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Detection of haemoglobinopathies and thalassemias inanaemicpatients in a tertiary care hospital

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Abstract
The hereditary disorders of haemoglobin such as thalassemias and other haemoglobinopathies are the most common single gene disorder throughout the world with the highest frequency in the tropics, subtropics, Mediterranean basin and Southeast Asia. There is regional variation in the distribution status of carriers and diseases in our country. The clinical presentation of these haemoglobin disorders varies from asymptomatic to severe transfusion dependent anaemia. Prevention is more cost-effective than treating thalassemia patients and reduction of birth of babies with haemoglobin disorders ensure better health care.This cross-sectional study involved a total of 734 patients and blood samples were collected foranalysis in the laboratory for haemoglobin electrophoresis. Other haematological parameters were also estimated in the laboratory. Various types of abnormal haemoglobins were detected in 122 cases (16.62%). The distribution of haemoglobin disorders wereobserved as the most predominant beta thalassemia trait(46.72%),followed by haemoglobin E trait(25.4%), α-thalassemia trait(20.4%), haemoglobin E- β- thalassemia(1.64%) and 0.81% were β-thalassemia major.The overall prevalence of β-thalassemia trait was highest (7.76%), followed by haemoglobin E trait (4.2%), α-thalassemia trait (3.4%), haemoglobin E-β- thalassemia (0.27%), haemoglobin E disease (0.27%) and β-thalassemia major (0.13%).The average haemoglobin concentration of the patients having haemoglobin disorders was 8.5 gm/dl and the averagehaemoglobin of other participants in the study was 10.4gm/dl. Relatively lower haemoglobin concentration were found in β-thalassemia major, β-thalassemia trait, α-thalassemia trait and haemoglobin E-β-thalassemia.

Keywords: *Anaemia, thalassemia, haemoglobinopathies, carriers, Hb electrophoresis.*

I. Introduction

The hereditary disorders like thalassemia is a major health problem throughout the world and these are increasing in developing countries.[1] The death rate of children from diarrhoea, respiratory tract infection and malnutrition has been reduced due to improved health service and diet and consequently more children are visiting the hospitals.[2]Haemoglobinopathies are responsible for considerable economic and psychological burden on the affected individuals, families, society and country.[3]The clinical presentation of haemoglobin disorders varies from asymptomatic to severe transfusion dependent anaemiasandaccording to various estimates, approximately 7% of the world population are carriers of haemoglobin disorders leading to high morbidity and mortality.[4]Investigations to determine the genetic burden of haemoglobin disorders are the need of the hour and prevention is more cost effective than treating thalassemic patients and reduction of birth of thalassemic childrenby population screening, genetic counselling and prenatal diagnosis ensure better health care.[5,6]The incidence of thalassemia may be the major health problem along with other genetic disorders and the need for genetic services will arise very soon in Bangladesh.[7]The gender and age group are important risk factors of anaemia and haemoglobinopathies among the children. More than half of the under-five children of Bangladesh are suffering from anaemia due to different causes and there are only a few studies available mentioning epidemiology and clinical aspects of haemoglobin disorders.[8] Screening and diagnosis of haemoglobin disorders require a comprehensive evaluation of the combination of clinical and family history, blood counts, red blood cell indices and molecular analysis.[9] There is no documentary register of thalassemia and other carrier status of haemoglobinopathy in different regions of our country,[10] therefore, it is important to investigate the carrier status and prevalence of diseases by regional study to formulate the basis of future planning for the control of thalassemias.

II. Materials and Methods

This cross-sectional and descriptive study involved a total of 734 patients with anaemia suspected to have Hb disorders attending the Khulna City Medical College Hospital during the period from July 2021 to June 2022.The patients who refused to participate in the study and who had history of blood transfusion at least two months back were excluded from the study. The need of Hb electrophoresis arises wherever an individual complains of fatigue, pale skin or other symptoms of anaemia, jaundice or conditions causing yellow colour eye or skin, growth problems in children, family history of Hb disorders and ethnic background. After approval of the study protocol by ethical committee of the institute and obtaining informed consent of the participants with consideration of the inclusion and exclusion criteria,5 c.c blood sample was collected from each patient aseptically. The collected blood samples wereanti-coagulated with EDTA and then it was sent to the laboratory for Hb electrophoresis in cellulose acetate medium at alkaline PH, and HPLC was performed using theBio-Rad variant D-10 instrument.The reports with chromatogram were generated for each case where different peaks were identified in defined windows with relevant information such as retention time, relative percentage of different haemoglobin fractions with total areas,and other haematological parameters were also performed by Sysmex XN-1000 automated analyser. Other tests like sickling test using sodium metabisulphite, HbH inclusions (Golf balls) demonstration using brilliant cresyl blue stain and Hb F estimation by Singer's method were performed in the laboratory. An elaborative haematological parameters including haemoglobin concentration, RBC count, RBC indices, (MCV=mean corpuscular volume, MCH=mean corpuscular haemoglobin, MCHC=mean corpuscular haemoglobin concentration & RDW-CV= Red cell distribution wide-coefficient of variation) were performed with all the cases by a 7-part differential automated haematology analyser[coulter LH 750 analyser]. Anaemia was defined as haemoglobin concentration less than 11 g/dl. adjusted for altitude and stratified into mild, moderate and severe as per WHO guideline. The detailed information of the patients such as age, sex, caste, ethnicity and place of origin were recorded in a prescribed proforma reviewed by the ethical committee and a unique laboratory number for each patient was assigned to ensure confidentiality and kept in a secured room. Parenteral screening was done to confirm the diagnosis wherever feasible.All data obtained from observations of the study subjects and diagnoses by laboratory findings of each individual patients were recorded and summarized to present in charts and tables. All the patients were stratified in different age groups and detection rate of different types of Hb disorders were calculated and tabulated. The data were calculated and analysed by using computer generated software 'The statistical package for the social sciences' (SPSS) version 20.

Results and observations:

A total of 734 blood samples from the patients of age ranging from 2 years to 52 yearswere received in the department of pathology for haemoglobin electrophoresis during the study period and among those, 122(16.62%) patients had Hb disorders. The distribution of patients according to different age groups and gender are shown in Table-1.

Table-1; Distribution of the patients having hereditary Hb disorders in different age groups and gender, (n=122).

Sex	Age	B-Thalassemia trait	α-Thalassemia trait	Hb E - trait	others
Male	2 yrs-37 yrs	17	5	7	3
Female	3yrs-52 yrs	40	20	24	6
Total	122	57(46.72%)	25(20.49%)	31(25.4%)	9

Haemoglobin disorders were found in 32(26.22%) male patients of age ranging from 2 years to 37 years and in 90 (73.77%) female patients of age ranging from 3 years to 52 years. The most prevalent Hb disorder wasβ-Thalassemia trait 57(46.72%), haemoglobin E trait 31(25.4%) and α-Thalassemia trait 25(20.49%) followed by otherabnormalities (Table-1).The β- thalassemia trait was predominantly found in female patients.

Table-2: Spectrum of thalassemia and other haemoglobinopathies with overall prevalence.

SL No:	Type of haemoglobin disorder	No. of Patients	Hb disorders%,(n=122) (abnormal Hb)	(overall),(n=734) Prevalence %
1	Beta- Thalassemia major	01	0.81%	0.13%
2	Beta- Thalassemia trait	57	46.72%	7.76%
3	Hb E disease	02	1.64%	0.27%
4	Delta-Beta-Thalassemia	01	0.81%	0.13%
5	Hb E beta thalassemia	02	1.64%	0.27%
6	Alpha- thalassemia trait	25	20.49%	3.4%
7	Haemoglobin E trait	31	25.4%	4.22%
8	Elevated Hb F	03	2.46%	0.4%
9	Normal (other participants)	612	00%	83.38%

10	Total	734	100%	100%

Among the patients having haemoglobin disorder, the highest proportion was the β-Thalassemia trait (46.72%), followed by Hb E trait (25.4%), and α-Thalassemia trait (20.49%). Overall prevalence of β-thalassemia trait was highest (7.76%), followed by haemoglobin E trait (4.2%), α- thalassemia trait (3.4%), haemoglobin E-β-thalassemia (0.27%), haemoglobin-E disease (0.27%), β-thalassemia major (0.13%) and δβ-thalassemia (0.13%),(Table-2).

Table-3: Haematological parameters of patients having Hb disorders and normal patients, (n=734).

variable	Free from Hb disorder	β-Thalassemia major	β-Thalassemia trait	Hb-E Disease	Hb E-β-thalassemia	α-Thalassemia trait	Hb-E Trait	δ-β-Thalassemia
Hb(gm/dl)	10.4±2.96	4.8	9.19±1.26	9.8±2.64	8.3±2.54	6.64±1.56	10.7±1.84	10.4
Hb A(%)	97±1.52	13.6	94.13±0.56	70.82±2.5	61.4±6.82	97.61±0.21	71.52±1.37	81.54
Hb A2(%)	2.1±1.3	3.24	5.31±0.54	3.52±1.22	2.84±0.56	1.92±0.07	4.14±0.39	2.62
Hb F(%)	≤0.50	83.16	≤0.90	≤0.50	3.54±0.82	≤0.90	≤0.90	15.84
Hb E(%)	-	-	-	26.54±1.33	32.4±2.54	-	23.87±1.12	-
RBC(10^9/µl)	4.5±0.98	2.90	4.80±0.65	4.20±0.40	3.44±0.87	3.65±0.92	4.80±0.85	3.76
HCT(%)	37±7.25	18.6	29.24±2.14	28.2±1.24	23.4±5.08	30.4±1.56	35.6±5.63	32.4
MCV(fl)	77.5±12.4	64.2	61.53±5.99	64.2±3.44	64.8±1.87	78.5±6.45	74.5±7.60	74.5
MCH(pg)	23.6±6.2	18.5	18.84±1.58	24.52±1.84	21.8±2.13	25.2±2.85	24.2±4.56	25.4
MCHC(g/dl)	29.5±5.75	24.8	30.66±1.05	28.64±2.64	27.5±2.25	30.5±1.78	31.4±4.24	30.4

The mean haemoglobin concentration of the patients having Hb disorders was 8.5 gm/dl and the mean concentration of haemoglobin of the patients without Hb disorder was 10.4 gm/dl which was slightly higher (Table-3).The mean red blood cell count of patients with Hb disorders were 3.92 million/µL,(lower than 4.5/µL in patients without Hb disorder, $p<0.01$). The mean MCV of the patients having Hb disorder was 68.89 fl (lower than 77.5 fl in the patients without Hb disorder, $p<0.01$). The mean Hb A was 94.13% in β-Thalassemia trait and 71.52% in haemoglobin E trait. The mean Hb A2 was 5.31% in β-Thalassemia trait and 4.14% in haemoglobin E trait. There wereslight rise of Hb F in α-Thalassemia trait, β-thalassemia trait, and haemoglobin E trait andit was not elevated in haemoglobin E disease and in patients without Hb disorder.

III. Discussion

Aim of the study was to investigate the distribution of haemoglobin disorders among the patients withanaemia suspected to have haemoglobinopathies and thalassemias with their prevalence so that we might become aware of these genetic abnormalities. The overall prevalence of haemoglobin disorder in the present study was 16.62% (Table-1), proportionately the incidence of beta thalassemia trait was highest (46.72%), followed by haemoglobin E trait (25.4%) and α- thalassemia trait (20.4%).The overall prevalence of β-thalassemia trait was 7.76%, haemoglobin E trait was 4.2%, α-thalassemia trait was 3.4%, haemoglobin E-β-thalassemia(0.27%), haemoglobin E disease (0.27%) and only one case of β-thalassemia major was detected (0.13%), (Table-2). The overall detection rate of haemoglobin disorders was slightly lower than that of a similar hospital based study in Nepal which reported that 27.71% of anaemic patients had haemoglobin disorder of all ages.Among the different haemoglobin disorders, beta thalassemia trait was with the highest proportion of 37.11%, followed by Sickle cell trait(16.49%), beta thalassemia major (9.28%), alpha thalassemia trait (6.19%), and haemoglobin E trait(6.19%),[3]and other discrepancies were such as the higher prevalence of Sickle cell trait with beta thalassemia major in Nepal and the α-thalassemia trait with Haemoglobin E trait were relatively lower than those observed in our study. The prevalence of β-thalassemia major were relatively lower (only one case was detected)observed in our studyand there was no sickle cell haemoglobinopathy but some cases showed elevated fetal haemoglobin which were suspicious and these were not categorized with definite diagnosis.Aretrospective study in India carried out by Narang et al,[4] reported that detection rate of Hb disorders was 8.45% in all patients of specific age group, among those, distribution of disorders were 72.2% Beta Thalassemia trait, 17.8% Hb D, 1.2% Hb D Iran trait, 1.8% Beta thalassemia major, 0.6% Hb E, and 1.2% of Hb S. The most common variant of abnormal haemoglobin was thalassemia trait and overall detection rate was much lower as because of the study was based on screening programme among the population, on the other hand, in the present study, we paid attention to involve the clinically anaemic patients attending the hospital having symptoms of varying degree and seeking advice. For this reason the prevalence is higher and there was a sampling bias in the present study. We also considered the impact of the study on clinical suspicion of having a haemoglobin disorder so that our observation might influence on enthusiasm for consideration of preventive measure with active participation of families and society and thereby increase the awareness about the disease in the population. Khan et al,[10]reported on prevalence of thalassemia among the school children(n=735) in Bangladesh that 4.1% of beta-thalassemia trait and 6.1% of haemoglobin E trait.The proportion of Hb E trait was the highest 41.7% and followed by haemoglobin E disease in 6.3% of tribal children. The prevalence of β-thalassemia trait was variable in different divisions from 2.9% to 8.1%, the highest inBarisal division (8.1%)

and the lowest in Khulna (2.4%). The prevalence of HbE trait was variable from 2.4% to 16.5%, the highest prevalence in Rajshahi division (16.5%), and the lowest in Khulna division(2.4%).

Another report from central India mentioned that prevalence of Hb disorders was 14.8% among 527 pregnant women, the highest incidence was 9.1% of sickle cell trait followed by sickle cell disease (3%), beta thalassemia trait (2.3%), and haemoglobin E trait (0.4%). About 85% pregnant women were found free of abnormal haemoglobin. Overall, 56.4% of total pregnant women had anaemia, and also Hb S was higher than Hb D Punjab and Beta Thalassemia trait. The values of haematological indices such as Hb, RBC count, Haematocrit (HCT) were reduced and fetal haemoglobin were elevated in sickle cell disease than women without Hb disorders.[11]Overall detection rate of the present study was similar but contrasts were higherprevalence of haemoglobin E trait and absence of Sickle cell haemoglobinopathy (Table-2). A study carried out by Sheikh et al,[12] emphasized on demographic distribution in different regions of Pakistan which revealed that out of 10,297 samples, 997 cases(9.7%) had Hb disorders of different variants. The most common variant was beta thalassemia trait, the regional distribution were, 57.8% in Punjab, 36.1% in KPK and 5.2% in Baluchistan, sickle cell trait was 26% in Punjab, 44% in KPK and 30% in Baluchistan. It was a community based study and for this reason the overall detection rate was lower than that of present study. A study carried out by Li CK,[13] observed that the incidence of beta thalassemia carriers is high in the regions such as the Mediterranean, the Middle East, the Indian subcontinent, Southeast Asia, and South China.

Most of the studies are on the basis of prevalence rate of different variants of Hb disorders with their distribution, but there is unavailability of studies providing data related to various hemoglobinopathies in terms of incidence, morbidity, and mortality.A population-based study (n=50,487) in rural area of west Bengal revealed the carrier rate of beta-thalassemia and Hb E to be 6.61% and 2,78% respectively.[14]Bangladesh shares linguistic and socio-cultural commonalities with East Indian region particularly West Bengal and largely genetic makeup is closely related in this part. Despite different religious background, the prevalence of haemoglobin disorder in Bangladesh could be similar.[15] Another study (n=9990) estimated the frequency of 3.64% for beta-thalassemia trait and 3.92% for Hb E trait.[16] and this was also a community based study and showed much lower incidence of Haemoglobin disorders than our observation. Hossain et al,[17] mentioned that despite of high prevalence of malaria, contrary to the certain part of Africa and India, the sickle cell anaemia is non-existent in Bangladesh. Although there is a strong association of haemoglobin S with Malaria, but this was found in Africa, not in Asia and America. Hb S was found in the western part of India particularly hilly areas and it was assumed that Hb S might be misdiagnosed as Hb D beta thalassemia.

The present study revealed that among the haematological parameters, Hb concentration, Total RBC count and MCV were 8.5gm/dl, 3.92 Million/µl and 68.89fl respectively, which were lower than those found in the patients without Hb disorder, (p=0.005). The fractions of Hb A were reduced in all the varieties of Hb disorders, and Hb A2 was increased in β-thalassemia trait and haemoglobin-E trait. There was mild increased Hb F in haemoglobin-E-β-thalassemia and α-thalassemia trait. The haemoglobin F was not elevated in Hb E disease and Hb E trait. A study to determine the reference values of haematological parameters and Hb analysis involving 224 subjects carried out by sari DP et al,[18] in Indonesia revealed that the cutoff point of MCH for β-thalassemia trait was 20.5pg (Sensitivity 85%, Specificity-90%) and that of MCV was 66.8fl (Sensitivity-87%, Specificity-87% p=0.005) which were apparently comparable to that of present study.The average haemoglobin concentration was ≤12.3 gm/dl (higher than that of present study). The haemoglobin A2 was ≥4.65%, and Hb F was ≥0.35%,(95% CI) which were comparable to that of the present study.

Bangladesh is one of the most densely populated countries in the world with a population over 160 million and over 70% of the population live in rural areas where health facilities are not available and most tertiary hospital providing diagnostic facilities are located in the big cities.[19,20]Despite the fact that Bangladesh is in the world's thalassemic belt but the information on different aspects such as its epidemiology, clinical course, mortality, complications and treatment outcomes of thalassemias is lacking. A study carried out by Rahman et al,[21] revealed that the highest prevalence of anaemia in Bangladesh is not associated with iron deficiency, the nationwide prevalence of anaemia which is 33.1% in children under five years of age and 26% in women are more than three times higher than that of iron deficiency in children (10.7%) and women (7.1%), which suggests that other determining factors which might be congenital haemoglobin disorder along with micronutrients such as dietary iron, vitamin A, folate and Zn deficiency. The patients having thalassemia should know that their disease is not due to iron deficiency and that iron supplement will not cure the anaemia, rather it will lead to more iron load if they have already receiving blood transfusion.[22]The present study has been devoted to depict the prevalence of different varieties of haemoglobin disorder and their distribution on regional basis to increase the awareness to prevent these genetic abnormalities.

IV. Conclusion

β-thalassemiatrait was the most prevailing haemoglobin disorder followed by haemoglobin E traitand these were found predominantly in females of age ranging from 3 years to 52 years. There was no sickle cell

haemoglobinopathy in the study population. The anaemic patients showed morphologically microcytic hypochromic features in their blood film.Relatively lowerconcentration of haemoglobin were found in beta thalassemia major, alpha thalassemia trait, beta thalassemia trait and haemoglobin-E-β-thalassemia. The population can be screened so that the carriers be detected and preventive measures could also be adopted in the form of genetic counselling and prenatal diagnosis.

What does this study add to existing knowledge: The most prevalent haemoglobin disorder was β-thalassemia trait and haemoglobin E trait. There was no sickle cell haemoglobinopathy detected in the study subjects.

Ethical clearance

The research protocol for this study and informed consent form were reviewed and approved by the ethical committee of Khulna City Medical College. The written informed consent was then obtained from each participant and a unique identification number was assigned and all records were kept in a secured room to ensure confidentiality.

Conflict of interest: None.

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