Genetic Mutation of Hb E/beta Thalassemia Patient in Bangladesh and Its Relation With Clinical Severity

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Abstract

Background: Hemoglobin E/β-thalassemia is a common inherited hemoglobin disorder among South Asian countries. The phenotypically diverse presentation of the disease is often attributed to coinheritance of β-globin (*HBB*) gene mutations. The current study described the phenotype and genetic basis of Hb E/β-thalassemia patients and assessed its relation with clinical severity.

Methods: A total of 32 patients were included in this cross-sectional study. Cases were confirmed by using capillary hemoglobin electrophoresis or high-performance liquid chromatography. Those with positive findings were further analyzed with clinical information and ancestral data either from the interview or medical records. Data collection was confined to May 2019 and July 2020. Gene sequencing was performed using Sanger's sequencing method for mutational analysis, and Mahidol scoring was used to grade clinical severity.

Result: A total of 13 heterozygous mutations were identified in the *HBB* gene. Of all, IVS-1-5 (G>C) (n=17, 53.1%) was the most common, and codon 30 (G>C) (n=4, 12.5%) was the second most common mutations. According to the Mahidol scoring system, 37.5% (n=12) were classified as phenotypically mild, 43.8% (n=14) as moderate and 18.8% (n=6) as severe. The IVS-1-5(G>C) mutation was found to be frequently associated with severe disease and showed no mild form.

Conclusion: The present study described the clinical severity and its association with genetic mutations in hemoglobin E/β-thalassemia patients. This finding could guide individually tailored management strategies for this particular group of patients.

Background

Thalassemia is one of the most common hemoglobin disorders worldwide. Each year, over 332,000 conceptions are affected, with 56,000 having clinically significant hemoglobinopathy [1]. One or more globin chains of the hemoglobin (Hb) tetramer are reduced or absent in this autosomal recessive disease, resulting in impaired production of hemoglobin [2]. Based on the globin chain involved, thalassemia is mainly classified as alpha (α) and beta (β) thalassemia. The beta form of the disease is most prevalent, affecting approximately 1.5% of the global population [3]. South Asia is a hotspot of β-thalassemia, and Hb E is the most prevalent variant in this region, with an estimated 30 million carriers and 1 million affected (homozygous) mutants [3]. In Bangladesh, 11.89% of people carry β-globin gene mutations, among whom 8.68% have Hb E traits, and 2.24% have beta-thalassemia traits [4].

Hb E and β-thalassemia alleles are frequently co-inherited in the South Asian population because of the high prevalence of both mutations in this region. Although Hb E itself produces a mild form of anemia, its interaction with various forms β-thalassemia mutations may produce a wide range of clinical syndromes [5]. The compound heterozygote Hb E/β-thalassemia frequently occurs in India, Bangladesh, and throughout Southeast Asian countries [6]. Hb E/β-thalassemia produces noticeable phenotypic plasticity ranging from mild to severe disease [6]. Many genetic and environmental factors have been implicated in

the clinical diversity of this disease. These include the type of β-thalassemia mutation coinherited with Hb E, the coinheritance of modifier mutations of hemoglobin F (HbF), age-related adaptive changes, the presence of malaria etc. [6, 7].

Despite a high prevalence of thalassemia in Bangladesh [4], there is a dearth of literature on the clinical and molecular characteristics of the disease in the country. Although several studies attempted to describe the clinical and mutational patterns of β-thalassemia [8–10], none of them characterized the severity of the disease. One study classified β-thalassemia intermedia patients according to the Mahidol scoring system, a scoring system to classify thalassemia into three grades of severity [11]. However, few attempts have been made to characterize only Hb E/β-thalassemia patients. This study aimed to explore the clinical pattern of Hb E/β-thalassemia patients and their association with accompanying β-globin gene (*HBB)* mutations in a tertiary care center of the country.

Materials And Methods

Study place and population: This was a cross-sectional study conducted in the Department of Hematology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, throughout May 2019 and July 2020. Diagnosed patients with Hb E/β-thalassemia (confirmed by capillary hemoglobin electrophoresis or high-performance liquid chromatography) coming to the outpatients of the hematology department were consecutively selected for inclusion. Patients with concomitant chronic disease (e.g., SLE, RA CLD) that might interfere with the severity of thalassemia were excluded.

Measures: Data were collected by two trained physicians using a semi-structured questionnaire consisting of two parts: a. demographic and clinical features and b. laboratory investigations, including genetic analysis.

Clinical features and severity scoring

Several scoring systems had been developed to classify the clinicopathological heterogeneity of thalassemia [12]. We used the Mahidol scoring system developed by Srichipai and colleagues from Mahidol University, Thailand [13], which is one of the earlier clinical severity scoring systems developed for thalassemia patients. It is a comprehensive scoring tool that takes into account the steady-state hemoglobin level, age of starting transfusions, number of transfusions required, spleen size, age at first presentation, and growth retardation of patients. A score of 0 – <4 is defined as mild, 4–7 as moderate and > 7– 10 as severe disease [14]. The details of the scoring are available in Supplementary Table 1. Transfusion dependency was defined as regular requirement of transfusion for survival [14, 15]. Growth was assessed using chart CDC growth charts [16]. Growth retardation was defined as weight for age and/or height for age below the 3rd percentile. Steady-state hemoglobin was calculated as the average hemoglobin level from previous records before receiving blood transfusion [13].
Laboratory investigations

Patients were investigated for hematological profile, capillary hemoglobin electrophoresis, and gene sequencing for DNA analysis and mutation detection by Sanger sequencing. A complete blood count (CBC) was carried out using an automated hematology analyzer (Pentra ABX-120 DX), hemoglobin electrophoresis was performed using a Sebia capillary electrophoresis system, and gene sequencing was conducted in a genetic analyzer machine (3500 Genetic Analyzer, CE, IVD, Applied Biosystems, USA).

Statistical analysis: All data were checked and entered into the statistical software SPSS version 26 (IBM©, Chicago, USA) for analysis. Categorical variables were expressed as frequencies (percentages), and continuous variables were expressed as the mean ± standard deviation or median (interquartile range) where appropriate. Bivariate analysis was conducted using Fisher's exact test. A p-value of < 0.05 was considered significant.

Ethical measures: Ethical clearance for the study was taken from the Institutional Review Board (IRB) and ethical review committee of BSMMU (No.BSMMU/2019/7579). All the study procedures were conducted in line with the ethical principles laid out by the Declaration of Helsinki. Informed written consent was taken from the patients or their guardians if the patient was a minor before inclusion.

Results

A total of 32 confirmed cases of Hb E/β-thalassemia were included in the study. According to the Mahidol scoring system, 37.5% (n = 12) were classified as phenotypically mild, 43.8% (n = 14) as moderate and 18.8% (n = 6) as severe (Fig. 1).

The median age of these patients was 20 years (IQR: 17–22.5). Confirmation of the diagnosis and first blood transfusion was reported at a median age of 9.5 (IQR: 5–19.5) and 11 (IQR 5–16) years, respectively. Patients diagnosed at a younger age commenced earlier first blood transfusion. A greater frequency of blood transfusions was associated with severe disease assessed by Mahidol criteria. Overall, 59.4% were male, and the proportion was higher in the moderate severity group. Only four patients (12.5%) had consanguinity among parents. However, 34.4% of patients were transfusion-dependent; and they transfused a median of 9.5 (IQR: 2.3–43.5) units of blood. The overall requirement of transfusion is higher according to the degree of severity. Fifteen patients had completed growth. The average steady-state hemoglobin was 7.6 ± 1.4 g/dl (Table 1).

Table 1
Demographic and clinical findings according to disease severity (n = 32)

Variable	Total	Severity of Hb E/β-thalassemia		
		Mild	Moderate	Severe
Age (years)	20 (17–22.5)	20 (14.5–23)	19.5 (17–21.3)	22 (17.3–33.3)
Age at diagnosis (years)	9.5 (5–19.5)	15 (12–22.8)	6 (5.8–19.3)	3 (2–5.3)
Age at which transfusion started (years)	11 (5–16)	15 (13–19.5)	8.5 (5.8–19.0)	3.5 (2.8–8)
Weight (kg)	45.9 ± 14.1	53.3 ± 15.3	42.1 ± 11.3	40 ± 13.4
Height (cm)	146.8 ± 24.5	153.7 ± 23.6	143.6 ± 26.1	140.3 ± 22.9
Sex				
Male	19 (59.4)	6 (50.0)	10 (71.4)	3 (50.0)
Female	13 (40.6)	6 (50.0)	4 (28.6)	3 (50.0)
Blood group				
A positive	6 (18.8)	5 (41.7)	1 (7.1)	0
B positive	12 (37.5)	1 (8.3)	8 (57.1)	3 (50.0)
O positive	7 (21.9)	1 (8.3)	3 (21.4)	3 (50.0)
AB positive	7 (21.9)	5 (41.7)	2 (14.3)	0
Consanguineous parents				
Present	4 (12.5)	2 (16.7)	1 (7.1)	1 (16.7)
Absent	28 (87.5)	10 (83.3)	13 (92.9)	5 (83.3)
Transfusion dependency*				
Transfusion dependent	11 (34.4)	1 (8.3)	5 (35.7)	5 (83.3)
Non transfusion dependent	21 (65.6)	11 (91.7)	9 (64.3)	1 (16.7)

Data are expressed as the median (IQR), mean ± SD and n (%) where appropriate.

* Transfusion dependency is defined as regular requirement of blood transfusion for survival

**Growth retardation is defined as weight for age and/or height for age below 3rd percentile

***Steady-state hemoglobin was calculated as the average hemoglobin level from previous records before blood transfusion.

Variable	Total	Severity of Hb E/β-thalassemia		
Number of transfusions required last year	3 (1–9)	0.5 (0–1.8)	4 (2.5–9.0)	14 (9–18.5)
Number of transfusions required in lifetime	9.5 (2.3–43.5)	1.5 (0–6.5)	23.5 (5.0–40.5)	175 (54.3–317.5)
Growth Retardation**				
Present	17 (53.1)	1 (8.3)	10 (71.4)	6 (100)
Absent	15 (46.9)	11 (91.7)	4 (28.6)	0
Hepatomegaly				
Present	17 (53.1)	2 (16.7)	9 (64.3)	6 (100)
Absent	15 (46.9)	10 (83.3)	5 (35.7)	0
Splenomegaly				
Present	28 (93.3)	11 (91.7)	12 (92.3)	5 (100)
Absent	2 (6.7)	1 (8.3)	1 (7.7)	0
Splenectomy				
Done	2 (6.3)	0	1 (7.1)	1 (16.7)
Not done	30 (93.8)	12 (100)	13 (92.9)	5 (83.3)
Steady State Hemoglobin (g/dl)***	7.6 ± 1.4	8.8 ± 1.0	7.2 ± 1.1	6.2 ± 0.8
Data are expressed as the median (IQR), mean ± SD and n (%) where appropriate.				
* Transfusion dependency is defined as regular requirement of blood transfusion for survival				
**Growth retardation is defined as weight for age and/or height for age below 3rd percentile				
***Steady-state hemoglobin was calculated as the average hemoglobin level from previous records before blood transfusion.				

Table 2 shows the clinical presentation of patients according to the Mahidol scoring system. The majority of patients had steady-state hemoglobin > 7.5 g/dl (53.1%), age at receiving first blood transfusion > 10 years (56.3%), occasional blood transfusion requirement (53.1%), spleen size > 10 cm (37.5%), age at thalassemia presentation > 10 and between 2–10 years (46.9% each), and growth and development < 3rd percentile (50.0%).

Table 2
Clinical presentation of patients according to the Mahidol Scoring System

Mahidol Criteria	Score	Total	Mild	Moderate	Severe
		n (%)	n (%)	n (%)	n (%)
Steady state Hemoglobin (g/dl)					
> 7	0	17 (53.1)	11 (91.7)	6 (42.9)	0
6–7	1	14 (43.8)	1 (8.3)	8 (57.1)	5 (83.3)
< 6	2	1 (3.1)	0	0	1 (16.7)
Age at receiving first blood transfusion (year)					
> 10	0	18 (56.3)	11 (91.7)	6 (42.9)	1 (16.7)
4–10	1	9 (28.1)	0	7 (50.0)	2 (33.3)
< 4	2	5 (15.6)	1 (8.3)	1 (7.1)	3 (50.0)
Requirement for blood transfusion					
None/rare	0	4 (12.5)	4 (33.3)	0	0
Occasionally	1	17 (53.1)	8 (66.7)	8 (57.1)	1 (16.7)
Regularly	2	11 (34.4)	0	6 (42.9)	5 (83.3)
Size of spleen (cm)					
< 4	0	9 (28.1)	5 (41.7)	3 (21.4)	1 (16.7)
4–10	1	11 (34.4)	6 (50.0)	4 (28.6)	1 (16.7)
> 10	2	12 (37.5)	1 (8.3)	7 (50.0)	4 (66.7)
Age at thalassemia presentation (year)					
> 10	0	15 (46.9)	10 (83.3)	5 (35.7)	0
2–10	0.5	15 (46.9)	1 (8.3)	8 (57.1)	6 (100.0)

*Percentile of growth development was assessed based on weight and height measurements plotted on a Centers for Disease Control & Prevention (CDC) standard growth chart.

Mahidol Criteria	Score	Total	Mild	Moderate	Severe
< 2	1	2 (6.3)	1 (8.3)	1 (7.1)	0
Growth and development*					
> 25th percentile	0	10 (31.3)	9 (75.0)	1 (7.1)	0
3rd to 25th percentile	0.5	6 (18.8)	2 (16.7)	4 (28.6)	0
< 3rd percentile	1	16 (50.0)	1 (8.3)	9 (64.3)	6 (100.0)
*Percentile of growth development was assessed based on weight and height measurements plotted on a Centers for Disease Control & Prevention (CDC) standard growth chart.					

In total, 13 different heterozygous beta-globin chain mutations were identified. Point mutation IVS-1-5 (G > C) (n = 17, 53.1%) and codon 30 (G > C) (n = 4, 12.5%) mutations were the most common. The genetic mutations of all patients are summarized in Table 3.

Table 3
Observed mutations in the Hb E β-thalassemia participants of the study

Allelic status	Mutation	Type of the mutation	n (%)
Heterozygous	IVS-1-5 (G > C)	Point mutation	17 (53.1)
Heterozygous	Codon 30 (G > C)	Point mutation	4 (12.5)
Heterozygous	Codon 15 (G > A)	Point mutation	1 (3.1)
Heterozygous	Codon − 90 (C > T)	Point mutation	1 (3.1)
Heterozygous	Codon 110 (T > C)	Point mutation	1 (3.1)
Heterozygous	IVS1-130 (G > C)	Point mutation	1 (3.1)
Heterozygous	IVS-2-1 (G > A)	Point mutation	1 (3.1)
Heterozygous	619-bp deletions	Deletion	1 (3.1)
Heterozygous	Codon 15 (-G)	Point deletion	1 (3.1)
Heterozygous	Codon 15 (-T)	Point deletion	1 (3.1)
Heterozygous	Codon 16 (-C)	Point deletion	1 (3.1)
Heterozygous	Codon 77/78 (+ C)	Point insertion	1 (3.1)
Heterozygous	Codon 41/42(-TTCT)	Deletion	1 (3.1)
IVS: Intervening sequesnce; bp: base pair;			

Of the 12 patients in the mild group, 3 were characterized by heterozygous codon 30 (G > C), and the rest were characterized by heterozygous codon − 90 (C > T), heterozygous codon 110 (T > C), heterozygous IVS-1-130 (G > C), heterozygous 619-bp deletion, heterozygous codon 15 (-G), heterozygous codon 15 (-T), heterozygous codon 16 (-C), heterozygous codon 77/78 (+ C), and heterozygous codon 41/42 (-TTCT). Of the 14 patients who were in the moderately severe group, most had heterozygous IVS-1-5 (G > C) (n = 11), one had heterozygous codon 30 (G > C), one had heterozygous codon 15 (G > A) and one had heterozygous IVS-2-1 (G > A). Of the 6 patients in the severe group, no other mutation was found except heterozygous IVS-1-5 (G > C), and all patients were characterized by this mutation. The heterozygous IVS-1-5 (G > C) mutation showed a significant association with the severity of disease (p < 0.001) (Table 4).

Table 4
Association of *HBB* gene mutations with clinical severity of Hb E/β-thalassemia

Mutations	**Clinical Severity**			**p-value**
	Mild	**Moderate**	**Severe**	
Heterozygous for IVS-1-5 (G > C)	0 (00.0)	11 (64.7)	6 (35.3)	< 0.001
Heterozygous for Codon 30 (G > C)	3 (75.0)	1 (25.0)	0 (00.0)	
Heterozygous for Codon 15 (G > A)	0 (00.0)	1 (100.0)	0 (00.0)	
Heterozygous for Codon − 90 (C > T)	1 (100.0)	0 (00.0)	0 (00.0)	
Heterozygous for Codon 110 (T > C)	1 (100.0)	0 (00.0)	0 (00.0)	
Heterozygous for IVS-1-130 (G > C)	1 (100.0)	0 (00.0)	0 (00.0)	
Heterozygous for IVS-2-1 (G > A)	0 (00.0)	1 (100.0)	0 (00.0)	
Heterozygous for 619-bp deletions	1 (100.0)	0 (00.0)	0 (00.0)	
Heterozygous for Codon 15 (-G)	1 (100.0)	0 (00.0)	0 (00.0)	
Heterozygous for Codon 15 (-T)	1 (100.0)	0 (00.0)	0 (00.0)	
Heterozygous for Codon 16 (-C)	1 (100.0)	0 (00.0)	0 (00.0)	
Heterozygous for Codon 77/78 (+ C)	1 (100.0)	0 (00.0)	0 (00.0)	
Heterozygous for Codon 41/42(-TTCT)	1 (100.0)	0 (00.0)	0 (00.0)	
Data are expressed as n (%) within rows;				
p-value was determined by Fisher's Exact test				

A detailed breakdown of clinical features (as used in the Mahidol scoring system) by HBB gene mutations shows that heterozygosity for IVS-1-5 (G > C) mutation was associated with a higher frequency of regular blood transfusion, enlarged spleen (> 10 cm), and growth retardation (< 3rd percentile). However, the steady-state hemoglobin of these patients remained mostly between 6 and 7.5 g/dl. See details in supplementary table 2.

Discussion

Hb E/β-thalassemia shows phenotypic heterogeneity with presentations ranging from mild to severe [17]. The reasons for such variability in presentations are not completely understood and are an active area of research [18]. To that end, we studied the clinical and genetic profile and explored their association in Hb E/β-thalassemia patients in Bangladesh from a specialized referral hematology unit of a tertiary care hospital.

We found that nearly two-thirds of patients had moderate to severe disease according to the Mahidol Scoring System. Our finding nearly corresponds to the original work that produced the scale at Mahidol University, Thailand, which found that more than two-thirds of patients had moderate to severe disease in a large sample of 950 patients [13]. However, an Indonesian study found that nearly four-fifth of patients had moderate to severe disease using the same scale, which is higher than that found in our study. Although no such classification was done previously among similar patients in Bangladesh, the Mahidol score was used to classify β-thalassemia intermedia patients in the country. The study by Mannan and his colleagues found that 35.3% had moderate disease, and 6% had severe disease. Various genetic interactions underlie intermediate forms of β-thalassemia and are less severe phenotypically than the forms compounded by hemoglobin E variants [17].

We noted a wide age range at which the diagnosis was made, and transfusions were started among the patients. Earlier age at presentation was usually associated with a higher severity score requiring a higher number of transfusions and a higher proportion of growth retardation and hepatosplenomegaly among Hb E/β-thalassemia patients. Olivieri and colleagues [6] extensively studied the phenotypic variability of such a group of thalassemic patients, which endorses our findings. Male patients were more frequent in our study, congruent with previous estimates [12].

The IVS-1-5 (G > C) mutation was the most common mutation found in more than 50% of the participants of this study. Mutational analysis of β-thalassemic individuals by Ayub et al. [9] reported that the splice site mutation IVS-1-5 (G > C) was the most common mutation in Bangladesh. Moreover, this mutation happens to be the most prevalent among thalassemic patients of South Asia [19]. However, unlike previous studies [6, 18] that argued against any association of the mutational specificity of the *HBB* gene with the phenotypic variability of Hb E/β-thalassemia, we noted that the IVS-1-5 (G > C) mutation was predominantly associated with moderate to severe disease. In particular, all severe phenotypes in our study were associated with this mutation. This relationship could be explained by the fact that IVS-1-5 (G > C) mutations produce β^{+}-thalassemia alleles leading to defective splicing of mRNA and impaired globin

chain production [20]. Which, when compounded by Hb E mutation (codon 26 G > A), leads to impaired Hb A production, increased Hb A2 and Hb E synthesis, and an overall decrease in hemoglobin level [21]. However, one might argue that the presence of IVS-1-5 (G > C) mutations across all severities of presentation in Hb E β-thalassemic patients, as described by Olivieri et al. [22], points against such an association. However, their findings could be explained by other secondary and tertiary modifiers, including other genetic and environmental attenuating factors which might be responsible for such phenotypic heterogeneity of the same mutation [7]. On the other hand, classification schemes used for severity grading might have affected the distribution seen in Olivieri et al. [22]. As we could not characterize the α-globin gene mutation among the participants, an exploration of the concomitant taming effect of alpha-mutation on the severity [6] was not possible in our study. Hence, further large sample studies are suggested to conclude any association.

As mild diseases could often be managed without transfusion preventing iron overload in these patients, a mutational analysis of the Hb E/β-thalassemia patients could guide individually tailored management for them.

Finally, one case among our participants needs particular attention. Interestingly, we noted that this case had a Mahidol score classified as severe but needed occasional blood transfusion only. This patient had a low steady-state hemoglobin of 5 g/dl, early age at first presentation (6 years), large spleen size (18 cm), severe growth retardation (< 3rd percentile), and an IVS-1-5 (G > C) mutation. Interestingly, the patient started blood transfusion at the age of 17 years, which indicates a lack of awareness and follow-up in the patient's part and might explain why the patient developed overt clinical presentation. However, a general lack of awareness about thalassemia and inadequate access to dedicated treatment facilities due to location and/or poor socioeconomic condition often led to delayed presentation or follow-up at hospitals. Hossain et al. [19], in a review of thalassemia in South Asian countries, noted that health awareness of the general population is very poor among the general population, and many thalassemia patients may die without knowing about their disease. However, this case also shows the variety of presentation Hb E/β-thalassemia patients can have and emphasize the importance of early detection and regular follow-up.

The major limitation of our study is the small sample size and characterization of patients from a single center. Another limitation is the inability to characterize α-globin gene mutations among the patients. However, our study is one of the earliest attempts to explore any association between phenotypic severity and mutations of the *HBB* gene among Hb E/β-thalassemia patients, and the results could aid in determining management strategies of patients carrying both Hb E and β-thalassemia mutations.

Conclusion

Hb E/β-thalassemia patients mostly present with moderate to severe disease as determined by the Mahidol scoring system. Splice site IVS-1-5 (G > C) is the most common mutation and is frequently associated with severe presentation. As one of the South Asian countries harboring a high burden of Hb E traits, the presence of a concomitant *HBB* gene is expected in Bangladesh. By showcasing the variability

in presentations of these patients, our findings could guide the screening and diagnosis of severe phenotypes and could elicit large-scale multicenter studies for further exploration.

List Of Abbreviations

BSMMU – Bangabandhu Sheikh Mujib Medical University

CBC – Complete Blood Count

HBB – β-globin gene

IVS – Intervening sequence

Declarations

Availability of data and materials:

Please contact author for data requests.

Author Contributions:

Conception and development of the idea:NM, ALK.

Methodology: NM, MAA, MAI, NJ.

Investigation: NM, MAA, MAI, NJ, MKHS, IAI, MRRC, MD.

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Writing - Original Draft Preparation: NM, MJH, MASK

Writing – Review & Editing: NM, MAA, MAI, NJ, MKHS, IAI, MRRC, MAZ, MKHS, MRRC, MJH, MASK

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Conflict of Interests:

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Ethical consideration:

Ethical clearance for the study was taken from the Institutional Review Board (IRB) and ethical review committee of BSMMU (No. BSMMU/2019/7579). All the study procedures were conducted in line with the ethical principles laid out by the Declaration of Helsinki. Informed written consent was taken from the patients or their guardians if the patient was a minor before inclusion.

Consent of Publication:

All authors agree to publish the article.

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Supplementary Tables

Supplementary Tables 1 and 2 are not available with this version.

Figures

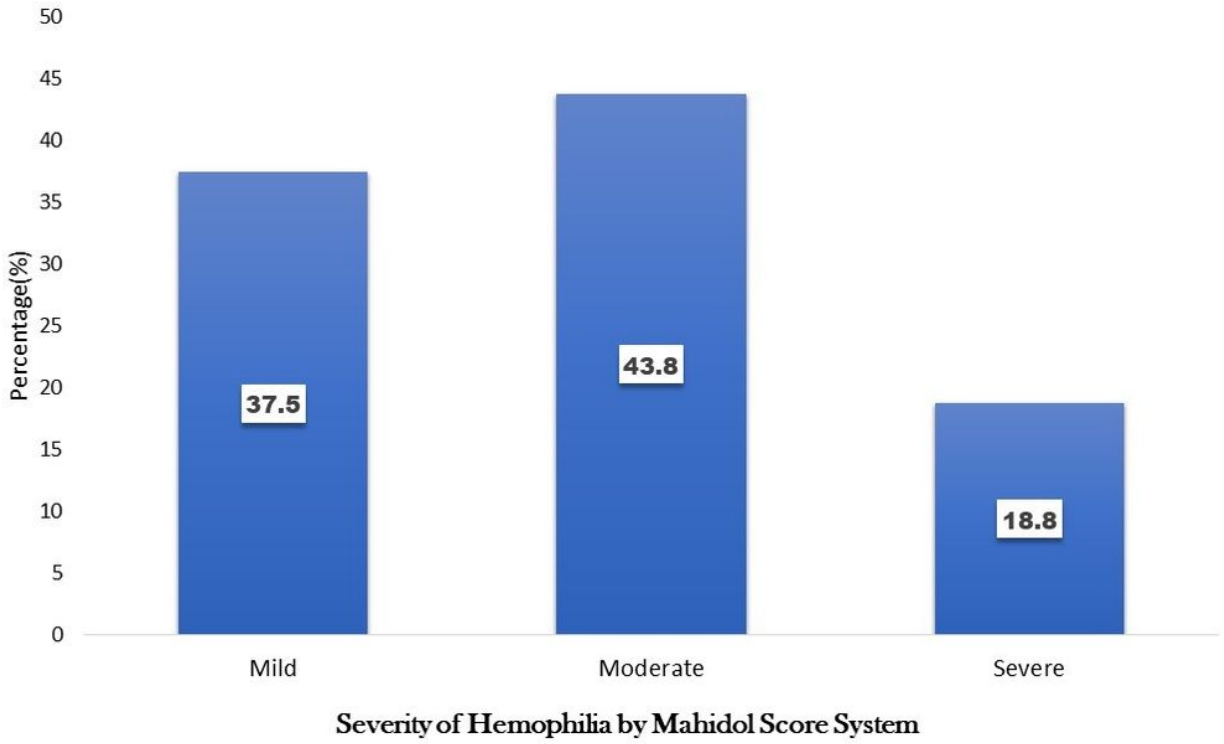

Figure 1

Severity of Hemophilia Mahidol Score System