ORIGINAL ARTICLE

PERIPHERAL BLOOD FILM PATTERNS IN THALASSEMIA AND HEMOGLOBINOPATHY CARRIER

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ABSTRACT

Thalassemia and hemoglobinopthies are the commonest genetic disorders of hemoglobin. The disease has autosomal recessive pattern of inheritance. Screening of thalassemia and hemoglobinopathy carriers is a burning issue to prevent new cases. Thalassemia and hemoglobinopathies can be prevented by genetic testing followed by genetic counseling, if we can detect carriers of thalassemia or hemoglobinopathies by an easy method. The objective of this study was to see the peripheral blood film pattern among the carriers of thalassemia and hemoglobinopathies. It was a cross-sectional study conducted from September 2015 to November 2015. A total of 437 random specimens were collected from three tribal groups. Two mL of venous blood were collected in EDTA tube. Hemoglobin variants were studied by HPLC method using Variant Hemoglobin Testing System (Variant II Beta Thalassemia Short Program). Statistical analysis was carried out using SPSS statistical package (version 23.0). Data were analyzed by frequency distribution. In this study, total 437 respondents were examined for blood test. Male to female ratio was 1:1. The study included cases from 2 years to 82 years where mean age was 18.3±12.0 years. In clinical analysis, Hb ranged from 8.5 to 19.0 gm/dL and 187 cases were found normochromic, 110 cases anisochromic 140 cases hypochromic. Among them, 38.2% were normal individuals, Hb E Trait were 34.8%, Hemoglobin E disease were 21.7% and Beta Thalassemia trait were 5.3%.Peripheral blood film and hemoglobin status may give an idea about thalassaemia and hemoglbinopathy trait or carrier. In the suspected cases, High Performance Liquid Chromatography (HPLC) may be done to confirm the diagnosis.

Key words: Peripheral blood film, Thalassemia, Hemoglobinopthies, Carrier, HPLC

Introduction

Thalassemia and hemoglobinopthies are commonest genetic disorders of hemoglobin[1]. Thalassemias are characterized by reduced rate of production of normal hemoglobin due to absent or decreased synthesis of one or more types of polypeptide chains. Hemoglobinopathies are characterized by the production of structurally defective hemoglobin due to abnormalities in the formation of globin moiety of molecule[2].

The disease has autosomal recessive pattern of inheritance. Screening of thalassemia and hemoglobinopathy carriers are burning issues to prevent new cases. Thalassemia and hemoglobinopathies can be prevented by genetic

testing followed by genetic counseling, if we can detect carriers of thalassemia or hemoglobinopathies by easy method. In this study we examined the Peripheral Blood Film pattern among the carriers of thalassemia and hemoglobinopathies.

Although hemoglobinopathies and thalassemia are two genetically distinct disease groups, the clinical manifestations of both include anemia of variable severity and variable pathophysiology. The thalassemias are characterized by a reduction in the amount of the normal globin chain produced. This diminution in globin chain production may result from gene deletion or from mutations that adversely affect the transcription or stability of mRNA products. The manifestations range from mild anemia with microcytosis (thalassemia trait) to fatal severe anemia (Hb Barts hydrops fetalis or beta-thalassemia major). The hemoglobinopathies, or structural Hb variants, are attributable to amino acid substitution in either beta or alpha chain. More than 700 hemoglobinopathies have been described to date, the majority of which are clinically benign and fortuitously discovered. The clinically significant hemoglobinopathies are attributable to amino acid substitutions, primarily in the beta chain, that bring about changes in the secondary and tertiary structure of the Hb tetramer. These substitutions are most common at positions in close proximity to either heme group or globin chain attachment sites[3].

These hereditary disorders are major public health problems in many countries of the world including Bangladesh. The clinical spectrum of the disorders varies from asymptomatic conditions to serious disorders like thalassemia major that requires regular blood transfusion and extensive medical care. World Health Organization (WHO) figures estimate that 7% of world population is carrier for hemoglobin disorders[4].

Beta thalassemia and HB-E beta thalassemia are common in our country. A conservative world health report has estimated that 3 percent are carriers of beta thalassemia and 4 percent are carriers of HB-E beta thalassemia (WHO 1994). A recent study on school children in different districts of Bangladesh has shown overall prevalence of beta thalassemia trait 4.1 percent and hemoglobin E trait 6.1 percent. This study also included frequency study of 44 tribal school children where 22% were Hemoglobin E trait and 3% were beta trait[5].

In this study tribal population were chosen as study population. The total population of indigenous ethnic minorities in Bangladesh were estimated to be approximately 1.58 million out of 152.52 million total populations in 2011 (last census) which represents 1.8% of total population[6]. They comprise of diverse ethnic communities including Australoid, Tibeto-Burman and Sino-Tibetan races. Different ethnic groups of Bangladesh and their colorful lifestyles have significantly enriched the entire culture of Bangladesh[7]. They are closely knit people marrying within their clans and therefore highly vulnerable to many hereditary disorders causing high degree of morbidity and mortality. As the frequency of thalassemia is increased by the consanguinity and endogamous mating, it may be assumed that the tribal communities are facing the problem at a very large scale. It is, therefore, very important to know their status of thalassemia and hemoglobinopathies.

In Bangladesh, there are about 45 different tribal groups spread across the country[8]. Chakmas are the largest ethnic group in Bangladesh. They live in Rangamati, Khagrachhari and Bandarban of Greater Chittagong Hill Tracts. They also live in Teknaf of Cox's Bazar. They are ancestors of mongoloids[9].

Marmas are the second largest ethnic group. Majority of them live in Rangamati, Khagrachhari and Bandarban, some of them also live in coastal area of Teknaf and Patuakhali[8]. Marmas belong to the mongoloid races[10].

Garo population is another large indigenous community in Bangladesh[11]. They live in the north-eastern part of Bangladesh, with the highest presence in Gazipur, Mymensingh, Netrokona, Tangail, Sherpur, Jamalpur and Sylhet districts[12]. There is no accurate information about the ancestosrs of Garos. But anthropologists presume that Garos belong to the Tibet-Burmese people of the Mongoloid race[9].

Cell counters are now widely used in our country for routine hematological parameters including hemoglobin, total count of WBC, differential count of WBC, platelet count along with RBC indices. Peripheral blood film examination also is routinely overviewed.

Microcytic hypochromic anemia is most common in our country[13]. Causes of anemia mainly includes iron deficiency, hemoglobin disorder like thalassemia, beta thalassemia trait, hemoglobin E trait. RBC indices like total count of RBC, MCV, MCH and RDW can be utilized for screening of trait and diagnosing of thalassemia. On the other hand, peripheral blood film examination can guide to diagnosis.

Final diagnosis can be made by HPLC or gel electrophoresis or capillary electrophoresis. With RBC indices values like MCV, 75 fL, MCH <26 pg are advised for hemoglobin electrophoresis or HPLC[14].

HPLC is a powerful, excellent diagnostic tool for direct identification of different hemoglobin variants with a high degree of precision in the quantification of major and minor, normal and abnormal, hemoglobin fractions. HPLC is suitable for routine investigation of thalassemia, hemoglobinopathies and hemoglobin variants[15].

Normal hemoglobin types include hemoglobin A (about 95−98%), HbA2 (about 2−3.5%), Hb F (up to 2%). Hb F production usually falls to a low level within a year after birth. Hemoglobin variants include Hemoglobin E, Hemoglobin S, Hemoglobin D, Hemoglobin C, Hemoglobin lepore, etc.[16].

Materials and Methods

It was a cross-sectional study. Study period was three months, from September 2015 to December 2015. Specimens were collected from three major tribal groups Chakmas, Garos and Marmas. The samples were tested in Dhaka Shishu Hospital Thalassemia and DNA Laboratory.

An educational session was given regarding thalassemia and importance to know about carriers of thalassemia and other hemoglobinopathies. The educational session also highlighted that marriage between carriers should be discouraged to prevent thalassemia.

Sample collection and preparation: Two milliliters (2 mL) of venous blood was collected in a tube containing EDTA which were stored at 2–8 degrees Celsius and tested within three days. No preparation was required. HbA2/F calibrators and normal and abnormal controls were analyzed at the beginning of each run.

Variant Hemoglobin Testing System (Variant II Beta Thalassemia Short Program, Bio-Rad Laboratories Inc., Hercules, CA, USA) under the experimental conditions specified by the manufacturer[17].

Hb estimation of 437 specimens were done by colorimetric method using drubkins reagent. Peripheral blood films were also prepared and observed under microscope.

HPLC were done by BIO-RAD D-10TM Dual program for determination of different fractions of hemoglobin level. Beta Thalassemia carriers was diagnosed by their Hb A2 levels, which were usually above 4%. However, a notable proportion of carriers had values between 3.5 and 4%. The presence of Hb variants interferes with the quantification of Hb A2 on dedicated HPLC systems. Hb E interferes with HbA2 because of co-elution. The highest Hb A2 level expected in beta thalassemia trait is 9%; the presence of 25−30% “Hb A2” indicated

Hemoglobin E trait. On the other hand, an elevated Hb A2 level was typical in HbE homozygotes, while in Hb E beta thalassemia had increased Hb F values. 10% to 90% Hb F was diagnosed for thalassemia. Beta thalassemia major was diagnosed by elevated or normal Hb A2 level and increased Hb F values[2].

Blood films were reviewed to correlate with HPLC before giving the final diagnosis. Patients' age, sex, hemoglobin status and HPLC reports were recorded in excel sheets. Total 437 cases were examined in the current study. Data were analyzed by SPSS, version 23.0. Approval from Ethical Review Committee was obtained prior to the study. Patients having previous transfusion at least within four months were excluded from the study.

Results

In this study, total 437 respondents are examined for blood test. Male to female ratio was 1:1. The study included cases from 2 years to 82 years where mean age was 18.3±12.0. In clinical analysis Hb ranges from 8.5 to 19.0 gm/dL and 187 cases was found normochromic, 110 cases anisochromic, 140 cases had hypochromic blood films. Among them 38.2% were normal individuals, Hb E trait were 34.8%, hemoglobin E disease were 21.7% and beta thalassemia trait were 5.3% (Table I).

Table I: Demographic and clinical presentations of the cases (n=437)

Characteristics	Number	Percentage
Genders (M:F)	49.9:50.1	
Age (years)		
Mean±SD	18.3±12.0	
Minimum-Maximum	2−82	
S. Hemoglobin (gm/dL)		
Mean±SD	12.3±1.8	
Minimum−Maximum	8.5−19.0	
Types of peripheral blood film		
Normochromic	187	42.8
Anisochromic	110	25.2
Hypochromic	140	32.0
Diagnosis according to HPLC of hemoglobin		
Normal	167	38.2
Hb E Trait	152	34.8
Hemoglobin E disease	95	21.7
Beta Thalassemia trait	23	5.3

Among the HbE traits 47.36% were male and 52.64% were female, among Beta Thalassemia trait 60.9% male and 39.1% female and for HbE diseases male were 51.58% and 48.2% female. Mean Hb for HbE was 12.0±1.7 gm/dL, for HbE disease 12.2±1.8 gm/dL and for Beta Thalassemia trait 12.3±1.9 gm/dL. Among the Beta thalassemia traits and Hb E trait 2/3 of them were either anisochromic or hypochromic (Table II and III).

Table II: Distribution of subjects according to sex and different thalassemia traits

Variables	Normal (167)	Hb E trait (152)	Hb E disease (95)	Beta thalassemia trait (23)	p values
Gender					
Male (218)	88 (52.7)	72 (47.36)	49 (51.58)	14 (60.9)	0.289
Female (219)	79 (47.3)	80 (52.64)	46 (48.42)	9 (39.1)	
S. hemoglobin (gm/dL)					
Mean±SD	12.8±1.9	12.0±1.7	12.2±1.8	12.3±1.9	0.123
Minimum-Maximum	8.5−19.0	8.5−17.0	8.6−16.5	8.5−16.0	
Types of Peripheral Blood Film					
Normochromic	104 (62.3)	53 (34.86)	29 (30.52)	8 (34.8)	
Anisochromic	41 (24.6)	24 (15.78)	12 (12.63)	6 (26.1)	0.001
Hypochromic	22 (13.2)	75 (49.34)	54 (56.84)	9 (39.1)	

Table III: Distribution of peripheral blood films among different thalassemia trait cases

Variables Peripheral blood film	Diagnosis according to HPLC of h emoglobin				Total
	Normal Number(%)	Hb E trait Number(%)	Hb E disease Number(%)	Beta thalassemia trait Number(%)	
Anisochromic RBC with anisocytosis	4 (57.1)	2 (28.57)	1 (14.28)	0 (0.0)	7 (100.0)
Anisochromic RBC with anisocytosis with few elongated cells	14 (42.4)	9 (27.27)	7 (21.21)	3 (9.1)	33 (100.0)
Anisochromic RBC with anisocytosis with few elongated cells with target cell	3 (33.3)	3 (33.33)	3 (33.33)	0 (0.0)	9 (100.0)
Anisochromic RBC with anisocytosis with few target cells	15 (28.8)	20 (38.46)	14 (26.92)	3 (5.8)	52 (100.0)
Anisochromic RBC with Anisocytosis with poikilocytes	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)
Anisochromic RBC with anisocytosis with poikilocytes with target cells	3 (42.9)	2 (28.57)	2 (28.57)	0 (0.0)	7 (100.0)
Mildly hypochromic RBC with mild anisocytosis with few elongated cells	5 (83.3)	1 (16.7)	0 (0.0)	0 (0.0)	6 (100.0)
Mildly hypochromic RBC with mild anisocytosis with few elongated cells with target cells	9 (23.1)	12 (30.77)	13 (33.33)	5 (12.8)	39 (100.0)
Mildly hypochromic with mainly target cells	6 (6.7)	48 (53.93)	32 (35.95)	3 (3.4)	89 (100.0)
Mildly hypochromic with mild anisocytosis	2 (33.3)	1 (16.67)	2 (33.33)	1 (16.7)	6 (100.0)
Normochromic normocytic RBC	95 (57.6)	40 (24.24)	24 (14.54)	6 (3.6)	165 (100.0)
Normochromic normocytic RBC with few target cells	6 (31.6)	8 (42.10)	3 (15.78)	2 (10.5)	19 (100.0)
Normocytic normochromic with elongated cell	3 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100.0)

Discussion

Thalassemia is an emerging health problem in developing countries like Bangladesh. As a result of fall of deaths from infectious diseases and better nutrition, more children are now attending hospital with congenital disorders like thalassemia. In this study, we found 5.3% beta thalassemia trait 34.8% hemoglobin E trait and 21.7% hemoglobin E disease. This is the most common haemoglobinopathy in our country. Khan et al[5] reported in a study in 2005 that, expected birth of hemoglobin E beta thalassemia in Bangladesh is 6443 and beta thalassemia major is 1044 in every year.

This study showed that there was high frequency of hemoglobin E trait and hemoglobin E disease in the tribal groups; beta thalassemia trait was also little bit higher in comparison to the randomly selected general Bangladeshi population. Result of the study showed 34.8% were hemoglobin E trait and 21.7% homozygous for hemoglobin E disease. Cumulative percentage for hemoglobin E inheritance were 56.5%.

A similar study in Indian tribals of Tripura showed Hb E carriers (45.8%) and homozygous E (14.83%). Chakmas who previously resided in Bangladesh showed 8 out of 9 children with Hb E trait while the Jamatias also showed 8 carriers of Hb E out of 9 children[18]. Another study in the population of Northern Region of West Bengal reveals Hb E trait was most common (34.4%) followed by Hb E disease (25.3%)[19]. Hb E disorders (92.7%) were observed mostly among Rajbangsi population while E-β-thalassemias (40%) in the Muslims and a heterogeneous pattern noted among tribals and mongoloids[19]. Hb E is common in the north-east India. About 50% Assam population have Hb E and Tripura have 55% of Hb E which is similar in our tribal population[20]. A previous study on school children of different districts showed carrier status of Hb E trait is 6.1% in randomly selected general Bangladeshi population[5,21]. In the same study, a sample of 44 tribal children showed 22% Hb E trait in Chakmas[5].

In this study, hemoglobin was not significantly different ($p < 0.123$) in both beta thalassemia trait and hemoglobin E trait. But peripheral blood film was significantly different ($p < 0.001$) among normal individuals, beta thalassemia trait, hemoglobin E trait and hemoglobin E disease.

Peripheral blood film and hemoglobin status may give an idea about thalassemias and hemoglobinopathy carriers. In the suspected cases HPLC may be done to confirm the diagnosis. This study reveals that there was high frequency of Hb E trait and Hb E disease in the tribal population of Bangladesh and its coinheritance with beta thalassemia trait will give birth to significant number of thalassemic children in this population.

Acknowledgement

The study was funded by Bangladesh Medical Research Council. Dhaka Shishu Hospital Thalassemia and DNA lab supported to do HPLC and related laboratory works.

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