

Spectrum of types of thalassemias and hemoglobinopathies: study in a tertiary level children hospital in Bangladesh

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Abstract

Thalassemias and hemoglobinopathies are the most common hemolytic congenital disorders in Bangladesh as in many parts of the world. This study was done to find the common types of thalassemias and abnormal hemoglobin variants seen in Bangladeshi populations. A total of 4813 samples were analyzed for hemoglobin disorders out of which 2308 (49.95%) showed abnormalities. The samples were analyzed by Bio Rad D 10 Analyzer in 3914 (81.32%) cases, BIORAD VARIANT β thalassemia short program using the principle of high performance liquid chromatography in 474 (9.85%) cases and by CAPILLARYS 2 FLEX PIERCING utilizing capillary electrophoresis in 425 (8.83%) cases. The samples were analyzed in the Department of Biochemistry and Molecular Biology of Dhaka Shishu (Children) Hospital, Dhaka, Bangladesh. The common hemoglobin disorders seen were β trait 863 (17.94%), Hb E trait 601 (12.50%), Hb E β thalassemia 524 (10.87%), β thalassemia major 192 (4.00%), Hb E disease 99 (2.05%). Other Hb abnormalities detected were Hb D trait 17 (0.35%), Sickle cell trait 4 (0.08%), hereditary persistence of fetal hemoglobin (HPFH) 2 (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%).

Introduction

An estimate of World Health Organization shows that 7% of the world population are a carrier for hemoglobin disorders and 300,000 to 500,000 children are born with clinically significant hemoglobin disorders every year and majority of them

are born in developing countries.¹ Inherited disorders of hemoglobin both structural and synthesis are an important cause of morbidity and mortality. World wide 3.4% of mortality rate in children under 5 years age are due to Hb disorders.² Birth of a single child with a hemoglobin disorder in a family poses enormous psychological trauma along with financial strain for treatment especially in developing countries like Bangladesh. The aim of this study was to determine the types and frequencies of thalassemias and hemoglobinopathies seen in a tertiary level children hospital (Dhaka Shishu (Children) Hospital) in Bangladesh from referred cases of subjects presenting with anemia, abnormal hemograms and for detection of carriers. It is important to know the common Hb variants for diagnostic and preventive planning and to create awareness of physicians to refer cases with abnormal hemograms to rule out hemoglobin disorders. Most of the samples were from children.

Materials and Methods

The study was conducted in the Department of Biochemistry and Molecular Biology of Dhaka Shishu (Children) Hospital, Dhaka, Bangladesh.

A total of 4813 samples were analyzed for hemoglobin disorders over the period of 4 years from August 2011 to September 2015. Two ml of blood were collected in EDTA, and RBC indices were done by using automated Hematology analyzer SIEMEN ADVIAi and Mytic 22AL. Hb A₂, HbF and hemoglobin variants levels were obtained by high pressure liquid chromatography (HPLC) by Bio Rad 10 analyzer, BIORAD VARIANT β thalassemia short program and by CAPILLARYS 2 FLEX PIERCING utilizing capillary electrophoresis and tests done according to procedures provided by manufactures. Samples from subjects below one year were excluded as the presence of Hb F, which is seen below one year, may interfere with interpretation of results. Also subjects with a history of recent blood transfusion were advised to come after four months from the last transfusion. Serum iron or ferritin levels were not done as samples were sent to the laboratory to do electrophoresis only by the referring physicians.

Results

HbE β -thalassemia was the most common type of thalassemia seen in 524 cases

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(10.87%) followed by β thalassemia major in 192 cases (4.00%). There were one case each of δ β thalassemia and Sickle cell β thalassemia (0.02%).

β thalassemia trait was the most common β chain carrier abnormality detected in 863 (17.94%) cases.

Hb E trait was the most common abnormal hemoglobinopathy detected in 601 (12.50%) cases.

Hb E disease was seen in 99 (2.05%) cases and Hb D trait in 17 (0.35%) cases. Other rare Hb abnormalities seen were Sickle cell trait 4 (0.08%) hereditary persistence of fetal hemoglobin (HPFH) 2 cases (0.04%), and Hb Lepore, double heterozygote for Hb E +D and Q band all one case each (0.02%) (Table 1).

Discussion

This study shows the types and frequencies of thalassemias and abnormal hemoglobins seen in subjects referred to a tertiary level children hospital for analysis of Hb disorders presenting with anemia, abnormal hemograms or for detection of carriers.

Hb abnormalities were detected in 2308 (47.95%) cases and thus signifying that disease of Hb disorders is a very significant genetic problem in Bangladesh.

Hb E β thalassemia was the most common type of thalassemia seen constituting

524 (10.87%) of cases. It was also detected to be the most common type of thalassemia in a similar study in Bangladesh by Uddin *et al.*³ Studies of pattern of hemoglobinopathies in West Bengal in India also found Hb E β thalassemia to be the most common type of thalassemia.^{4,6} Hemoglobin E β thalassemia shows a varied presentation from a mild type of thalassemia to severe type like beta thalassemia major.^{7,8}

β thalassemia major was the second most common type of thalassemia seen. It was seen in 192 cases (4.00%) which was higher than in comparison to the study by Uddin *et al.* (0.5%) in Bangladesh and in West Bengal by Mondal *et al.* and Chaudhury *et al.*^{3,5,6} It may be due to the fact that most of our samples were from children and its presentation is earlier than Hb E β thalassemia. Thalassemia major occurs due to mutations of β globin genes resulting in the absence or decreased production of the globin genes.

There was one case (0.02%) each of sickle cell β thalassemia and $\delta\beta$ thalassemia. $\delta\beta$ thalassemia was diagnosed in one subject when HPLC report showed complete absence of Hb A and A2 with 100% Hb F. Hb Analysis of parents revealed increased level of Hb F in both with low MCV and MCH and was diagnosed as $\delta\beta$ thalassemia. It needs to be confirmed by DNA analysis, which we could not do due to lack of facilities in our laboratory.

Sickle cell β thalassemia was seen in only 1 case (0.02%). Uddin *et al.* did not find a single case in his study in Bangladesh.³ while it was found in 1.07%, 0.2% and 3.67% in three studies in Eastern India.^{4,6} HbS gene is mainly seen in Central India like Maharashtra, Madhya Pradesh and Gujarat while it is rare in other states of India.⁹ Hb S gene is characterized by replacement of glutamine to valine at position 6 of beta globin chain.¹⁰

$\delta\beta$ thalassemia was presumably diagnosed in 1 case only (0.02%) while Uddin *et al.* reported in 3 cases (0.5%) but interestingly it was not reported in three large study of hemoglobin pattern in Eastern India.^{4,6}

β thalassemia trait was seen in 863 cases (17.94%) and its frequency was rather high as most of the cases referred had low Hb or abnormal RBC indices and specially samples were sent to rule out β thalassemia trait from iron deficiency. Nearly 1.5% of the world's population are carriers of β trait.¹¹ High frequency of β trait was also detected by Jain *et al* and Chaudhury *et al* in their study as their samples were also referred cases with abnormal hemogram.^{4,6} In β trait Hb may be lower than normal with

elevation of RBC count and low MCV and MCH and a HbA₂ level typically between 4-6%. The blood film shows microcytosis, anisocytosis, poikilocytosis and hypochromia.¹²

Hb E is one the most common and important mutations in the world. It was seen in 601 cases (12.50%) in this study. It is caused by the substitution of lysine for glutamic acid at position 26 of the beta globin chain.¹³ Hb E traits is mainly seen in the eastern half of Indian sub-continent, Bangladesh, Myanmar and east and south east Asia.¹⁴ In Hb E trait carriers the red blood cells are either normal or mildly microcytic and few target cell may be seen. The frequency of β trait and Hb E trait in Bangladeshi population is 4.1% and 6.1%.^{12,15}

Hb E disease was detected in 99 cases (2.05%) The frequency of Hb E disease detected was rather high in the study by Uddin *et al.* 9.2%, while other studies from West Bengal by Mondal *et al.* and Chaudhury *et al.* found 0.39% and 0.12% respectively.^{3,5,6} Hb E disease is clinically normal, but the blood film shows hypochromia with variable number of target cells. HPLC shows the major hemoglobin to be Hb E constituting 85% to 99% of the total hemoglobin the rest being hemoglobin F. The differential diagnosis is with Hb E β thalassemia and needs the help of clinical features, family studies and sometimes DNA analysis.¹⁶ Most of our cases were confirmed by testing of parent's blood showing Hb E trait in both the parents.

Hb D trait also known as D Punjab was

detected in 17 (0.35%). Hb D Punjab is seen in northwest India, Pakistan and Iran.¹⁷ It is also a common abnormal Hb variant in the population of Denizli of South Eastern Turkey and also in Xinjiang province of China.^{18,19} Hb D Punjab differs structurally from normal Hb A at 121 position of beta globin chain where glutamine replaces glutamic acid.²⁰ Heterozygous Hb D Punjab is clinically normal and blood film may show some target cells. However its association with Hb S results in moderate to severe clinical manifestations.²¹ This mutation may have come from migration of people from western part of India during Muslim rule in Bengal.

Other rare Hb disorders detected in this study were sickle cell trait, sickle cell anemia and Hb Lepore and hereditary persistence of fetal hemoglobin (HPFH). HPFH was reported when HbF level between 15% to 30% with normal hemogram.

Conclusions

This study shows that hemoglobin disorders is a significant genetic problem in this country Measures needs to be taken to prevent the births of thalassemic children with hemoglobin disorders like thalassemia by creating awareness, education of medical doctors, screening of population and prenatal diagnosis. Furthermore physicians should be made aware of the fact that all abnormal hemograms shown in cell counter should be referred to laboratories to detect Hb disorders and not brushed aside as iron

Table 1. Spectrum of thalassemias and hemoglobiopathies.

	Number	Frequency
Normal	2505	52.05%
Hb E β thalassemia	524	10.87%
β thalassemia major	192	4.00%
Sickle cell β thalassemia	1	0.02%
Delta β thalassemia	1	0.02%
β thalassemia trait	863	17.94%
Hb E trait	601	12.50%
Hb E disease	99	2.05%
Hb D trait	17	0.35%
Sickle cell trait	4	0.08%
Sickle cell disease	1	0.02%
HPFH	2	0.04%
Hb Lepore	1	0.02%
Hb E+D	1	0.02%
Q Band	1	0.02%
Total	4813	100%

deficiency as this being very common in Bangladesh.

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