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**ORIGINAL RESEARCH**

# Distribution of hemoglobinopathies in pediatric population: A tertiary care experience

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**Abstract**

**Introduction:** Anemia is defined as a decreased concentration of hemoglobin as compared to the values in the age matched controls.Anemia in children is one of the major problems in India and many parts of the world, since anemic children have reduced exercise capacity, slower capacity of growth, impaired cognitive development, and delayed wound healing.

**Aims and objectives:** The aim of this study was to screen the anemic population for the thalassemia and hemoglobinopathies by HPLC/electrophoresis for hemoglobin variations and to offer counselling accordingly.

**Material and methods:** The present study is a cross sectional, descriptive study conducted on 100patients in the age group of 6months to 18years, who were admitted to the pediatric ward of Sri Aurobindo Medical College and PG Institute, Indore with anemia.

**Results:** HPLC/electrophoresis was performed in all the microcytic anemia cases without iron deficiency to differentiate the sickle cell anemia, thalassemia trait and thalassemia major. Out of 27 microcytic hypochromic patients with normal iron levels sickle cell anemia was found in 10 cases. 10 patients were thalassemia trait and 7 were thalassemia major. Accordingly sickle cell anemia patients were further classified into sickle cell homozygous anemia, sickle cell trait and sickle beta thalassemia.

**Discussion:** In the present study sickle cell anemia and thalassemia trait haemoglobinopathies had equal incidence seen in 20 (37% each) children followed by thalassemia major seen in 7(25.9%) childrens. In the Bangalore study, thalassemia major was the most common type of hemoglobinopathy seen in 9 (54%) children followed by thalassemia minor among 2 (30%) children and equal incidence of about 1 (8%) child each suffering from sickle cell anemia, and sickle cell thalassemia. In the present study, homozygous thalassemia was the most common. In a study conducted by Mitra, HbE was found to be the most common, followed by homozygous thalassemia and least common was

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HbDE disease.
**Conclusion:** HPLC is a reliable method for screening of thalassemia. Screening should be done to all suspected patient to find out the exact prevalence of thalassemia. Treatment should be start as soon as thalassemia positive detected before the end organ damage. Genetic council the patient family for next pregnancy for avoid birth of a thalassemia child in their family.
**Keywords:** anemia, thalassemia, children

## Introduction

Anemia is defined as a decreased concentration of hemoglobin as compared to the values in the age matched controls. Anemia in children is one of the major problems in India and many parts of the world, since anemic children have reduced exercise capacity, slower capacity of growth, impaired cognitive development, and delayed wound healing. Anemic children are also at increased risk of dying due to complication associated with malnutrition and infection. Anemia is an important indicator of nutritional status within the pediatric population. As many as 20% children in the United States and 80% of the children in the developing countries are anemic at some point. Because of these factors, the study of the etiopathogenesis of anemia in infancy and childhood has attracted wide attention in the recent years in India[1].

Thalassemia and Hemoglobinopathies are hereditary anemia resulting from defects in hemoglobin production[9]. Thalassemia syndrome is an autosomal recessively inherited group of hemoglobin synthesis disorder characterized by the absence or reduction in output of one or more of the globin chains of hemoglobin. Depending upon the globin gene affected thalassemia is mainly of two types namely β thalassemia and α thalassemia[2].

β-Thalassemia, is caused by a decrease in the production of β-globin chains. It affects multiple organs and is associated with considerable morbidity and mortality. It is a complex group of disorder because of the genetics of hemoglobin production and structure of hemoglobin molecule in which the normal hemoglobin protein is either not produced or produced in lower amounts than usual. Beta-thalassemia is prevalent in Mediterranean countries, the Middle East, Central Asia, India, Southern China, and the Far East as well as countries along the north coast of Africa and in South America. The highest carrier frequency is reported in Cyprus (14%), Sardinia (10.3%), and Southeast Asia[3].

It is estimated that world over there are more than 200 million (1.5% of the world's population) carriers of β-thalassemia gene, out of which about 40 million (20%) are in South-East Asia, and 20 million of them are in India alone. Every year approximately 100,000 children with thalassemia major are born world over, of which 10,000 are born in India[4-5].

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**Aims and objectives**

The aim of this study was to screen the anemic population for the thalassemia and hemoglobinopathies by HPLC/electrophoresis for hemoglobin variations and to offer counselling accordingly. To fulfil this aim following objectives were studied-

1. To find out causes of anemia in patients visiting department of Pediatrics, SAIMS (Sri aurobindo institute of medical sciences), Indore.
2. To find the contribution of thalassemia and other hemoglobinopathies among these anemic children.
3. To establish correlation of simple clinical and laboratory parameter like RBC indices with Hemoglobin abnormalities.

**Material and methods**

The present study is a cross sectional, descriptive study conducted on 100patients in the age group of 6months to 18years, who were admitted to the pediatric ward of Sri Aurobindo Medical College and PG Institute, Indore with anemia. The children with hemoglobin values of less than 11gm/dl in the age group of 6months to 6years those with hemoglobin values of less than 12gm/dl in the age group of 6 to 18years, were included in the study which was conducted from March2013 to June2014. Patients who do not give consent or have received blood transfusion within 1 month, leukemia or any other malignancy or any chronic illness were excluded from the study.

In the present study, WHO criteria was employed for grading and categorization.

A detailed history was elicited, a thorough clinical examination undertaken and the data recorded in the proforma. The collected blood was analyzed using **coulter LH 750 analyzer**, having three part differentials, from which the following parameters were obtained. We used **ClinReP HPLC Complete Kit** for the determination of haemoglobin variants and for the screening of β-thalassemia in whole blood.

**Statistics and Analysis**

Statistical analysis will be done using SPSS software version 15. Quantitative variables will be analyzed by student t test whereas Chi Square test will be used to estimate difference in frequencies of discrete variables.

**Results**

Out of 100 subjects 47 were females and 53 males as shown in **fig.-1**

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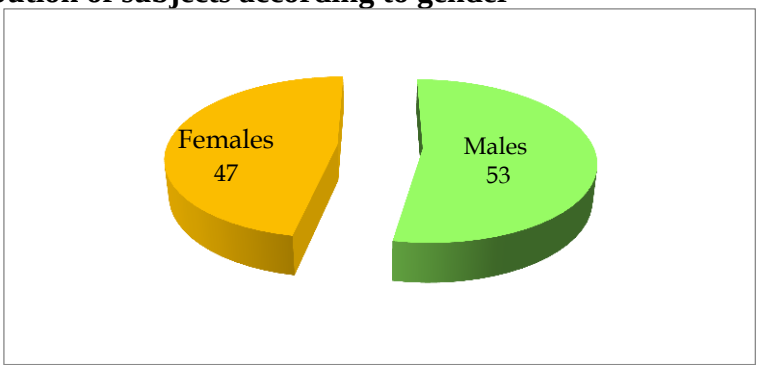
**Figure 1: Distribution of subjects according to gender**




The mean age of patients was 4.63±4.18 years (range 6 months to 15 years) the thalassemia major patients develop symptoms as early as 6 months in our study. The mean age of Thalassemia major patients was 2.70±2.44 years and it was minimum in all studied cases. Ten patients were found as carrier for thalassemia gene. The mean age of thalassemia trait patients was 10.0±3.6 years **(table 1).**

Sickle cell disease was observed in 10 cases with a mean age of 6.14±4.35 years (15 months to 14 years).

**Table 1: Age and sex distribution in different anemic groups**

| Parameter | No. | Age | Male | Female |
|---|---|---|---|---|
| B12 Deficiency | 12 | 5.41±5.2 | 5 | 7 |
| IDA | 61 | 3.59±3.41 | 32 | 29 |
| SCA | 10 | 6.14±4.35 | 6 | 4 |
| TM | 7 | 2.70±2.44 | 3 | 4 |
| TT | 10 | 10.0±3.6 | 7 | 3 |

Mean corpuscular hemoglobin was decreased in all type of anemia except Vitamin B12 deficiency patients. It was least in iron deficiency anemia cases. Of the hemoglobinopathies patient the MCH values were lowest in thalassemia trait patients **(table 2).**

**Table 2: MCV and MCH in different types of anemia**

| Diagnosis | MCV | MCH |
|---|---|---|
| B12 Deficiency | 100.7±13.9 | 26.3±3.8 |
| IDA | 59.2±5.8 | 19.3±2.6 |
| SCA | 63.3±10.6 | 21.5±3.1 |
| TM | 69.5±5.2 | 20.3±4.4 |
| TT | 64.4±8.2 | 19.7±3.8 |
| Hemoglobinopathy (SCA+TM+TT) | 65.3±8.6 | 20.5±3.6 |

When we compared MCV and MCH values in hemoglobinopathis and Iron Deficiency anemia group, significant difference was found in MCV values only (P value <0.0001). MCH values were almost similar in IDA and hemoglobinpathy group (p =0.079).

However there was no significant difference in MCV and MCH values of thalassemia major, thalassemia trait and Sickle cell anemia patients.

Therefore on the basis of MCV and MCH values alone we cannot differentiate between the types of hemoglobinopathy.

Reticulocyte count was also found normal in vitamin B12 deficiency cases (0.95±0.64%) and it was near normal in iron deficiency anemia patients (2.0±0.86%). Highest reticulocyte count was observed in sickle cell anemia patients showing highest degree of hemolysis on sickle patients **(Table 3).** Reticulocyte count was significantly higher in hemoglobinopathies group than iron deficiency and Vitamin B12 deficiency group (p<00001).

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**Table 3: Reticulocyte count in different anemic patients**

| Diagnosis | Reticulocyte Count |
|---|---|
| B12 Deficiency | 0.95±0.64 |
| IDA | 2.0±0.86 |
| SCA | 4.6±2.5 |
| TM | 3.85±2.11 |
| TT | 4.0±2.10 |
| Hemoglobinopathy (SCA+TM+TT) | 4.18±2.23 |

Serum Iron and Total iron binding capacity was measured in all the microcytic hypochromic anemic patients to differentiate the Iron deficiency and hemoglobinopathies or any other cause. The percent transferrin saturation was calculated as Serum Iron/TIBC *100. If percent transferrin saturation was less than 16 then patient was classified as Iron deficiency anemia. The mean % transferrin saturation in IDA patients in our study was 4.9±3.1% whereas in hemoglobinopathy group it was 51.9±123.8%**(Table 4).**

HPLC/electrophoresis was performed in all the microcytic anemia cases without iron deficiency to differentiate the sickle cell anemia, thalassemia trait and thalassemia major. Out of 27 microcytic hypochromic patients with normal iron levels sickle cell anemia was found in 10 cases. 10 patients were thalassemia trait and 7 were thalassemia major. Accordingly sickle cell anemia patients were further classified into sickle cell homozygous anemia, sickle cell trait and sickle beta thalassemia **(Table 5).**

**Table 4: Iron Profile in all patients**

| Diagnosis | SI | TIBC | % Transferrin |
|---|---|---|---|
| B12 Deficiency | 57.8±15.5 | 247.1±42.2 | 23.17±2.6 |
| IDA | 22.98±10.1 | 508.40±92.4 | 4.9±3.1 |
| SCA | 36.80±20.2 | 340.80±153.3 | 21.5±10.6 |
| TM | 98.8±512.8 | 398.2±168.9 | 25.1±8.6 |
| TT | 108.4±109.7 | 250.8±143.8 | 116.1±213.8 |
| Hemoglobinopathy (SCA+TM+TT) | 81.3±73.7 | 329.9±157.0 | 51.9±123.8 |

**Table 5: HPLC pattern in different hemoglobinopathy group**

| Diagnosis | HbA | HbA2 | HbF | HBS |
|---|---|---|---|---|
| TM(7) | 3.6±1.5 | 2.45±0.69 | 92.57±1.6 | - |
| TT(10) | 92.37±1.61 | 5.13±0.61 | 2.15±1.24 | - |
| Sickle homozygous(4) | 9.8±9.7 | 2.01±0.71 | 20.2±9.6 | 72.31±15.2 |
| Sickle beta thalassemia(4) | 18.7±2.4 | 4.75±0.35 | 16.0±0.0 | 60.75±1.76 |
| Sickle cell trait(2) | 33.4±1.9 | 2.6±0.56 | 3.0±1.41 | 60.75±1.76 |

## Discussion

The inherited disorders of hemoglobin, particularly the β- thalassemia and their interaction with hemoglobin S (HbS) are a considerable health problem in India and contribute significantly to morbidity and mortality. Earlier studies have shown that the overall prevalence of β-thalassemia is 3–4 % with an estimate of around 8,000 to 10,000 new births with major disease each year[6]. Most of these children have a severe clinical presentation but are managed sub-optimally due to lack of financial resources in majority of the families. Thus preventing the birth of affected children is the best option for India. A prerequisite for this is the knowledge of the prevalence of β-thalassemia and other hemoglobinopathies in different regions of the country and in particular in different ethnic groups[7].

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In the present study 57% of male were anemic and 43% of female were anemic with male to female ratio of 1.3:1. Most common age group affected were toddler age group(1-3 years) constituting 37%, followed by > 5 years age group constituting 27% followed by infant age (6 months to 1 years) constituting 19% with least in preschool age group(3 years to 5 years) constituting 17 % .Among hemoglobinopathy age group most commonly affected was more than 5 year(46%) and least affected was age group 6 months to one year (6%)[8].

In a similar study conducted at multispecialty hospital Bangalore suggestive of 58% male were anemic and 42% female were anemic with male to female ratio of 1.4:1. Most common age group affected were infants (6 month -1 year) constituting 33%., followed by school-going children (6 years-12 years, 26%), toddlers (2 years-3 years, 25%), and preschool children (4 years-5 years, 16%).Of the non hemoglobinopathies, (33%) children in the age group 6 months-1 year were most affected and (16%) in the age group 4-5 years were least affected. Among hemoglobinopathies, (54%) children in the age group 6-12 years were most affected, followed by (30%) children in the age group 2-3 years and equal occurrence of 1 (8%) in both the age groups 6 months-1 year and 4-5 years[9].

According to the NFHS 1998-99, 74% of the children in the age group of 6-35 months were anemic. In the study conducted by Gomber *et al.*, 76% of children were anemic in the age group of 3 months-3 years. Osσrio *et al.*, have noticed the incidence of anemia to be 40.9% in the age group of 6-59 months[10].

**Severity**

Our study depicted that severity and morphological types in all age groups most children suffered from severe anemia and microcytic hypochromic anemia being the most common with the highest number of 33% in 1 to 3 years age groups. Macrocytic anemia was the least common morphological type of anemia in all age group (12%).

In comparison Study conducted at multispecialty hospital Bangalore depicted that most children in all the age groups suffered from moderate anemia and microcytic hypochromic anemia being the commonest with the highest number of (29.39%) found among children of age group 6 months to 1 year and least number of (11.92%) in the 4-5 years age group. Macrocytic anemia was the least common morphological type of anemia in all the age groups (10%).In comparison, the study conducted by Gomber *et al*, among children aged 5-5.9 years, mild anemia was found in 28.9% and moderate anemia in 2.9% of children. In another study conducted by Vishwanath *et al*it was found that of the 100 children evaluated 89 children had iron deficiency anemia and 48% had mild, 42% had moderate, and 10% had severe anemia[11].

**Morphological types of anemia**

In the present study ,Microcytic hypochromic was the most common 76% followed by normocytic normochromic (20%) picture followed by macrocytic anemia .This in comparison to bangalore study MCHA (49%) was most common followed by NNA (22%), and macrocytic (4%). This is in comparison to Kapoor *et al* study in which MCHA was most common (43.2%) followed by equal incidence of NNA and dimorphic anemia (27%), while the least common was macrocytic anemia (2.7%). Gomber *et al*have adopted an etiological classification of anemia rather than morphological classification. They found iron deficiency anemia to be the most common (41%) and folate deficiency to be least common (2.2%).Difference in our study is due to as we excluded dimorphic anemia[12].

**Red cell indices studied**

In the present study, Mean Hb concentration in studied patients was 6.54±1.63 g/dl. The mean Hb level in thalassemia major patients was 6.28±1.75 gm/dl and it was minimum 3.2 g/dl in a 2.5 year old female child. In Sickle cell anemia patients the mean Hb level was

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6.5±1.7gm/dl. The mean Hb levels were almost similar in all the type of anemia (p value 0.471). We observed that iron deficiency patient had lowest MCV values (59.15±5.8 fl). MCV values were almost similar in thalassemia major, thalassemia trait and sickle cell anemia patients[13].
In the Bangalore study mean Hb, MCV was 8.5 g/dL, 75.08 fl, respectively in comparison to study conducted by Geibel Herbert N which had a mean Hb, MCV of 9.78 g/dL, 64.34 fl, respectively.In the study conducted by Osorio, mean hemoglobin was found to be 11 g/dL[14].

**Hemoglobinopathies**
Patients with hemoglobinopathy syndrome are commonly encountered in hematology clinic. Of these, the commonest disorder of hemoglobinopathy syndrome in India is thalassemia
The following findings of the present study are discussed comparing with other studies.

**Distribution among different age groups**
Of the 27 children with haemoglobinopathies, 16 were male and 11 were female .Thus the male to female ratio was found to be 1.4:1.
In Bangalore studyout of 13 children with hemoglobinopathies, 8 were males and 5 were females. Thus, the male to female ratio was found to be 1.6:1[15].

**Distribution among different age groups**
In our study out of 27 children of hemoglobinopathies majorities were in the age group of >5 years 11(40.7%) followed by3 to 5 years age group 9(33.3%) least in infants age group.
In a study by Saba et al out of the 13 children with hemoglobinopathies, majority were in the age group of 6-12 years 7 (53%) followed by 2-3 years 4 (31%). There was an equal occurrence among 6 months-1 year 1 (8%) and 4-5 years 1 (8%)[16].

**Distribution of types of hemoglobinopathies**
In the present study sickle cell anemia and thalassemia trait haemoglobinopathies had equal incidence seen in 20 (37% each) children followed by thalassemia major seen in 7(25.9%) children.In the Bangalore study, thalassemia major was the most common type of hemoglobinopathy seen in 9 (54%) children followed by thalassemia minor among 2 (30%) children and equal incidence of about 1 (8%) child each suffering from sickle cell anemia, and sickle cell thalassemia. In the present study, homozygous thalassemia was the most common. In a study conducted by Mitra, HbE was found to be the most common, followed by homozygous thalassemia and least common was HbDE disease[17].

**Red cell indices**
Among non hemoglobinopathies parameters Hb, and MCV were evaluated as follows: Mean Hb was found to be 8.5 g/dL, with a maximum value of 11 g/dL, minimum of 3.2 g/Dl, and a standard deviation of 1.3466. Mean MCV was 75.08 fl, with a maximum of 105 fl, minimum of 49 fl, and a standard deviation of 9.9348.
Among hemoglobinopathies Hb MCV, reticulocyte count, and HbF were evaluated as follows: Mean Hb was 6.23 g/dL, minimum was 4.3 g/dL, and maximum was 10.5 g/dL with a standard deviation of 1.8931. Mean MCV was 65.04 fl with a minimum of 52 fl, maximum of 78.90 fl and a standard deviation of 6.7106. Mean reticulocyte count was $0.163 \times 10^{12}$ with a minimum of $0.08 \times 10^{12}$, maximum of $0.36 \times 10,^{12}$ and a standard deviation of 0.343. Mean HbF was 31.69, with a minimum of 3.00, a maximum of 90.10, and a standard deviation of 23.
Thalassemia major was the most common type of hemoglobinopathy seen in nine (54%) children followed by thalassemia minor among two (30%) children, and equal incidence of

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about one (8%) child each suffering from sickle cell anemia and sickle cell thalassemia[17].

**Conclusion**

A total of 100 subject were included in the study following are the main finding of our study with prevalence of thalassemia major was found 7% thalassemia trait 10% and sickle cell anemia 10%.The mean age of presentation of thalassemia major was 2.70±2.44 years thalassemia trait was 10.0±3.6 years and sickle cell anemia 6.14±4.35 years. Splenomegaly was observed in all thalassemia major 30% patient of thalassemia trait and 40% patient of sickle cell anemia. Mean Hb conc. was 6.2±1.7gm/dl in thalassemia major, 6.06±1.30gm/dl thalassemia trait, and 7.17±2.8 sickle cell anemia.Mean MCH was found in 20.3±4.4pg thalassemia major, 19.7±3.8pg thalassemia trait, and 21.5±3.1pg sickle cell anemia.Mean reticulocyte was found 3.85±2.11 in thalassemia major, 4.0±2.10 and 4.6±2.5 in sickle cell anemia.Mean HbA2 level was 2.45±0.69, in thalassemia major, 5.13±0.61 in thalassemia trait, and 2.01±0.71 in sickle homozygous.Mean HbF level was 92.57±1.6 in thalassemia major, 2.15±1.24 in thalassemia trait and 20.2± 9.6 in sickle homozygous.Blood transfusion frequency was more in thalassemia major as compared to sickle cell disease.HPLC is a reliable method for screening of thalassemia. Screening should be done to all suspected patient to find out the exact prevalence of thalassemia. Treatment should be start as soon as thalassemia positive detected before the end organ damage. Genetic counselingthe patient family for next pregnancy for avoid birth of a thalassemia child in their family.

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