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Types of Inherited Hemoglobin Disorders Among the Patients Attending Hematology Outdoor of a Tertiary Care Hospital in Bangladesh

Nur Mohammad Rakib[1], Mahbub Mayukh Rishad[2], SM Kawser Zafor Prince[3], Miftahul Jannat[4], Tazbiha Rahman Khan[5], Talha Islam Zinan[6], Tanvir Jeshan[7], Md. Alamgir Kabir[8]

Abstract

Background: *Inherited Haemoglobin (Hb) disorders are the most frequent genetic hereditary disorders with an increasing global health burden, especially in low- and middle-income countries. This disorder is mostly caused by a point mutation on a globin gene resulting mostly in single amino acid substitutions and consequently defective globin chain production or reduced production of normal haemoglobin. Despite the limited study of this country regarding this issue, the number of individuals suffering from inherited Hb disorders is not negligible. Thus, the study was designed to evaluate the types of inherited haemoglobin disorders among the patients attending the haematology outdoor of Dhaka Medical College Hospital (DMCH).*

Methods: *This hospital-based cross-sectional study was conducted at the Department of Haematology in DMCH, for a period of 6 months (June 2022 to November 2022. People attending the Haematology outpatient department and diagnosed with a case of inherited Hb disorder were approached for inclusion. Written informed consent was taken from each subject. Ethical issues were ensured properly. For the study, a total of 100 patients were interviewed for less than 30 minutes of duration in each case. Relevant investigations such as, Hemoglobin (Hb), Red Cell Count (RCC), MCV, MCH, MCHC, RDW, Reticulocyte, Serum ferritin were done in the Haematology lab of DMCH, and data were collected in a semi-structured questionnaire. Collected Data were analyzed by SPSS 21.*

Results: *Out of 100 inherited Hb disorder subjects, the majority (60 or 60%) were from the age group of 14 to 24 years. The mean age of patients was 38.78 ± 6.09 with range 15-51 years. The male-female ratio was 1:1. The majority (58%) came from rural areas and 42% came from urban areas. Mean value of Hb was 10.54; subsequently, MCV (63.25), MCH (22.18), S ferritin (140.43). The majority of patients had their onset of clinical presentation of illness for 5 years. Among total subjects, two-thirds of the patients (66 or 66%) had Hb E Disease followed by 14% Beta thalassaemia trait, 14% Hb E trait, and 6% β thalassaemia major. No association was noted between the type of thalassaemia with age & sex of the subjects.*

Conclusion: *Irrespective of age and sex variations, Hb E disease is the most frequent inherited haemoglobin disorder among the patient attending the haematology outdoor of a tertiary care hospital.*

Keywords: *Inherited Haemoglobin Disorders, Beta Thalassaemia, Haematological Malignancy,*

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1. Medical Officer, National Institute of Kidney Diseases and Urology
2. Registrar of Medicine, Popular Medical College
3. Lecturer, Department of Community Medicine, Popular Medical College
4. Registrar of Medicine, Ahsania Mission Cancer and General Hospital
5. Lecturer, Department of Biochemistry, Popular Medical College
6. Medical Officer, Bangabandhu Sheikh Mujib Medical University Hospital (BSMMU)
7. Registrar of Cardiology, Enam Medical College Hospital
8. Professor, Department of Haematology, Dhaka Medical College Hospital

Corresponding author: Dr. Nur Mohammad Rakib, Medical Officer, National Institute of Kidney Diseases and Urology, Email: rakibcmc2006@gmail.com

Background

Inherited haemoglobin disorders are emerging as a global public health concern.[1] These disorders are a heterogeneous group of mendelian disorders referred to haemoglobinopathies, characterized by structurally abnormal haemoglobin variants and thalassaemia by partial or total suppression of normal peptide chains of haemoglobin molecules.[2] Majority of these results from single amino acid substitution in one or other of the globin chains.[3] An estimated 320,000 babies (83% sickle cell disorders, 17% thalassaemias) are born globally each year with a clinically significant hemoglobin disorder.[4] Nearly 80% of these births occur in developing countries.[5] Most conservative estimates suggest that at least 5.2% of the world population (over 360

million) carry a significant hemoglobin variant and in excess of 100 million beta-thalassemia carriers with a global frequency of 1.5%.[4,5] Homozygous or compound heterozygous states between certain variants can lead to clinical manifestations of hemoglobinopathies which makes the burden on the family socially, psychologically, and economically, especially in low-resource settings.[1,6] Moreover, it is responsible for a significant number of mortality. World statistics suggest level about 3.4% of mortality in children under 5 years of age is due to Hb disorders.[4]

According to the study 'The inherited diseases of hemoglobin are an emerging global health burden' by Weatherall et al.[7] showed 'breakdown of the annual number of births with the different hemoglobin disorders are 22 989, 19128, 9568, 5183, 217331, 54736 in β thalassemia major, Hb H disease, Hb Bart hydrops, SS disease, S β thalassemia, and SC disease respectively'. Among single-genedisorders, inherited beta thalassemias including sickle cell anemia and hemoglobin E (HbE) disorders are the most frequent single-gene disorders globally.[1,4,7] Surprisingly, the serious forms of β thalassemia are equally divided between β thalassemia major and hemoglobin E (HbE) β thalassemia.[7] However, hemoglobin E disease occurs at a high frequency in parts of the Indian subcontinent, Bangladesh, Myanmar, and throughout Southeast Asia, whereas, a higher frequency of severe forms ofá thalassemia is confined only in Southeast Asia.[7]

South Asia, including Bangladesh, is a hotspot of hemoglobinopathies.[5] Data suggest that it is home to 23% of the world's population (approximately 1.7 billion).[8] Most information on thalassemia in South Asia comes from studies conducted in India.[1,9,10] The overall prevalence of beta-thalassemia carriers has been estimated to be between 2.78 to 4% (approximately 30–48 million) in India[9,11] and 5–7% (approximately 5–12 million) carriers in Pakistan.[5,12] The information on the prevalence of hemoglobinopathies in Bangladesh is scarce due to a lack of population-based data. The only published report available on the prevalence of thalassemia among (n = 735) school children in Bangladesh showed a 4.1% prevalence of the beta-thalassemia trait and a 6.1% prevalence of the HbE trait.[1] Another estimate done by World Health Organization (WHO) evidenced, approximately 3% of the population are carriers of beta-thalassemia and 4% are carriers of hemoglobin E (HbE) in Bangladesh.[11] However, these estimates must be interpreted with caution since the data was mainly based on studies conducted in 1980, and a small number of non-representative samples obtained from treatment centers were analyzed.[7] Most recently Khan et al, studied to find the common types of thalassemia and abnormal hemoglobin variants seen in Bangladeshi populations and revealed 49.95% abnormalities among his 4813 samples.[7] The common hemoglobin disorders were β trait (17.94%). On the contrary, the frequency of other hemoglobinopathies like the Hb E trait was 12.50%, Hb E β thalassemia 10.87%, β thalassemia major 4.00%, and Hb E disease was 2.05%. Other Hb abnormalities detected were Hb D trait 0.35%, Sickle cell trait 0.08%, hereditary persistence of fetal hemoglobin (HPFH) 0.04%, and Hb Lepore, δ β thalassemia, sickle cell β thalassemia, Sickle cell disease, compound heterozygote for HbE+D and Hb Q band one case each 0.02%. Considering the importance of the issues and the of the different studies, the study is planned to evaluate the types of inherited haemoglobin disorders among the patients attending haematological outdoor of DMCH.

Materials and Methods:

The study was a descriptive cross-sectional study conducted in the Department of Hematology at Dhaka Medical College Hospital over a period of six months after approval of the protocol. The study population consisted of patients attending the Hematology outpatient department in DMCH and the sample size was determined to be 280. However, due to time and resource constraints, only 100 subjects were included in the study, selected through purposive convenient sampling.

Inclusion criteria included patients aged 14-70 years, of both genders, suffering from haemoglobinopathies previously diagnosed according to Hb electrophoresis. Exclusion criteria included critically ill patients needing immediate hospitalization, extremes of age, patients already admitted, and those with haematological malignancy.

Data was collected through face-to-face interviews using a semi-structured questionnaire containing socio-demographic parameters and relevant information regarding inherited Hb disorder. Relevant information was also collected from the hospital record form. Relevant investigations such as, Hemoglobin (Hb), Red Cell Count (RCC), MCV, MCH, MCHC, RDW, Reticulocyte, Serum ferritin were done in the Haematological lab of DMCH. The researcher kept recorded all the information into the case-record form. After compilation, data were analyzed by SPSS 21.

Written informed consent was obtained from every patient and ethical issues related to this study were carefully addressed. The research protocol was approved by the ERC of DMC and precautions were taken to protect the confidentiality of the participants. The study subjects or legal guardian were informed about the nature and purpose of the study, benefits and hazards to all participants, and that the

data obtained from the study would be used only for research purposes. The participants' basic human rights would not be violated in any way.

Data Processing and Analysis:
After collection of all the required data, these were checked, verified for consistency and tabulated using the SPSS (v. 21) software. Statistical significance is set as 95% confidence level at 5% acceptable error level. Data were presented as the proportion of valid cases for discrete variables and as means ± standard deviations and/or medians with interquartile ranges for continuous variables. Differences and/or relation in baseline characteristics were compared using necessary statistical test. A p value of <0.05 was considered significant.

Result
Out of 100 with, majority (66/60%) were from age group 14 to 24 years. The mean age of patients was 38.78 ± 6.09. Minimum age of the patients was 15 and maximum age of the patients was 51 (Figure 1).

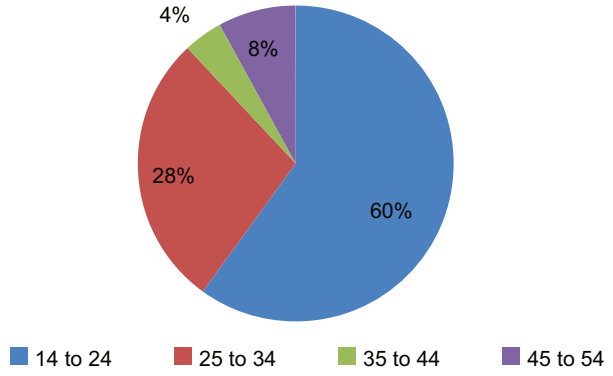

Figure-1: *Distribution of patients according to their age (n=100)*

Fifty percent patients were male and another 50% were female (Figure 2).

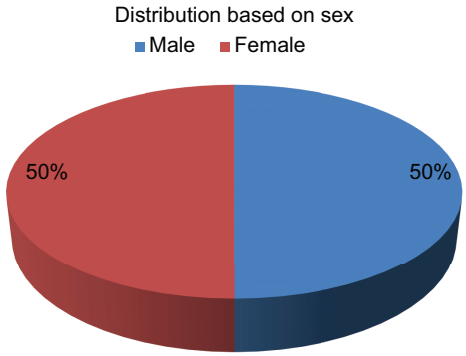

Figure-2: *Distribution of patients according to their sex (n=100)*

Among 100 patients, majority (58%) came from rural area and 42% came from urban area (Figure 3).

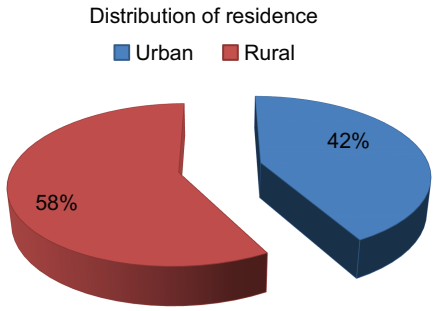

Figure-3: *Distribution of patients according to their residence (n=100)*

The most common clinical symptom was weakness (86%), followed in second and third by dizziness (30%) and palpitation (30%). The most common clinical sign was anemia (82%) followed by splenomegaly (60%) (Table 1).

Table-1: *Clinical presentation of patients (n=100)*

Clinical presentation	Percentage (%)
Weakness	86
Anemia	82
Splenomegaly	60
Dizziness	30
Palpitation	30
Headache	6
Yellowish sclera	6
Abdominal pain	6
Leg swelling	6
Jaundice	6
Fever	4
Abdominal discomfort	4
Loss of appetite	4
Shortness of breath	2
Per vaginal bleeding	2

All of the patients included in the study had thalassaemia. Among them majority (66%) had Hb E Beta thalassaemia followed in decreasing order by 14% Beta thalassaemia trait, 14% Hb E trait and 6% β thalassaemia major (Figure 1).

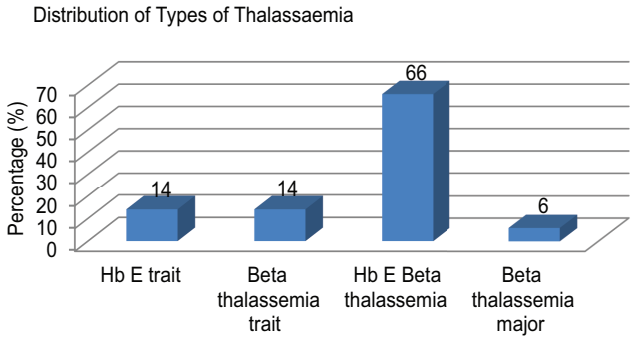

Figure-4: *Distribution of patients according to their thalassaemia types inherited (n=100)*

Majority patients had their onset of clinical presentation of illness for > 5 years. Rest of the distribution is shown in pie chart below (Figure 5).

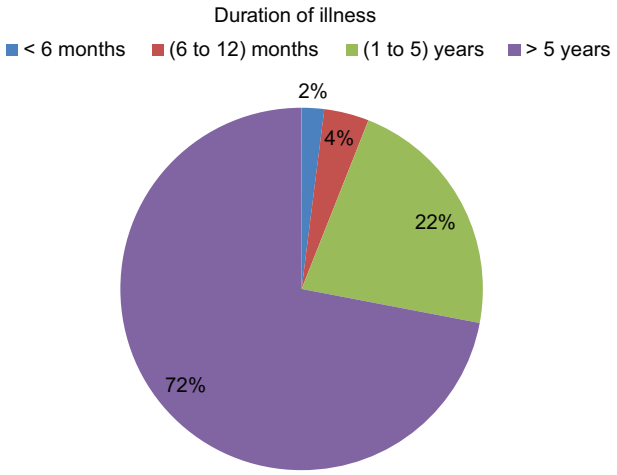

Figure-5: *Distribution of patients according to their duration of illness (n=100)*

Mean hemoglobin and MCH was lower in thalassaemia patients in comparison to thalassaemia trait patients. Reticulocyte and ferritin was higher in thalassaemia patients in comparison to thalassaemia trait patients (Table 2).

Table-2: *Red cell indices of study population (n=100)*

Red Cell indices		Diagnosis			
		Hb E trait	Beta thalassemia trait	Hb E Beta thalassemia	Beta thalassemia major
Hemoglobin (Hb),	Mean	10.54	10.80	7.56	7.57
g/dl	SD	1.83	1.52	1.54	0.74
Red Cell Count	Mean	5.23	4.97	4.09	NA
(RCC), $x10^6/cm^3$	SD	1.34	0.60	0.62	
MCV, fl	Mean	63.25	71.64	65.32	78.73
	SD	5.96	11.21	7.92	4.56
MCH, pg	Mean	22.18	23.56	22.19	17
	SD	2.45	7.50	4.16	12.71
MCHC, g/dl	Mean	27.60	29.70	27.63	30.85
	SD	3.21	3.74	5.32	2.32
RDW	Mean	15.17	15.23	20.23	20.60
	SD	2.56	4.16	6.65	0.01
Reticulocyte, %	Mean	1.55	1.58	3.33	8.52
	SD	0.25	0.39	4.57	7.47
Serum ferritin,	Mean	140.43	211.18	4866.12	4866.12
ng/ml	SD	88.63	102.01	2717.95	2717.95

NA = Not available.

Table 3 enlists the hemoglobin electrophoresis findings. Mean fraction of hemoglobin A was lower in Hb E Beta thalassaemia patients than beta thalassaemia patients. Hb A2 level was high in Hb E trait and Hb E beta thalassaemia patients. Hemoglobin F was more common in full blown thalassaemias in comparison to traits.

Table-3: *Electrophoretic profile of study population*

Electrophoretic profile		Diagnosis			
		Hb E trait	B thalassemia trait	Hb E B thalassemia	B thalassemia major
Hemoglobin	Mean	72.08	83.07	25.08	37.84
(HbA), %	SD	1.42	28.31	30.87	48.28
HbA2, %	Mean	14.21	4.85	14.84	4.20
	SD	22.12	0.67	19.48	0.01
HbE, %	Mean	22.47	0	51.28	19.14
	SD	2.93		27.86	
HbF, %	Mean	8.67	0.90	25.81	15.89
	SD	13.97	0.01	16.15	9.91

Please recheck the data of Hb A2 value of E trait

No association was noted between type of thalassaemia and age of the patients (p>0.05) (Table 4).

Table-4: *Association of type of thalassaemia with age (n=100)*

Clinical Diagnosis	Age (14 to 24) years N (%)	Age(25 to 34) years N (%)	Age(35 to 44) years N(%)	Age(14 to 24) years N(%)	P-value
Hb E trait	2 (28.6)	2(28.6)	1(14.3)	2(28.6)	
Beta thalassemia trait	2(28.6)	4(57.1)	0	1(14.3)	0.14
Hb E Beta thalassemia	24(72.7)	7(21.2)	1(3)	1(3)	
Beta thalassemia major	2(66.7)	1(33.3)	0(0)	0(0)	

p-value is determined by chi-square test, p >0.05 is insignificant

No association was noted between types of thalassaemia and sex (p>0.05) (Table 5)

Table-5: *Association of type of thalassaemia with sex (n=100)*

Clinical Diagnosis	Male (n=50) N (%)	Female (n=50) N (%)	P-value
Hb E trait	4(57)	3(43)	
Beta thalassemia trait	2(28.6)	5(71.4)	0.56
Hb E Beta thalassemia	18(54.5)	15(45.5)	
Beta thalassemia major	1(33.3)	2(66.7)	

p-value is determined by chi-square test.p>0.05 is insignificant

Discussion

Haemoglobinopathies are inherited disorder of globin chain synthesis. It either reduced rate of synthesis or structurally abnormal globin chain leading to abnormal haemoglobin molecule synthesis. A patient of haemoglobinopathy and the family undergoes through a socio-economic strain and ultimately causes the burden for the whole community. Knowing the importance this study was designed to assess the type of hemoglobinopathies in outdoor of DMCH.[47,48]

Out of 100 patients with, majority were from age group 14 to 24 years. The mean age of patients was 38.78 ± 6.09. Minimum age of the patients was 15 and maximum age of

the patients was 51. In the study of Uddin M et al, out of 100 patients, majority were from age group 11-20 years[49] and the patients' mean age was found 7.8 by the study of Karim F et al.[50]These results varies with ours as those studies were conducted according to their study planning.

Fifty percent patients were male and another 50% were female in our study. Goswami BK reported, out of his 890 abnormal cases 54.5% and 45.5% were males and females respectively.[51]

In our study among 100 patients, majority (58%) came from rural area and 42% came from urban. Majority patients were educated below SSC (54%), followed in decreasing order by 24% completing SSC, 8% completing HSC, 8% completing graduation and 6% were illiterate. Though there has no linked between hemoglobinopathies and patients' residence, education or occupation but these studies can be compared with others as these results reflects the socioeconomic status of a study population and sometimes of that region. In the study of Malik S et al majority was from rural background (74%), a good percentage of them (75%) had completed primary education, 48% were still students but 37% of the thalassaemia patients were not engaged in any activity, either educational or livelihood earning and among them 16% were housewives.[52]

Among of our patients all, 52% had monthly income <10000 taka, 46% had income between 10000 to 20000 taka and only 2% had income above 20000 taka. Verma P et al reported among his patients 5.3% were in high income group, 10.1% were in middle income group and 17.3% were in low income group.[53]

The most common clinical symptom was weakness (86%), followed in second and third by dizziness (30%) and palpitation (30%). The most common clinical sign was anemia (82%) followed by splenomegaly (60%). In the study of Uddin M el al the most common symptom was weakness (70.47%). Jaundice was in second (55.71%) and hepatomegaly found in third (40.00%). Others followed in decreasing order by splenomegaly (60.09%) fever (36.19%) retardation of growth (12.89%) pallor (2.85%) leg ulcer (2.38%) bony change (1.48). Another study can be mentioned which was done by Chattopadhyay K et al. He found that hepatomegaly was the most common presenting complaint among the study population (66.3%), followed by jaundice (53.9%) and thalassemic facies (53.2%). Ascites and edema were found to be least common complaint among them.[54] It appears from the studies that clinical spectrum of thalassemia is widely variable in all of its subtypes. Clinical features of beta thalassemia are usually manifested in younger age group starting below 5 years of age and become more severe with advancing age. Conditions like hepatic dysfunction, portal hypertension and other organ involvement causing functional impairment are found in the advanced age group HbE Beta Thalassemia appears to be less severe clinically. But the patients in this variety may show clinical features resembling those of thalassemia major even in infantile age. In most cases of HbE Beta Thalassemia, clinical severity increases with age and complications like those of Beta Thalassemia eventually develops. Sometimes these patients manifest clinical feature during adolescence with delayed puberty and undeveloped secondary sex characters. All this findings are corroborative to the findings of previous workers.[54,55]

All of the patients included in the study had thalassaemia. Among them majority (66%) had Hb E Beta thalassaemia followed in decreasing order by 14% Beta thalassaemia trait, 14% Hb E trait and 6% betathalassaemia major. Study from India by Chattopadhyay K et al reported that the commonest congenital hemolytic anemia in the present study is HbE Beta Thalassemia (41.1%) followed by Beta Thalassemia (23.6%), Beta Thalassemia trait (13.5%), HbE disease (7.4%), HbE trait (5.1%), sickle Beta Thalassemia (4.7%) and sickle Thalassemia trait (4.7%).These study results are consistent with ours.[54] This signifying that disease of Hb disorders is a very significant genetic problem in Bangladesh and the higher incidence of HbE Beta Thalassemia can be explained by the fact that these cases having a milder clinical course and thus presenting at a later age compared to other group of patients of congenital hemolytic anemia, live longer and also get the opportunity to come under medical attention.[54] The population of West Bengal, Assam and Myanmar share the same ancestry with that of Bangladesh. In North Eastern, the HbE gene reaches the frequencies of about 7.5%9, in Myanmar it is about 10-20% and in Assam it is 30%. As Bangladesh is situated in Between all these area and the people might have been migrated from these areas to Bangladesh in decades earlier.[49,56] Another study from Pakistan byHussan J et al reported that among the hemoglobin disorders the most common disorder was Beta thalassemia major in 87 (38.3%) patients followed by sickle cell disease in 73 (32.16%) and Beta thalassemia trait in 42 (18.5%) patients. Beta thalassemia intermedia was diagnosed in 13 (5.73%) patients, Sickle cell trait in 6 (2.65%), HbE and HbD/Beta thalassemia in 2 (0.88%) cases each and

Sickle cell/ Beta thalssemia and HbD trait in one (0.45%) case each.[57] This study can be a comparative one to ours.

Mean hemoglobin and MCH was lower in thalassaemia patients in comparison to thalassaemia trait patients. Reticulocyte and ferritin was higher in thalassaemia patients in comparison to thalassaemia trait patients. Chattopadhyay K et al found that the mean hemoglobin was lowest in patients of â thalassemia and HbE â thalassemia (5.4 gm/dl). The mean serum ferritin value in highest in HbE â Thalassemia patient (1802 ng/ml) followed by â Thalassemia (1266.5 ng/ml). The mean total serum bilirubin was found to be highest among â Thalassemia patients (3.4mg/dl).[54] This study correspond with our study. Clinical data confirm that the decrease of the haemoglobin level is accompanied by a decrease in the number of erythrocytes and diminished values of their specific indexes (MCV, MCH, HCT, etc). An increase in serum iron and ferritin level thalassemia patients have been observed in this study, which is consistent with several other studies. In case of betathalassemia patients, absence of beta globin chains lead to accumulation of unpaired alpha globin chains. Excess presence of the alpha globin chains is a primary reason for the cellular oxidative damage and also iron overload. Higher ferritin content was directly linked to the accumulation of reactive iron in the tissues of these patients. Iron overload starts another pathological mechanism leading to oxidative damage of erythrocyte membranes, the so-called "second disease".[58,59]

In table 3 the hemoglobin electrophoresis findings are enlisted. Mean fraction of hemoglobin A was lower in Hb E Beta thalassaemia patients than beta thalassaemia patients. Hb A2 level was high in Hb E trait and Hb E beta thalassaemia patients. Hemoglobin F was more common in full blown thalassaemias in comparison to traits. Chandrashekar V found in his study that he average value of HbA2 was 5.4% in â-thalassemia traits. HbF was minimally elevated in 42% of his â-thalassemia trait patients and was around 1.4%. HbF ranges from 10 to 90% in âthalassemia major. In his study, the HbF average was 88%. HbA2 was elevated in â-thalassemia major and 15% of the patients with â-thalassemia major were seen to have elevated HbA2 and the average value of HbA2 was around 3.1%.[60]

We have found no association among the types of thalassaemia, age and sex. But Chattopadhyay K et al reported, in his study it is evident that 72.9% patients of â-thalassemia belong within 17 years, but â thalassemia trait was mostly (57.5%) within 18-20 yrs. HbE trait was commoner in patients above 20 years. Sickle cell â thalassemia was solely present within 17 years of age. In the perspective of sex he noted that except â thalassemia trait all other congenital hemolytic anaemia were commoner among males than females and the difference was found to be statistically significant.[8] These data varies with us but all these data point to the more severe nature and earlier age of manifestation of Beta Thalassemia.

Conclusion

In this study, the majority of the patients had Hb E disease. However other disorders like Beta thalassemia trait, Hb E trait, and beta-thalassemia major were not uncommon. Further analysis showed that there was no association between the type of thalassemia and the age & sex of the patients. However, further larger studies are required to finalize the findings and get an original picture of inherited Hb disorders among the Bangladeshi population.

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

The Principle Investigator, Dr. Nur Mohammad Rakib, oversaw all activities related to the conduct of the study and contributed to the study idea and writing of the manuscript. Dr.Mahbub MayukhRishad, Dr. S M Kawser Zafor Prince, Dr. Miftahul Jannat, Dr. Tazbiha Rahman Khan, Dr. Talha islamZinan, Dr.Tanvir Jeshan and Prof. Md. Alamgir Kabir contributed to the study idea, data collection, and literature review. All authors accepted the final version. All authors read and approved the final manuscript.

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