A RETROSPECTIVE STUDY ON PATTERN OF THALASSEMIA AND OTHER HAEMOGLOBINOPATHIES IN PAEDIATRICS AGE GROUP USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN JORHAT DISTRICT OF NORTH EAST INDIA

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ABSTRACT

BACKGROUND

In India, haemoglobinopathies and thalassemias contribute in a significant number of paediatric cases with anaemia. As corrective treatment like bone marrow transplantation is costly, prevention through population screening, and genetic counselling is the best possible strategy.

AIMS

Data pertaining to the pattern of these disorders is scarce in this region and hence it was considered worthwhile to study these disorders using a large series of patients referred to clinical diagnostic laboratory.

METHOD

A retrospective study was done on 800 patients who were referred for Hb variant analysis to two clinical diagnostic laboratories in Jorhat District in upper Assam region of North East India between the age group of 7 months to 18 years. This study was performed by high performance liquid chromatography (HPLC) using BIORAD (D-10) variant Hb typing system.

RESULT

Out of 800 patients, abnormal Hb fractions were seen in 417 (51.9%) patients. HbE gene was detected in 373 patients of which HbE trait was seen in 243 (58.7%) followed by HbE disease in 100 (23.9%). There were 30 (7.19%) HbE beta thalassemia cases. Out of 46 tea garden community cases, 41 showed HbS gene of which 12 (2.87%) each in HbS trait and HbS thalassemia groups and 17 patients in sickle cell anaemia group (4.07%). HbE thalassemia was mainly found in Chutia (1.92%), Ahom (1.43%) and Mishing (1.67%) communities.

CONCLUSION

A high incidence of haemoglobinopathies and thalassemias and their combinations is unique for this part of the country particularly the high prevalence of HbE thalassemia. HbS gene is mainly found in tea garden community.

KEYWORDS

Haemoglobinopathies, Thalassemia, High Performance Liquid Chromatography, Paediatrics, North Eastern India.

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INTRODUCTION

Thalassemia and other haemoglobinopathies are a group of hereditary disorders of haemoglobin (Hb) where there is quantitative and qualitative abnormal production or structure of Hb molecule,[1,2] a major public health problem in many parts of India.[2]

There are over 25 million carriers of this disease in India[3] and beta thalassemia and sickle cell disease are the most frequent among them.[2,4] The clinical presentation may vary from asymptomatic carrier state to more serious condition like thalassemia major, which requires regular blood transfusion[2] and yearly 10,000 children are born with beta thalassemia major in India, which contributes to 10% of the total number of thalassemia major in the world.[5] Indian Council of Medical Research (ICMR) in their multicenter study have found the incidence of beta thalassemia to be 2.78%.[6] The prevalence of sickle cell anaemia and beta thalassemia trait in India varies between 1-44% and 3-17% and respectively.[1,2]

In different parts of India, the incidence of haemoglobinopathies also differs. In Orissa, HbS is common[7] whereas in West Bengal the commonest one is HbE.[8] ICMR multicenter study showed that HbE trait was mainly seen in Kolkata in West Bengal (3.92%) and in Dibrugarh in Assam (23.9%). Among the six ethnic groups from Dibrugarh Assam, the prevalence of HbE trait varied from 41.1% to 66.7%.[6] The incidence of HbE gene in the North Eastern region of India is one of the highest in the world.[9] A huge migrant tea garden

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population also shows a high incidence of HbS in North East India.[10,11]

The curative treatment is costly and these children require frequent blood transfusion. The best preventive measure is through population screening and genetic counselling.[12]

As the exact data regarding the pattern of thalassemia and other haemoglobinopathies in paediatrics age group of different ethnic groups in this region is limited as majority studies were adult case based. We consider it important to find out the extent of burden of these conditions in anaemic children of various age group, sex, and community.

MATERIAL AND METHOD

A retrospective study was done in paediatrics population between the age group of 7 months to 18 years. As the prevalence of thalassemia varies with different geographical areas of India.[1,2,7,8] we took the prevalence of 66.7% among the six ethnic groups from Assam[6] while calculating the sample size. Taking relative error of 5% of prevalence, the sample size calculated was 800 cases. Paediatrics patients who were referred for Hb variant analysis to two clinical laboratories by clinicians as a workup for anaemia and for confirmation of clinically suspected patients of haemoglobinopathy or thalassemia in Jorhat district in upper Assam region of North East India were included in the study. The patient predominantly belongs to people of upper Assam, which is considered a mosaic of people with diverse ethnicity and cultural identity.[11]

The study was done between April 2013 and January 2016. 2 ml EDTA blood samples were collected by venepuncture from each subject. All these samples were analysed for haemoglobin disorders by using High Performance Liquid Chromatography (HPLC) based upon the principles of cation exchange chromatography using the Bio-Rad (D-10) variant Hb typing system. Though HPLC helps in the establishment of diagnosis in such cases the ultimate diagnosis is made by identification of abnormal gene using polymerase chain reaction.[13]

Details of subjects were recorded in a proforma, which included age, sex, caste/ethnic groups, and pattern of Hb fraction on HPLC. Major communities/ethnic groups of the region were included. Those groups where the community/ethnic groups were not mentioned were pooled together under the 'others' group. Data was compiled in excel sheet and statistical analysis was done with appropriate statistical methods.

RESULTS

Out of 800 cases, 417 (51.9%) displayed abnormal haemoglobin fractions on HPLC. The major abnormality observed in thalassemia cases was high HbA2. A cutoff over 3.9% was taken for diagnosis of beta thalassemia trait.[14]

Table 1 shows the sex distribution, out of 417 cases 230 (55.15%) were male and 187 (44.84%) were female and the difference was found to be statistically significant.

Table 2 shows the pattern of various haemoglobinopathies and thalassemia in our study. Out of 417 cases 243 cases showed HbE trait (58.2%) followed by HbE disease (23.9%), E. thalassemia (7.19%), Sickle cell anaemia (4.07%), S. trait (2.87%), S. thalassemia (2.87%), beta thalassemia and beta thalassemia trait (0.23%).

Table 3 shows the age distribution of cases. Out of 417 cases, 32.4 % cases were of 10-18 years age group followed by 1-3 yeas (19.4%), 4-6 years (19.2%), and 7-10 years (18.5%), and between 7-12 months, it was (10.6%). Table 3 also shows the pattern of haemoglobinopathies and thalassemia in different age group. HbE trait (18.22%), HbE disease (7.67%), S. trait (1.67%), and S. thalassemia were most common in 10-18 years age group. Most of the cases of E. thalassemia (2.39%) were found in 7-12 months age group. Sickle cell anaemia was commonly seen in 1-3 years and 10-18 years age group. One case each of beta thalassemia was noted in 7-12 months and 7-10 years age group. Age-wise distribution of cases found to be statistically significant.

Table 4 shows the distribution of haemoglobinopathies and thalassemia in different community/ethnic groups. In the present study, HbE trait (58.27%) was the most common type of haemoglobinopathy. Second most common pattern noted was HbE homozygous (HbE disease) with 23.9%. Among different haemoglobinopathies HbE trait, HbE disease, and HbE thalassemia showed significant statistical difference among different communities.

The most significant finding of our study was the high incidence of HbE beta thalassemia (7.19 %). The HbS gene was detected in 9.81% of patients of which sickle cell anaemia was the predominant form (4.07%) followed by HbS trait (2.87%) and S. beta thalassemia (2.87%). Children with sickle cell anaemia showed high level of foetal Hb level, which ranges between 12.2% and 24.4%.

Among the ethnic groups, HbE trait is most common among Ahom (13.66%), Chutia (15.5%), and Mishing (6.23%) communities. HbE disease was mainly found in Ahom (6.47%) and Chutia (5.9%) communities. HbE thalassemia was mainly found in Chutia (1.92%), Ahom (1.43%), and Mishing (1.67%) communities.

In our study, HbS was mainly restricted to tea garden community. Total 46 patients from tea garden community were included in this study out of which 17 (36.95%) cases were found to have sickle cell anaemia and 12 (26%) cases each in HbS trait and S. thalassemia groups. Two patients found to have B thalassemia major and beta thalassemia trait in one patient.

Sex	Male	Female
No. of cases	230 (55.15%)	187 (44.84%)
χ^2 =4.434; df=1; P value=0.0352		

Table 1: Sex Distribution of Haemoglobinopathies and Thalassemia (n=417)

df=degree of freedom

Variant Hb	No.	Percentage
HbE Trait	243	58.27%
HbE Disease	100	23.9%
E Thalassemia	30	7.19%
B Thalassemia	2	0.47%
B Thalassemia Trait	1	0.23%
S Trait	12	2.87%
S Thalassemia	12	2.87%
Sickle Cell Anaemia	17	4.07%

Table 2: Type Wise Distribution of Various Haemoglobinopathies and Thalassemia (n=417)

Age Group	Hb E Trait	Hb E Disease	E Thalassemia	B Thalassemia	S Trait	S Thalassemia	Sickle Cell Anaemia	Beta Thalassemia Trait	Cases %
7-12 M	26 (6.23%)	5 (1.2%)	10 (2.39%)	1(0.23%)	1 (0.23%)			1 (0.23%)	44 (10.6%)
1 yrs.-3 yrs.	43 (10.31%)	23 (5.51%)	8 (1.91%)		1 (0.23%)		6 (1.43%)		81 (19.4%)
4-6 yrs.	52 (12.47%)	20 (4.79%)	2 (0.47%)		1 (0.23%)	3 (0.71%)	2 (0.47%)		80 (19.2%)
7-10 yrs.	46 (11.03%)	20 (4.79%)	4 (0.95%)	1(0.23%)	2 (0.47%)	1 (0.23%)	3 (0.71%)		77 (18.5%)
10-18 yrs.	76 (18.22%)	32 (7.67%)	6 (1.43%)		7 (1.67%)	8 (1.91%)	6 (1.43%)		135 (32.4%)
Total	**243 (58.27%)**	**100 (23.9%)**	**30 (7.19%)**	**2 (0.47%)**	**12 (2.87%)**	**12 (2.87%)**	**17 (4.07%)**	**1 (0.23%)**	**417**
χ^2 =56.1; df=32; P value=0.005									

Table 3: Age Wise and Type Wise Distribution of Cases of Haemoglobinopathies and Thalassemia (n=417)

Community/ Ethnic Groups	HbE Trait	HbE Disease	E Thala	Beta Thala Major	Beta Thala Trait	S Trait	Sickle Cell Anaemia	S Thalassemia	Cases %
Brahmin	9 (2.15%)	4 (0.95%)							13 (3.1%)
Kalita	8 (1.91%)								8 (1.91%)
Koch	7 (1.67%)	4 (0.95%)							11 (2.63%)
Keot	2 (0.47%)								2 (0.47%)
Muttok	1 (0.23%)	1 (0.23%)							2 (0.47%)
Sunni	10 (2.4%)	1 (0.23%)	1 (0.23%)						12 (2.87%)
Ahom	57 (13.66%)	27 (6.47%)	6 (1.43%)						90 (21.58%
Chutia	65 (15.5%)	25 (5.9%)	8 (1.92%)		1 (0.23%)				99 (23.74%
Shut	1 (0.23%)	1 (0.23%)							2 (0.47%)
Mech	3 (0.71%)	2 (0.47%)							5 (1.19%)
Kaibarta	19 (4.55%)	2 (0.47%)	2 (0.47%)	1 (0.23%)					24 (5.75%)
Bodo	1 (0.23%)								1 (0.23%)
Kachari	14 (3.35%)	13 (3.11%)	3 (0.71%)						30 (7.19%)
Deori	3 (0.71%)	1 (0.23%)	1 (0.23%)						5 (1.19%)
Mishing	26 (6.235)	15 (3.6%)	7 (1.67%)						48 (11.5%)
Tea Garden Community	2 (0.47%)		2 (0.47%)	1 (0.23%)		12 (2.87%)	17 (4.07%)	12 (2.87%)	46 (11.0%)
other	15 (3.59%)	4 (0.95%)							19 (4.55%)
Total	**243 (58.27%)**	**100 (23.9%)**	**30 (7.19%)**	**2 (0.47%)**	**1 (0.23%)**	**12 (2.87%)**	**17 (4.07%)**	**12 (2.87%)**	**417**
t test	3.1004	2.7778	2.7133	1.4606	1.0000	1.0852	1.0609	1.0852	3.3607
p value	0.0069	0.0134	0.0153	0.1635	0.3332	0.2939	0.3045	0.2939	0.004

Table 4: Community/Ethnic Group Wise Distribution of Haemoglobinopathies and Thalassemia (n=417)

DISCUSSION

Thalassemia and other haemoglobinopathies are autosomal recessive disorders, which are mainly confined to certain areas, religions, cast, and tribes predominantly because of migration of population from one place to another and marriage between different communities.[15]

The results of present study were compared with those of other studies. Majority studies were adult-case based. There are limited studies, which were done on paediatrics population.

Maximum numbers of cases were between 10-18 years (32.4%) followed by 1-3 years (19.4%), 4-6 years (19.2%), 7-10 years (18.5%), and between 7-12 months, it was (10.6%). Shah SJ et al. found 34.3% cases in 10 or more years of age group followed by 31.4% cases between 1-3 years of age.[16]

Regarding the sex distribution, out of 417 cases 230 (55.15%) were male and 187 (44.84%) were female. Shah SJ et al. found male predominance with 74.3% and male to female ratio being approximately 3:1.[16] Coelho G et al. found 70.6% males and 29.4% females in their study.[17] Karthika M et al. found male predominance in their study (56.86% vs 43.13%).[18]

In the present study, HbE trait (58.27%) was the most common type of haemoglobinopathy. In ICMR multi-centre study, which included Kolkata in West Bengal in the east, Dibrugarh in Assam in the North East India among six cities showed a prevalence of HbE trait as 3.92% in Kolkata and 23.9% in Dibrugarh. In six ethnic groups from Assam, the prevalence of HbE trait varied from 41.1% to 66.7%.[6] Earlier studies on HbE have shown that the prevalence is very variable with some population groups like the Mizos from Mizoram in the north east having the prevalence of HbE trait of 1.5%[19] while among different tribal groups in Tripura and among the Mishings of upper Assam and Phayengs and Khurkhvels of Manipur the prevalence of HbE trait ranged from 16.2% to 47.3%.[20,21,22] The Bodo-Kacharis were shown earlier to have one of the highest reported prevalence of HbE trait (64%).[9] These findings were similar to our study.

Second most common pattern noted was HbE homozygous (HbE disease) with 23.9%. Baruah MK et al. reported similar findings with 21.07% of patients had HbE disease in a study done in upper Assam, India.[23] Aggarwal S et al. reported an incidence of 16.6% of HbE disease.[24] Chattopadhyay K et al. reported a prevalence of 7.4% of HbE disease.[25]

The most significant finding of our study was the high incidence of HbE beta thalassemia (7.19%). Most of the studies from the region showed low incidence of Hb E-beta thalassemia. Baruah MK et al reported a low incidence of HbE thalassemia (1.2%).[23] ICMR study reported a incidence of 1.44% in general population.[6] This finding is important as early report from North East India reported only seven cases.[26] The high incidence in our study could be because of few factors, first one was more interaction and marriage between different ethnic groups in this region as migration of various communities overtime, second it was a laboratory-based study as opposed to a population-based study and we analysed those referred children who were anaemic.

The HbS gene was detected in 9.81% of patients, of which sickle cell anaemia was the predominant form (4.07%) followed by HbS trait (2.87% and S beta thalassemia (2.87%). Baruah MK et al. reported HbS gene in 5.11% of patients of which sickle cell anaemia was the predominant form (2.26%) followed by 2.10% for HbS trait and 0.59% for S. beta thalassemia.[23] As it is a laboratory-based study, the high incidence of sickle cell anaemia maybe because of this. Children with sickle cell anaemia showed high level of fetal Hb level. Baruah MK et al. reported similar finding in their study.[23] This may be due to the prevalent haplotype in India is Saudi Arabia/Indian haplotype. This haplotype is associated with higher levels of HbF, which reduces the clinical severity of the disease.[27]

Among the ethnic groups, HbE trait is most common among Ahom (13.66%), Chutia (15.5%), and Mishing (6.23%) communities. Earlier studies by ICMR from the region reported that though the HbE trait had been detected across all ethnic groups in Assam. It was most commonly found in Ahom, Koch, Chutia, Muttock, Deori, and Mishing communities.[6] In our study, we found few cases in Muttock, Deori, and Koch communities probably because less population of these communities in this region. HbE disease was mainly found in Ahom (6.47%) and Chutia (5.9%) communities. HbE thalassemia was mainly found in Chutia (1.92%), Ahom (1.43%), and Mishing (1.67%) communities.

In our study, HbS gene was mainly restricted to tea garden community. Total 46 patients from tea garden community were included in this study out of which 17 (36.95%) cases were found to have sickle cell anaemia and 12 (26%) cases each in HbS trait and S. thalassemia groups. A similar finding was reported by Sharma SK et al. in tea garden community, a group of population brought to Assam by the British colonial tea planters from central, Eastern and Southern India during mid-19th century.[11] The sickle cell anaemia and sickle cell trait are found in relatively high frequency among the endogenous population of India. The highest of 22.2% has been found in Lohars of Orissa followed by Mahars of Madhya Pradesh (20%), and Kinkars of Assam (18.3%). The average sickle cell gene frequency is highest in Orissa (9.1%) followed by Assam (8.3%).[28]

HPLC helps in the establishment of diagnosis in these types of cases. The confirmatory diagnosis is made by identification of abnormal gene using polymerase chain reaction.[13] As these facilities are not available in our setup, we could not carry out these tests in our study.

Prevention programmes are important for these conditions. In countries like Cyprus, Italy, and Greece control of thalassemia is achieved through various prevention programmes. In these countries, population education, and screening forms the most important part.[29,30,31] In country like India, which carry remarkable diversity in frequency of thalassemia and other haemoglobinopathies accurate micro mapping is important while estimating the disease burden for planning the preventive programmes.[32]

HbE thalassemia is highly prevalent in this part of North-East India along with other HbE variants. Compared to other studies from this region, we found high prevalence of HbE thalassemia. HbS gene is mainly found in tea garden community. Because of costly curative treatment like bone marrow transplantation and other medical care, the best way to prevent this condition is through population screening and genetic counselling.

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