18.1±3.6 (*P*=0.88)
3-4 months	6.37±1	4th month±2 weeks	19.19±11.74		-
4-5 months	6.4±2.08	5th month±2 weeks	9.01±7.54		-
5-6 months	3.6±1.04	6th month±2 weeks	4.08±1.62	6 months	3.2±1.1 (*P*=0.52)
6 months-1 year	0.9±0.39	8th month±2 weeks	3.47±1.92	9-10 months	2.6±1.4
		10th month±2 weeks	2.53±1.58		
		12th month±2 weeks	3.16±1.86		
1-2 years	1.01±0.53		-	1 year	1.4±0.6
2-12 years	0.44±0.23		-	-	-

HPLC: High-performance liquid chromatography, Hb: Hemoglobin, HbF: Fetal hemoglobin

Table 3: Comparison of hemoglobin A_2 between our study and study from Thailand and Italy

Our study (Hb HPLC)		Thailand, Wong *et al*.[7] (Hb HPLC)		Italy, Mosca *et al*.[8] (Hb HPLC)	
Age	**HbA_2 (%)**	**Age**	**HbA_2 (%)**	**Age**	**HbA_2 (%)**
Cord blood	0			-	-
New born	0.04±0.12	At birth/cord blood	0.32±0.19 (*P*=0.006)	At birth	0.4±0.2 (*P*=0.001)
1-15 days	0.83±0.57		-		-
15 days-1 month	0.96 ±0.26	1st month±2 weeks	0.55±0.68 (*P*=0.18)	-	-
1-2 months	1.32±0.36	2nd month±2 weeks	1.40±0.54 (*P*=0.73)	-	-
2-3 months	2.04±0.41	3rd month±2 weeks	2.14±0.47 (*P*=0.7)	3 months	1.7±0.3 (*P*=0.11)
3-4 months	2.45±0.46	4th month±2 weeks	2.09±0.57 (*P*=0.31)		-
4-5 months	2.56±0.15	5th month±2 weeks	2.65±0.37 (*P*=0.69)		-
-5-6 months	2.52±0.22	6th month±2 weeks	2.78±0.25 (*P*=0.08)	6 months	2.5±0.3 (*P*=0.90)
6 months-1 year	2.52±0.3	8th month±2 weeks	2.98±0.17	9-10 months	2.5±0.4 (*P*=0.90)
		10th month±2 weeks	2.67±0.21		
		12th month±2 weeks	2.78±0.20		
1-2 years	2.28±0.26		-	1 year	2.5±0.3 (*P*=0.16)
2-12 years	2.65±0.29		-	-	-

HPLC: High-performance liquid chromatography, Hb: Hemoglobin

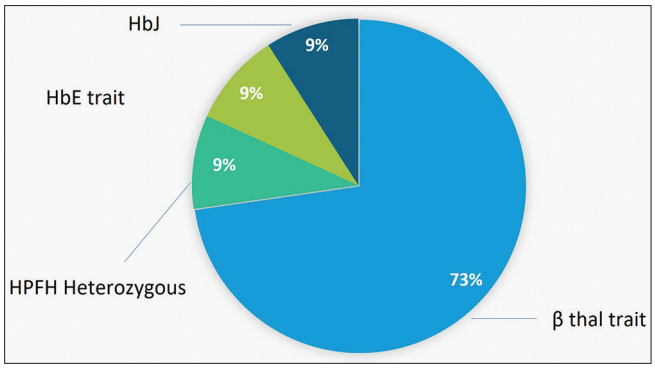

Figure 2: Types of hemoglobinopathies detected in this study

However, in other categorized age groups, the levels were similar to referred literature [Table 3]. Causes of elevated HbA2 include β thalassemia, Hb Lepore, hyperthyroidism, megaloblastic anemia, antiretroviral therapy, HbS, some unstable hemoglobin variants, triplicate α gene (ααα), pseudoxanthoma elasticum, and hypertrophic osteoarthropathy.[8]

India with a huge population of 1.3 billion has considerable ethnic diversity. Not only thalassemias and sickle cell disorders but other Hb variants are also prevalent. The cumulative gene frequency of hemoglobinopathies is around 4.2% in India,[10] and the prevalence of pathological hemoglobinopathies in India is 1.2/1000 live births.[11]

Taking into account such a huge burden of hemoglobinopathies in India, which is still a developing nation, prevention through screening programs is certainly a superior and economical strategy. Apart from identification of at-risk pregnancies and antenatal screening, newborn screening (NBS) is an additional scheme within prevention program. Fresh cord blood analyzed within 24 h gives the best separation patterns. Dried blood spots collected from a heel-prick within 1 week after birth can also be used. Sickle cell disease and β^0-thalassemia major are recognized immediately with 100% sensitivity and high specificity. Presence of ~20% HbS indicates homozygous HbS disease. The presence of Hb Bart identifies α thalassemia at birth. Carriers of β thalassemia cannot be diagnosed by their elevated HbA2 expression at birth; however, low HbA expression could give an indication, which can be further verified by molecular study and family study.

In Canada, the UK, and other European countries, prenatal screening is linked to NBS, while in the US, it is selectively performed.[12] In India, prenatal screening program has started with the support of Ministry of Health and Family Welfare and Delhi Government and is being performed in selective cases, though NBS for hemoglobinopathies has not been yet established.

There are some limitations in this study, as it is a small study and the recruited outpatient samples may not be a true representation of the reference population. Therefore, larger population-based screening programs are recommended.

Conclusion

HbF levels show a more dynamic change in the first 6 months of life than later half. It reaches adult levels by age of 2 years. HbA_2 levels reach a plateau at around 6 months of age. HbA levels rise substantially until 6 months of age and remain relatively stable thereafter. The data in this study can serve as a quick practical reference guide for analysis of Hb fractions in Indian pediatric population. Neonatal screening programs can be a part of the national thalassemia program so as to have our own registry and to decrease the burden of hemoglobinopathies in our country.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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