THE TRENDS OF HEMOGLOBINOPATHIES IN THE MITHILA REGION OF BIHAR

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Abstract:

Hemoglobinopathies are caused by mutations in the globin genes that encode for the protein that synthesizes the hemoglobin chains, which is one of the most commonly occurring monogenic diseases in the world. In thalassemia, hemoglobin production is reduced, or abnormal hemoglobins are formed, resulting in a wide range of disorders. Globally, hemoglobinopathies have become increasingly common over the last few decades. Due to the growing burden on healthcare delivery systems, it is especially important for developing countries like India as well as the Indian states. The overall prevalence of β thalassemia carriers in India is 3-4%, according to the 2011 Census of India. While the demographic situation in Bihar has improved in recent years, the state's socioeconomic status remains low, and the incidence of a number of diseases has remained high despite these improvements.

In our study, we found that hemoglobinopathies are a major problem in this region and we need to take precautionary measures to ensure the safety of our Mithila people, who have limited resources. There is no existing study in the Mithila region of Bihar State that uses standardized guidelines and methodology to screen for hemoglobinopathies, and this is the first study to do so at the Thalassemia clinic at the DMCH.

Keywords: Hemoglobinopathies, Thalassemia, Diagnosis, Bihar

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1. Introduction:

The term hemoglobinopathies refers to hemoglobinopathies such as thalassemia and variant hemoglobin. As a single gene abnormality, hemoglobinopathies are among the most prevalent in the human population. A higher prevalence of hemoglobinopathies can be observed in tropical countries because carriers have partial protection against falciparum malaria morbidity and mortality. In spite of the fact that there are several hundred different types of these disorders, sickle cell, and thalassemia disorders are the most prevalent among them. Recent advances in the field of hemoglobin structure and genetics have led to a significant improvement in our understanding of these disorders as a result of recent research.

Severe hemoglobinopathy's major classifications and the most common causes
Thalassemia major: Hereditary HBB gene thalassemia (and/or severe δβthalassemia) mutations.
Sickle cell syndromes: Hb S/Hb S, Hb S/C, Hb S/b-thalassemia, Hb S/D^{Punjab}, Hb S/O^{Arab}, Hb S/Lepore, and Hb S/Hb E are a few interactions that might occur.
Hb E thalassemia: Inheritance of thalassemia mutations in the HBB gene
Hb Bart's hydrops fetalis syndrome (and very rarely Hb H hydrops fetalis syndrome): Severe homozygous mutations in the HBA gene (often deletions of α^0 thalassemia or severe nondeletion mutations)

There are over 400,000 newborns who are born with hemoglobinopathies every year, and approximately 270 of them are carriers per million. In the last few decades, hemoglobinopathies have become an increasingly common condition across the globe. It is especially important for developing countries because of the increased burden on healthcare delivery systems.

As a matter of fact, B thalassemia and sickle cell anemia are two of the most serious health problems in India. According to the 2011 Census of India, the average prevalence of b thalassemia carriers is 3-4%, translating to 35 to 45 million carriers in our multicultural, culturally, and linguistically diversified population of 1.21 billion people, which also includes about 8% of tribal communities. The frequency is significantly greater (4–17%) in some ethnic groups [Madan N et al. 2010, Colah RB et al. 2014].

In India, genetic disorders account for a large proportion of all genetic diseases, making them particularly important in public health. The most common thalassaemic disorder in India is β-thalassemia, which is not associated with any morbidity. Nonetheless, α-thalassemia occurs more often in tribal populations. As far as abnormal hemoglobin molecules are concerned, there are several types, but the three most prevalent ones in India are Hb E, Hb H, Hb S, and HbD [Kar BC et al. 1991, Deka R et al. 1988, Colah R et al. 1998, Sarnaik SA. et al. 2005, Balgir RS et al. 1996].

As a single-gene disorder, alpha thalassemia is one of the most common disorders worldwide. Chromosome 16 contains a cluster of genes encoding the globin protein that consists of two copies of the globin gene (α1 and α2). The majority of α-thalassemia is caused by the deletion of one or both of these cis-linked genes [Higgs, D.R et al. 2009].

α-thalassemia is a condition that is predominant in tribal populations in Gujrat and Andhra Pradesh with a prevalence ranging from 1 to 40% [Saha N et al. 1990]. In addition, the A+ genotype has been identified as the dominant genotype of α-thalassemia in our country, and it is clinically silent in both heterozygous and homozygous states. Therefore, It doesn't have any morbidity [Saha N et al. 1990].

Our country has a range of prevalence rates of carriers ranging between 1% and 18% among non-tribal populations to as high as 90% among some tribal groups[Mukherjee MB et al. 1997]. The cumulative gene frequency of hemoglobinopathies in India has been estimated to be 4.2% [Agarwal MB et al. 1982]. In the country in general, some 42 million people are carriers of hemoglobinopathies, and approximately 12,000 babies are born each year who have a major hemoglobinopathy[[Agarwal MB et al. 1982]. Out of these, β-thalassemia major and clinically significant sickle cell disorders account for almost equal numbers.

In spite of the fact that beta-thalassemia is present in nearly all Indian populations, it is more common in the northwest and far east of the country. There is a high proportion of β-thalassemia in India, mostly in Punjabi, Sindhi, Bengali, Gujarati, and Muslims [Agarwal MB et al. 1982]. In India, there are between 1 and 17 % and an average of 3.2% carriers[Agarwal MB et al. 1982].

Other types of hemoglobin and thalassemia have also been reported in our country, including rare hemoglobin variants and thalassemia type. They include beta-thalassemia, hereditary persistent fetal hemoglobin, Hb Lepore, Hb M Hb Q, Hb K, Hb J, Hb Chandigarh, Hb Sun Prairie, Hb Koya Dora, etc. The majority of them are rare and have little clinical impact [Sarnaik SA et al. 2005].

This four-year research examined all patients who had a clinical or familial suspect of hemoglobinopathy and a hemoglobinopathy work-up was sought for diagnostic purposes. It was conducted at the prominent Darbhanga Medical

College and Hospital (DMCH) in north Bihar. There have not been any large studies published on hemoglobinopathies in Bihar up until now. While there hasn't been any significant research on the range of hemoglobinopathies in Bihar that has been published, our research project began as there was limited information about these disorders in Bihar at the time this study was started.

2. Methods and Materials:

This study was conducted between 2019 and February 2023 at the Thalassemia Clinic, Department of Pathology, Darbhanga Medical College and Hospital (DMCH), Darbhanga, Bihar. Blood samples from suspected patients who were referred by various clinics in the Mithila region over the previous four years were used to gather data on the prevalence of clinical and sub-clinical deficiencies from 2019 to the present. In the present study, a G8-HPLC analyzer and a BioRad D-10 analyzer were used for the detection of hemoglobinopathies and thalassemia in patients. For all patients, complete blood counts (Councell-21, Tulip diagnostic) were performed, peripheral smears were conducted for the determination of red blood cell morphology, and an HbH preparation was performed to display inclusions in the HbH. In order to prepare a HbH smear, equal volumes of blood were added to new methylene blue for two hours at 37°C. This process is similar to that used to measure reticulocyte count. The blood samples were diluted and loaded into the analytical cartridge of the D-10 analyzer (Bio-Rad Laboratories, Hercules, CA) after being collected in an EDTA vacutainer.

The hemoglobins subsequently elute out of the cartridge as a result of their ionic interactions with the phosphate buffers, which are then injected into the cartridge in increasing strength. The filter photometer's flow cell measures the elute's absorbance at 415 nm as it passes through. The HbA2/HbF/HbA1c dual software is used to produce a chromatogram for each sample.

There are several components included in each kit, including the elution buffers, calibrators, calibration diluents, primers for whole blood, and sample vials. In addition to anemia and hemoglobinopathy, bleeding disorders, such as platelet and coagulation disorders, as well as other rare conditions such as thrombosis and eosinophilia, were analyzed as diseases.

3. Results and Discussion:

Over the span of four years, an estimated 225 samples were collected from suspected patients in this study. 202 patients were diagnosed with hemoglobinopathy and the rest 23 were found free of these disorders. Among 202 patients, 105 were male, and 97 were female. The age of the patients with hemoglobinopathies ranged from 3 months to 66 years with a mean of 11.97 years. The male-to-female ratio was 1: 0.92

Table: 1- Prevalence frequency of Hemoglobinopathies

Age in years	No. of patients	Male	Female	Hindu	Muslim
0-5	104	64	40	73	32
5-10	15	10	5	11	4
10-15	12	6	6	6	6
15-20	12	6	6	12	0
20-25	26	5	21	22	4
25-30	13	6	7	10	3
30-35	7	4	3	6	1
35-40	2	0	2	1	1
40-45	4	0	4	4	0
45-50	2	1	1	2	0
50-55	1	1	0	1	0
55-60	2	1	1	2	0
60+	1	1	0	1	0
TOTAL	202	105	97	151	51

A summary of the distribution of males and females by gender, age group, and community can be found in Table 1. The majority of the participants were males (51.98%, mean: 9.6 years) and females (48.01%, mean: 15 years), with Hindus constituting 151 (74.75%, mean: 13.56 years) and Muslims constituting 51 (25.24%, mean: 8.07 years), respectively, constituting the two most common communities among the participants found. Based on gender, there were a greater number of male patients than female patients. Similar to the Muslim community, the Hindu community has a high incidence. Patient ages ranged between 3 months and 66 years. The mean age was found to be 11.97 years. Among the patients of all age groups, 51.48% were children of less than 5 years (mean: 1.73 years), the age group of 5-10 years constitute 15 (7.42%, mean: 7 years), 10-15 years 12 patients

(5.94%, mean: 11.5 years), 15-20 years 12 patients (5.94%, 7.83 years), 20-25 years 26 (12.87%, mean: 22.73 years), 25-30 years 13 (6.43%, mean:28.15 years), 30-35 years 7 (3.46%, mean: 33.85 years), 35-40 years 2 (0.99%, mean: 40 years), 40-45 years 4 (1.98%, mean: 42.75 years), 45-50 years 2 (0.99%, mean: 47 years), 50-55 years 1 (0.49%, mean: 53 years), 55-60 years 2 (0.99%, mean: 55.5 years), above 60 only 1(0.49%, mean: 66 years) were having hemoglobinopathies.

According to our study, children who are under the age of five years have a high prevalence of hemoglobinopathy, with 52% of those children being diagnosed with hemoglobinopathy between the ages of three months and five years old, which is a very worrying sign (Figure-1). A comparison of hemoglobinopathies is also shown in Table 1 between the two communities. The community with the highest number of hemoglobinopathies was the Hindu community, with 152 (74.75%), followed by the Muslim community with 51 (25.25%).

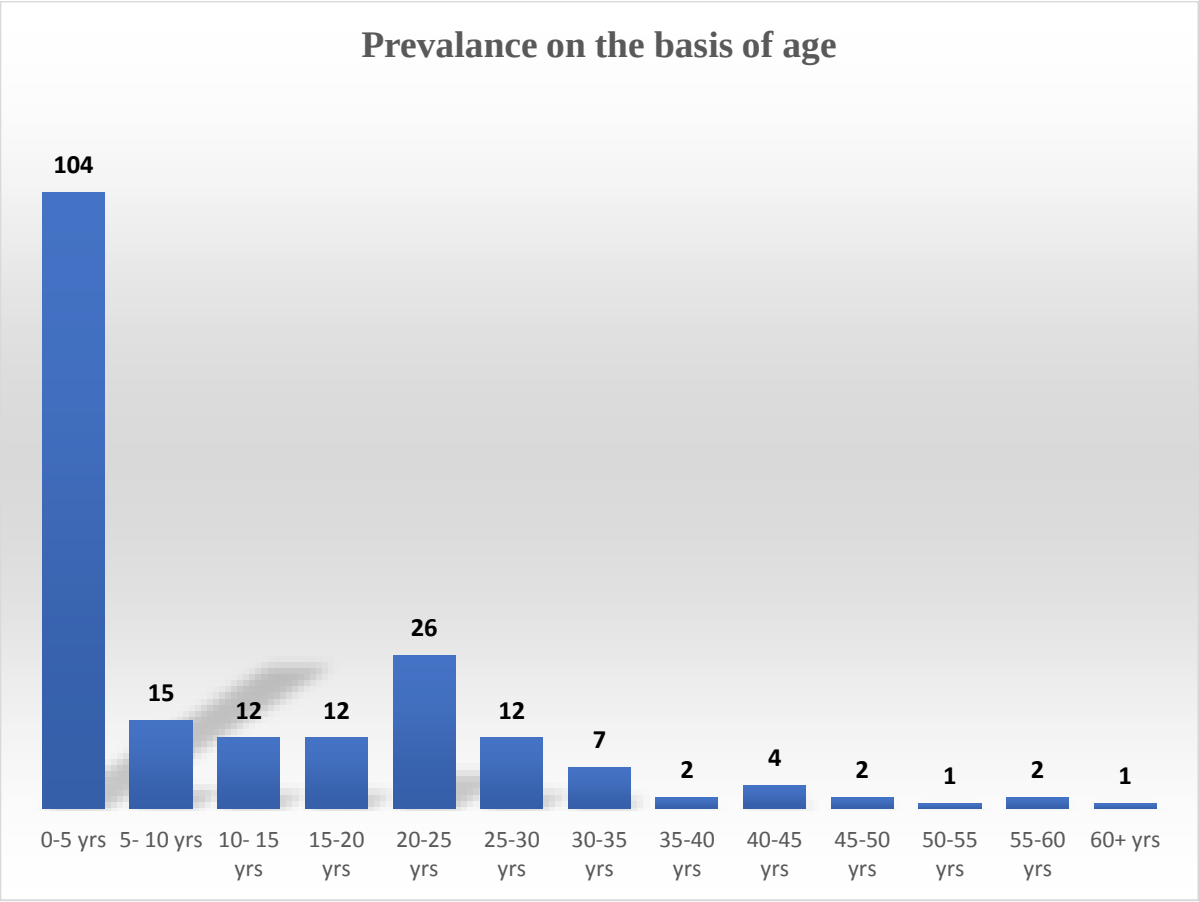

Figure 1: Age-wise distribution and Prevalence of hemoglobinopathies

Alpha thalassemia is one of the most common single-gene disorders worldwide. Located on chromosome 16, globin is encoded by a cluster of two genes (α1 and α2). As many as 90% of tribal groups have carriers, while non-tribal populations have prevalence rates ranging from 1% to 18%.[9] α-thalassemia is prevalent among tribal populations in Gujrat and Andhra Pradesh, with a 1 to 40% prevalence.[10] Furthermore, both in heterozygous and homozygous stages, the A+ genotype of -thalassemia has been found as the dominant genotype in our nation. Hence, it has no morbidity [Mukherjee MB et al. 1997].

There have been many previous studies on the Indian subcontinent that have identified the different patterns of hemoglobinopathy according to the tribes, castes, and communities living there [Deka R et al. 1998, Agarwal MB et al.1982, Mohanty D et al. 2002]. It is important to note that there are various types of abnormal hemoglobin molecules, but the most prevalent three in India are: Hb E, Hb H, Hb S, and HbD [Kar BC et al. 1991, Deka R et al. 1988, Colah R et al. 1998, Sarnaik SA. et al. 2005, Balgir RS et al. 1996]. Hemoglobin E (HbE) is a condition that affects the majority of tribal communities in the northeast, which is caused by a wide range of α- and β-thalassemia gene combinations [Das BM et al. 1971, Flatz G et al.1972, Das BM et al. 1975, Das BM et al. 1980, De M et al. 2006]. The first report of Hb Sallanches was reported in a French family, in which a homozygous condition led to hemolytic anemia and low Hb H levels. The homozygous

condition of this variant was also reported in Indian and Pakistani populations in the following years [Wajcman, H. et al. 2008]. Hb S was first described by Lehman and Cutbush in 1952 by tribals in southern India [Lehman H et al. 1952]. It has since been reported that the disease is found in many Indian states, communities, and ethnic groups at an average frequency of 4.3% (range : 0-44%) [Chui, D.H et al. 2003].

The range of hemoglobinopathies was recently revealed in a cohort study carried out from 1994 to 2003 by the Regional Medical Research Centre (ICMR) in Bhuvneshwar, Odisha, an eastern state of India. It is indeed interesting to note that sickle cell trait is the most prevalent hemoglobinopathy (29.8%), followed by beta-thalassemia trait (18.2%), sickle cell disease (7.6%), thalassemia major (5.3%), sickle cell-β-thalassemia (1.7%), thalassemia intermedia (0.9%), hemoglobin E trait (0.9%), and E-β-thalassemia (0.7%) [Balgir RS et al. 2005]. This report emphasizes once again the high prevalence of clinically significant hemoglobinopathies in eastern central India. The occurrence of thalassemia and Hb E in the eastern coastal region of Orissa and that of Hb S in the central, western, and southern regions of Orissa has significant historical value since it is based on the migration or flow of various populations from different parts of the country.

According to a report which has been submitted in the year 2017, collectively evaluated by ICMR Delhi, Public health foundation of India, and the Institute For Health Metrics And Evaluation, anemia is the 4th leading cause of death in Bihar and the largest death caused by different diseases in children under the age of five.

In spite of the fact that there are a few studies available, a lot of information is still lacking regarding the hemoglobinopathies in Bihar and its associated clinical course.

This study was done at the Thalassemia Clinic, Department of Pathology DMCH for four years between Jan 2019 to Feb 2023 and analyzed to better understand the hemoglobinopathies prevalent in Mithila patients visiting the hospital's outpatient department. Disease characterization has been done for the suspicion of hematological disorder on the basis of diagnosis, family history, and symptoms. Most of the patients visit for a consultation. The most common reason for consultation in the hematology OPD was anemia with nutritional anemia being the most common cause. Due to the COVID-19 pandemic, the center was not functional hence the patients and data were not available for the year 2021.

There was an increased risk of hemoglobinopathy in our study participants as a consequence of the differences in age, gender, and community distribution of the participants. In our study, the oldest participant was 66 years old and the youngest was only 3 months old.

On the basis of various physical blood analyses such as complete blood count (CBC), peripheral blood smears, iron deficiency studies, and Hb-HPLC into suspected anemic individuals knowing the health history, which led my research to the conclusion that the defects in genes of hemoglobin are found mostly in children under five years of age where typically it is detected around 52% and the hemoglobinopathies are regardless of major or minor thalassemia.

4. Conclusion:

Specifically, we found that beta thalassemia major is the most common inherited disease in children under five years of age. Symptoms of hemoglobinopathies are more apparent and unbearable in children than in adults, who often avoid visiting healthcare providers and show less concern about their health. Because of being a carrier or having the trait of hemoglobinopathies in order to keep a check on these diseases, it is much recommendable to go for pre-wedding screening of both genders, so that inheritance of defective genes can be avoided. One more step that can be taken in this context is to avoid marriages between very close relationships if they have any family history of any such diseases.

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Authors' Contributions: S. M. acquired the data. S. M. and M. S. A. analyzed the data, wrote the manuscript, and prepared the table. A.N.J. revised the manuscript and A. K. C. approved the data. All authors have read and agreed to the published version of the manuscript.

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