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Pattern of thalassemia and hemoglobinopathies among anemic under-five children of Northern Bangladesh: a hospital based cross sectional study

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ABSTRACT

Background: *Congenital hemoglobin defects are significant global health concern affecting more than 330,000 newborns every year. In Bangladesh, more than half of the under-five children are anemic, but the contribution of hemoglobin disorders to childhood anemia is quite unexplored. Therefore, the objective of the present study was to investigate the prevalence and pattern of hemoglobinopathies among anemic under-five (age 6-59 months) children visiting the hematology department of Rajshahi Medical College Hospital (RMCH).*

Methods: *This was a retrospective cross-sectional study among anemic patients aged between 6 to 59 months conducted in the hematology department of RMCH from July 2018 to June 2020. The guideline of WHO was used to define the severity of anemia. Hemoglobinopathies was measured using standard methods. Chi-square test was used to find the association between two categorical factors.*

Results: *We found that female and younger children were more likely to get moderate to severe anemic compared to their counterparts. Out of 534 patients 183 were suffering from different types of hemoglobinopathies (prevalence 34.3%). E-β-Thalassemia was the most common type of hemoglobinopathy (14%) followed by Hemoglobin E trait (11%) and β-Thalassemia minor (5.6%). Prevalence of these hemoglobin disorders was comparatively higher among female and older children ($p<0.05$). Patients with E-β-Thalassemia and β-Thalassemia major of our study were mostly suffering from severe form of anemia while patients with hemoglobin E trait and β-Thalassemia minor were mostly suffering from mild anemia ($p<0.01$).*

Conclusions: *A remarkable number of children were suffering from severe anemia and different types of hemoglobinopathies. Gender and age group were the risk factors of anemia and hemoglobinopathies among under-five children. Health authorities of Bangladesh should especially take care of anemic children in this country.*

Keywords: *Hemoglobinopathy; Thalassemia; Under-five children; Anemia; Bangladesh*

INTRODUCTION

Congenital hemoglobin defects are significant global health concern. Every year more than 330,000 babies are born with a clinically significant hemoglobin disorders which is accountable for almost 3.4% of under-five deaths all over the world (Modell & Darlison, 2008). It is reported that over 360 million people (5.2% of the world population) carry a clinically significant hemoglobin variant. Among these variants, inherited beta thalassemia, sickle cell disease and hemoglobin E disorders are the most common single gene disorders (Modell & Darlison, 2008; Weatherall, 2010). These hemoglobinopathies are most prevalent in African, Mediterranean, Middle Eastern, and Southeast Asian countries (Weatherall, 2010).

The epidemiological pattern of hemoglobinopathies varies widely in different regions of the world. According to evidences, beta thalassemia and hemoglobin E disorders are more commonly found in the south-east Asian countries including Bangladesh (Olivieri et al., 2011; Hossain et al., 2017). There are only a few studies reporting the epidemiology and clinical aspects of hemoglobinopathies in Bangladesh. A study has indicated that about 28% of assessed rural women were sufferers or carriers of beta thalassemia or hemoglobin E disorders (Merrill et al., 2012) while another population based study reported that 11.89% of the adult population had β-globin gene mutations (Noor et al., 2020). Among healthy school children the prevalence of beta-thalassemia trait was reported as 4.1% and hemoglobin E trait as 6.1% (Khan et al., 2005). However, most of these studies were conducted among healthy population and so, the percentage of the hemoglobinopathies among the anemic patients are quite unclear. A study conducted in this context among the anemic patients reported that the most common form of hemoglobin disorder is β-thalassemia minor (21.3%) along with E-β-Thalassemia and hemoglobin E trait. Other forms of hemoglobin disorders are hemoglobin E disease, hemoglobin D/S trait, β-thalassemia major, and δ-β-thalassemia (Uddin et al., 2012). More than half of the under-five children of Bangladesh (52.1%) suffers from anemia due to different causes (Yusuf et al., 2019). The contribution of congenital hemoglobinopathies to this disease burden is still unexplored.

Therefore, the aim of the present study was to investigate the prevalence and pattern of hemoglobinopathies among anemic under-five (age 6-59 months) children visiting the hematology department of Rajshahi Medical College Hospital.

METHODS

Setting and participants: This was a retrospective cross sectional study conducted in the hematology department of RMCH from July 2018 to June 2020. All the recorded data of the patients aged between 6 to 59 months visiting both outpatient and inpatient of this department during this period with the diagnosis of anemia were included in the study. Anemic patients suffering from malignancy were excluded.

Definition of anemia: Anemia was defined as a haemoglobin concentration less than 11 g/dL, adjusted for altitude and classified as mild if Hb was between 10 and 10.9 mg/dl, moderate if between 9.9 and 7 g/dl,

and severe if <7 g/dl as per the WHO guideline (https://www.who.int/data/gho/indicator-metadata-registry/imr-details/4801; https://pubmed.ncbi.nlm.nih.gov/16817681/).

Data collection procedure: 0.5 to 2 ml intravenous blood samples were collected according to age and body weight after obtaining informed consent of the parents of the child using EDTA as anticoagulants by disposable syringes and needles from each individual free of blood transfusions. The Sysmex XE-2100 system Hematology analyzer (Sysmex Corporation, Kobe, Japan) was used to determine peripheral cell count and red blood cell indices using standard procedure. Hemoglobin electrophoresis of the collected blood sample was carried out on agarose gel using the Hydragel K20 System (Sebia, Issy-les-Moulineaux, France). The resulting electropherograms were evaluated visually for pattern abnormality. Scanning densitometry was used to determine the relative concentration of individual hemoglobin fraction. Results were interpreted according to manufacturer's recommendations, where normal phenotype had between 96.8-97.8% of hemoglobin A, 2.2-3.2% of hemoglobin A2 and less than 0.5% of hemoglobin F.

Statistical Analysis: All the statistical analysis was carried out using SPSS version 25.0. Frequency distribution with percentage was used for nominal variables and mean with SD was used for continuous variables. Chi-square test or Likelihood ratio test was selected to find the association between two categorical variables. We used Chi-square test if the frequency of each cell in cross table was greater or equal to 5, otherwise we used Likelihood ratio in Chi-square test. A value of $p<0.05$ was considered as statistically significant in the analysis.

RESULTS

Table 1: Demographic variable and anemia status of the patients (n=534)

Variable	Group	N (%)
Age (months)	6-11	159(29.8)
	12-59	375(70.2)
Sex	Female	267(50.0)
	Male	267(50.0)
Anemia	Mild (10-10.9 g/dl)	192(36.0)
	Moderate (7-9.9 g/dl)	201(37.6)
	Severe (<7 g/dl)	141(26.4)

Patients' demographics and anemia status: A total of 534 anemic children were included in the study, 30% aged below 12 months and half of them were female. Among the participants 26% were suffering from severe anemia while others were suffering from mild to moderate anemia (Table 1).

It was noted that female children had more number of severe anemic (30.7%) than male children (20.6%) while male had more number of moderate anemic (44.6%) than female (32.6%). The Chi-square test demonstrated that the association between gender and anemia status of children was statistical significant ($p<0.01$). Younger children (age, 6-11 month) had more number of severe (32.7%) and moderate (41.5%) anemic than older children, the association between these two factors was significant ($p<0.01$) (Table 2).

Table 2: Association between gender, age group and anemia status of the patients (n=534)

Variable	**Group**	**Anemia status of children**			**Chi-square value**	**p-value**
		Mild, N(%)	Moderate, N(%)	Severe, N(%)		
Gender	Female	98(36.7)	87(32.6)	82(30.7)	10.423	0.005
	Male	93(34.8)	119(44.6)	55(20.6)		
Age (month)	6-11	41(25.8)	66(41.5)	52(32.7)	11.197	0.004
	12-59	150(40.0)	140(37.3)	85(22.7)		

Table 3: Pattern of hemoglobinopathies among the patients, and association with gender and age (n=534)

Hemoglobinopathy	N (%)	Age		Sex	
		6-11 months	12-59 months	Female	Male
α-Thalassemia	3(0.6)	3 (1.9)	0 (0.0)	3 (1.1)	0 (0.0)
β-Thalassemia major	3 (0.6)	0 (0.0)	3 (0.8)	0 (0.0)	3 (1.1)
β-Thalassemia trait	30 (5.6)	6 (3.8)	24 (6.4)	15 (5.6)	15 (5.6)
E-β-Thalassemia	75 (14.0)	24 (15.1)	51 (13.6)	39 (14.6)	36 (13.5)
E-β-Thalassemia trait	3 (0.6)	0 (0.0)	3 (0.8)	3 (1.1)	0 (0.0)
Hemoglobin E disease	9 (1.7)	0 (0.0)	9 (2.4)	3 (1.1)	6 (2.2)
Hemoglobin E trait	60 (11.2)	12 (7.5)	48 (12.8)	30 (11.2)	30 (11.2)
No hemoglobinopathy	351 (65.7)	114 (71.7)	237 (63.2)	174 (65.2)	177 (66.3)
Likelihood ratio in Chi-square test		Value=23.696, p-value=0.001		Value=13.642, p-value=0.045	

Out of 534 included patients 183 were suffering from different types of hemoglobinopathies (prevalence 34.3%). These disorders had greater contribution in causation of anemia among older children aged between 12 to 59 months (36.8%) compared to younger children aged between 6 to 11 months (28.3%). Beside this, hemoglobinopathies were more prevalent among female anemic children (34.8%) compared to their male

counterpart (33.7%). Likelihood ratio in Chi-square test demonstrated that both of these associations were statistically significant ($p<0.01$). E-β-Thalassemia was the most common type of hemoglobinopathy (14%) followed by Hemoglobin E trait (11%) and β-Thalassemia minor (5.6%). Prevalence of these hemoglobinopathies in different age and sex groups were different (Table 3).

Patients with E-β-Thalassemia were suffering from most severe form of anemia (mean Hb 6.5 g/dL) followed by β-Thalassemia major (mean Hb 7.3 g/dL) and Hemoglobin E disease major (mean Hb 7.9 g/dL). Patients with Hemoglobin E trait and β-Thalassemia minor were mostly suffering from mild anemia (mean Hb 9.6 and 9.3 g/dL respectively). The association between hemoglobinopathy and anemia status of children was significant ($p<0.01$) (Table 4).

Table 4: Anemia status in different hemoglobinopathies, and association between these two factors among the patients (n=534)

Hemoglobinopathy	Hb, mean (SD)	Mild	Moderate	Severe
α-Thalassemia	8.2 (1.9)	1 (33.3)	1 (33.3)	1 (33.3)
β-Thalassemia major	7.3 (1.4)	0 (0.0)	2 (66.7)	1 (33.3)
β-Thalassemia trait	9.3 (1.6)	10 (33.3)	17 (56.7)	3 (10.0)
E-β-Thalassemia	6.5 (2.1)	6 (8.0)	21 (28.0)	48 (64.0)
E-β-Thalassemia trait	8.5 (1.5)	1 (33.3)	1 (33.3)	1 (33.3)
Hemoglobin E disease	7.9 (1.8)	3 (33.3)	6 (66.7)	0 (0.0)
Hemoglobin E trait	9.6 (1.4)	39 (65.0)	18 (30.0)	3 (5.0)
No hemoglobinopathy	8.8 (2.1)	132 (37.6)	141 (40.2)	78 (22.2)
Likelihood ratio in Chi-square test		Value=109.417, p-value=0.001		

DISCUSSION

Our study provides baseline information about the prevalence and pattern of hemoglobinopathies among under-five anemic children of northern Bangladesh. The overall prevalence of hemoglobinopathies among them was 34.3%. The prevalence could not be compared with other studies from Bangladesh due to lack of evidences. However, a study conducted among anemic patients of all ages in a hospital of Dhaka reported that 57.8% anemic patients had hemoglobin disorders with an equal incidence in both males and females (Uddin et al., 2012), though we found that prevalence of hemoglobinopathies was slightly higher among female children of our included study population. An outpatient based study from neighboring India reported that 22% of the anemic children had some sort of hemoglobinopathies (Konar et al., 2018).

E-β-Thalassemia was the most common type of hemoglobinopathy (14%) followed by Hemoglobin E trait (11%) and β-Thalassemia minor (5.6%). Similar pattern of hemoglobinopathies were found in studies from this region. For example, among school-going children of Bangladesh β-thalassemia trait and hemoglobin E trait were reported as most frequent hemoglobin variants (Khan et al., 2005). Another study including thalassemia affected children in a referral center of Dhaka also reported that E-β Thalassemia, β Thalassemia and hemoglobin E disease were commonest hemoglobin disorders (Tahura, 2017). Among the adult anemic patients the most common form of hemoglobin disorder was β-thalassemia minor, E-β-Thalassemia and hemoglobin E trait. Other minor hemoglobin disorders were hemoglobin E disease, hemoglobin D/S trait, β-thalassemia major, and δ-β-thalassemia (Uddin et al., 2012). In West Bangal, the neighboring state of India, E-β-Thalassemia, hemoglobin E trait and β-Thalassemia were most prevalent hemoglobinopathies (Konar et al., 2018).

Patients with E-β-Thalassemia and β-Thalassemia major of our study were mostly suffering from severe form of anemia while patients with hemoglobin E trait and β-Thalassemia minor were mostly suffering from mild anemia. As E-β-Thalassemia and β-Thalassemia major are homozygotic disorders of β-chain of hemoglobin, production of hemoglobin is extremely low, resulting in severe anemia. On the other hand, the patients suffering from hemoglobin E trait and β-Thalassemia minor inherit one gene of β-thalassemia, they are manifested as either asymptomatic or mild to moderate anemia. Similar pattern of anemia was reported among thalassemia patients of Bangladesh in different studies (Tahura , 2017; Karim et al., 2016; Khan et al., 2005).

Strength and Limitations of the study

The present study provides a bird's eye view about the pattern of thalassemia and other hemoglobinopathies among the anemic under-five children attending the study site. Despite the fact, a number of limitations would be worth mentioning. Firstly, the study was a facility based study that included only the anemic children visiting the study center, mostly for treatment of anemia. As a result, the anemia status and prevalence of hemoglobinopathies do not represent the community picture. Moreover, detailed socio-demographic, clinical and laboratory parameter data of the patients was not available. Further community based studies including detailed socio-demographic and clinical factors is suggested for the better understanding of the epidemiology of hemoglobinopathies in the respective region.

Conclusions

Thalassemia and other hemoglobinopathies are some of the major causes of anemia among under-five children of Bangladesh. Our study found that more than one-third of the under-five anemic children are suffering from some sort of hemoglobin disorders. E-β-Thalassemia, hemoglobin E trait and β-thalassemia minor were the most common type of hemoglobinopathies in the study site. Further exploration in the

epidemiology and risk factors of hemoglobinopathies in this region will guide evidence-based action plan to decrease the disease burden.

List of abbreviations

EDTA: Ethylenediaminetetraacetic acid; Hb: haemoglobin; SD: Standard deviation; SPSS: Statistical Package for the Social Science Software; WHO: World Health Organization.

Consent for publication

Not applicable.

Availability of data and materials

Aggregate data may be made available through a formal request to the corresponding author.

Conflicts of interest

The authors declare that they have no competing interest.

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Ethics statement

The study was conducted in accordance with the Declaration of Helsinki of involving human participants. The ethical approval of the study was obtained from the ethical review committee of Rajshahi Medical College, Rajshahi-6000, Bangladesh (Ref: RMC/ERC/2017-2019/198/178). All authors provided consent for publication. As study subjects were children, informed written consent was taken from the parent of the children before collecting the blood sample.

Author Contributions

Concept, design: MAR. Data acquisition: MZM. Data analysis: MAR, MGH. Data interpretation: MAR, MZM, MGH. Primary draft: MAR, MZM. Critical appraisal and important intellectual content: MZM, MGH. All authors read and approved the manuscript.

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