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ORIGINAL RESEARCH

Clinical and Genetic Pattern of β-Thalassemia Major in East Java, Indonesia

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Background: Beta thalassemia major is the most common monogenic mutation disorder in Indonesia, with steadily increasing frequency. However, there are limited studies regarding genetic distribution and its relationship with the patient's clinical manifestation. This study aimed to identify the genetic mutation frequency and its association with the clinical phenotype pattern among β-thalassemia major patients in East Java, Indonesia.

Methods: In this observational study, we include subjects who have diagnosed with β-thalassemia previously through Hb electrophoresis. Demographic distribution with several ethnicities of Javanese, Sundanese, Chinese, Maduranese, and Batak was recorded. From each subject, a total of 6 mL of blood sample was collected and divided into two ethylene diamine tetraacetic acid (EDTA) tubes for CBC and DNA extraction. DNA samples were analyzed by PCR and followed by Sanger sequencing.

Results: A total of 91 subjects were included in this study, with a median age of 22.25 ± 7.56 years old; consisting of 52 females and 39 males, with Javanese as the most common ethnicity. There are 22 types of mutation were identified through Sanger sequencing. The most common mutation was IVS-1-5/CD 26 and the CD 35/CD 26 observed in 36 (39.5%) and 19 (20.8%), respectively. While 9 subjects (9.8%) had no mutation detected. Several clinical phenotypes, including iron overload, short stature, severe anemia, and splenomegaly, were most prevalent among the two most common genetic mutations.

Conclusion: There is variability in clinical phenotype in β-thalassemia observed in several types of genotype mutations. Among all the mutations found in East Java, the genotypes IVS-1-5/CD 26 and CD 35/CD 26 were the two most frequent genotypes. Those genotypes are linear with the severity of the phenotype in β-thalassemia, such as severe anemia, iron overload, short stature, and splenomegaly.

Keywords: thalassemia, genetic, mutation, multiethnic, iron overload

Introduction

β-Thalassemia is one of the most common monogenic disorders genetically inherited in an autosomal recessive pattern.[1] It has a negative impact on hemoglobin synthesis and is very prevalent in Southeast Asia, the Middle East, and the Mediterranean Basin.[1,2] Based on data from the Indonesian Thalassemia Foundation, cases of thalassemia have been continuously escalating. Thalassemia cases in Indonesia accounted for 4,896 cases until June 2021.[3]

Point mutations (substitution, small deletions, or insertion) on the genes coding for the globin synthesis caused impairment in hemoglobin (Hb) production, leading to chronic and severe anemia. The genes that encode the globin proteins are located on the α- and β-globin gene clusters located on chromosomes 16 and 11, respectively. While the expression of each gene varies depending on embryonic and foetal development.[4] These mutations result in reduced production of the β-globin chain and HbA. There are more than 350 β-thalassemia mutations known, with varied severity indexes.[5]

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Chronic anaemia in β-thalassemia major patients makes them undergo blood transfusions regularly if there is any sign of increasing oxygen demand in the organs. Several pathophysiologies, such as chronic anemia,[6] ineffective erythropoiesis,[7] and accumulated iron from hemolysis[8] and transfusion, lead to iron overload conditions in thalassemia patients. Excess iron accumulates in several organs, such as the heart, spleen, muscle, endocrine glands, bone marrow, and mostly in the liver.[9] Accumulation of iron in those organs can lead to organ failure, thus increasing the mortality rate of β-thalassemia major patients.

Studies investigating the genetic abnormalities in haematology abnormalities in Indonesia have been conducted.[10–13] However the gap occurs between genetic studies and clinical application, as they did not correlate the genetic abnormalities that occur with various clinical complications in multiple organ systems (eg, endocrine, kidneys, heart, etc) that could develop in patients. This study aimed to investigate the correlation of genetic profiles of β-thalassemia major patients in East Java with the propensity of individual clinical manifestations. Genetic results can help clinicians optimize treatment for patients with β-thalassemia major and anticipate the most incurred complication, in addition to providing regular blood transfusions and lifelong iron chelation therapy.

Method

To support community-based research on b-thalassemia in East Java, which is based at Airlangga University Hospital, we conducted research involving 105 β-thalassemia patients from the community, and then we carried out genetic examinations to look for genetic mutations in b-thalassemia patients. This research was conducted in a longitudinal, multicenter observational manner involving several regions in East, Central, and West Indonesia. We are collaborating with several educational centers in Eastern, Central, and Western Indonesia. Clinical and demographic profiles and blood samples from β-thalassemia patients in East Java were sent to Airlangga University Hospital for us to carry out laboratory examinations and genetic profiles. All participants included in this study had been previously diagnosed with transfusion-dependent β-thalassemia major during childhood and had been receiving regular blood transfusions at our center. The diagnosis was established based on clinical transfusion dependence in conjunction with hemoglobin electrophoresis findings, which constitute the standard diagnostic approach at our institution.

For tracking the genetic abnormalities of β-thalassemia patients, a comprehensive consultation has been carried out to confirm family history (pedigree). No multigenerational pedigree analysis or genetic ancestry assessment was performed. The clinical profile, laboratories, and genetic data were presented as descriptive and processed in bivariate and multivariate analysis to determine the pattern of thalassemia. The study was conducted over a six-month period, during which participants were consecutively included until the predetermined sample size was achieved. The minimum required sample size (n = 91) was calculated using a cross-sectional sample size formula to ensure adequate statistical power. All participants included in this study had been receiving regular iron chelation therapy with good compliance for more than ten years.

Laboratory Test

Blood samples were taken from all patients on their follow-up prior to their blood transfusion. A total of 6 mL of blood was divided into two ethylene diamine tetraacetic acid (EDTA) tubes. The first tube was analyzed for complete blood count (CBC), while the other tube was for DNA extraction using the NEXprep Blood DNA mini Kit lot no. 1E0620-02. Anemia was assessed hemoglobin parameter in CBC. All subjects were classified as having mild or severe anemia based on a hemoglobin cutoff value of 8 g/dL. DNA samples were stored at −80°C before subsequent analysis, ie, polymerase chain reaction (PCR), followed by Sanger sequencing on these DNA samples Molecular analysis was carried out at the Research Laboratory of Universitas Airlangga Hospital, and Sanger sequencing was carried out at the Universitas Gajah Mada (UGM) Integrated Research and Testing Laboratory.

Genotype Determination

In this study, we performed polymerase chain reaction-amplification-refractory mutation system (PCR-ARMS) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods to detect any mutation, followed by Sanger sequencing. Extracted DNA samples were subjected to PCR and Sanger sequencing.

PCR Arms

PCR-ARMS consists of two amplifications in the same reaction mixture using the same genomic DNA as substrate. The mixture contained 12.5 µL (NEXpro HS PCR 2X Master), 3 µL (8.6–284.3 ng/µL) of genomic DNA, and two pairs of primers (control primers D and E and common primer B and mutant IVSI-5), two pairs of primers (control primer forward and reverse and control primer forward and mutant Cd35 reverse), and/or two pairs of primers (control primers D and E and common primer B and mutant IVSI-1/T), and/or another two pairs of primers (control primers D and E and common primer B and mutant IVSI-1/A) at a concentration of 1.25 µM (0.625 µL) each, as shown in Table 1. The PCR reaction was performed using a Bio-Rad CFX-96 C1000 Touch Thermal Cycler. The gel preparation is 1% agarose gel in 0.5x Tris-Borate-EDTA buffer. Electrophoresis was conducted for 30 min at 100 volts and then viewed under a UV transilluminator prior to documentation.

Interpretation for IVSI-5 is as follows: all samples showed a band appearing at 861 bp as a positive control of β-thalassemia. While positive mutation of IVSI-5 along with the band at 285 bp in the electrophoresis gel is negative and only appears at 861 bp. Interpretation for CD35 is as follows: all samples showed a band that appears at 804 bp as a positive control of CD35, while a positive mutation of CD35 along with the band at 475 bp and a negative result only showed a band at 804 bp. Interpretation for IVSI-1/T or IVSI-1/A is as follows: all samples showed a band appearing at 861 bp as a positive control of β-thalassemia, while a positive mutation of IVSI-1/T or IVSI-1/A along with a band at 281 bp in the electrophoresis gel and a negative only appears at 861 bp.

PCR-RFLP

In this PCR-RFLP reaction, the mixture contained 12.5 µL (NEXpro HS PCR 2X Master), 3 µL (8.6–284.3 ng/µL) of genomic DNA, and a pair of primers (HbE primer forward and reverse) at a concentration of 1.25 µM (0.625 µL) each. RFLP analysis does not utilize primers as templates. Instead, genomic DNA was digested using the restriction enzyme **MnlI**, which recognizes and cleaves both wild-type DNA and DNA with codon 26 mutation, in either homozygous or heterozygous states, resulting in fragments of defined base-pair lengths. The PCR reaction was performed using the Bio-Rad CFX-96 C1000 Touch Thermal Cycler. The PCR cycle reactions were as follows: an initial 2 min of denaturation at 95°C; 15 s of denaturation (35 cycles) at 95°C; 30 s of annealing at 65°C; 30 s of extension at 72°C; 3 min of post-extension at 72°C; and 1 min of cooling at 100°C. Subsequently, 5 µL of the amplified product was aliquoted prior to visualization using gel electrophoresis. The gel preparation is 1% agarose gel in 0.5x Tris-Borate-EDTA buffer.

Electrophoresis was conducted for 30 min at 100 volts and then viewed under a UV transilluminator prior to documentation. Interpretation for HbE, also known as Cd26, is as follows: all samples showed a band appearing at

Table 1 Primer Sequences and PCR Product Size

Primer	Primer Sequence	Size (bp)
D E	5′ GAG TCA AGG CTG AGA GAT GCA GGA 3′ 5′ CAA TGT ATC ATG CCT CTT TGC ACC 3′	861
B IVSI-5	5′ ACC TCA CCC TGT GGA GCC AC 3′ 5′ CTC CTT AAA CCT GTC TTG TAA CCT TGT TAG 3	285
Control F Control R	5′ TCC AAC TCC TAA GCC AGT GC 3′ 5′ CGA TCC TGA GAC TTC CAC ACT G 3′	804
Control F Cd 35 R	5′ TCC AAC TCC TAA GCC AGT GC 3′ 5′ GAA CCT CTG GGT CCA AGG T 3′	475
IVSI-1/T B	5′ TTA AAC CTG TCT TGT AAC CTT GAT ACG AAA3′ 5′ ACC TCA CCC TGT GGA GCC AC 3′	281
IVSI-1/A B	5′ TTA AAC CTG TCT TGT AAC CTT GAT ACC GAT 3′ 5′ ACC TCA CCC TGT GGA GCC AC 3′	281

Abbreviations: PCR, Polymerase Chain Reaction; bp, base pair.

804 bp as a positive mutation of Cd26. Furthermore, the amplified DNA (PCR-RFLP product) was digested by restriction endonuclease Mnl1 enzyme; the mixture contained 2 µL (10X buffer G), 1 µL (10 U/µL) of Mnl1 enzyme (Thermo Fisher), and 10 µL of PCR-RFLP product of genomic DNA. The PCR-RFLP reaction was performed using a Biorad CFX-96 C1000 Touch Thermal Cycler. Electrophoresis was conducted for 30 min at 100 volts and then viewed under a UV transilluminator prior to documentation.

Interpretation for PCR-RFLP is as follows: the amplified normal DNA would be fragmented into three bands, which are showing at 171 bp, 114 bp, and 50 bp. Furthermore, the genotype of the CD26 homozygote would be fragmented into two bands, which are showing at 221 bp and 114 bp. Meanwhile, the genotype of the CD26 heterozygote would be fragmented into four bands, which are showing at 221 bp, 171 bp, 114 bp, and 50 bp.

Sanger sequencing is carried out if no mutations are found using the PCR ARMS and RFLP methods. In this PCR reaction, the mixture contained 12.5 µL (Bioline My Taq HS Red Mix), 50–150 ng of genomic DNA, and two pairs of primers (Table 2) at concentrations of 0.4 µM each. The PCR reactions were performed using Bio-Rad T100 (Bio-Rad Laboratories, Inc., USA electrophoresis). The gel preparation was as follows: 1% agarose gel in 1× Tris-Borate-EDTA buffer. Electrophoresis was conducted for 25 min at 100 V and then viewed under a UV transilluminator prior to documentation.

DNA Sequence Analysis

The PCR products encompassing the β globin genes were amplified with double reaction PCR. The amplified fragments were subsequently sequenced by Sanger methods by DNA Sequencing Services (UGM Integrated Research and Testing Laboratory). The sequences were analyzed and aligned using the Benchling web service (https://www.benchling.com/) with the reference sequence from NCBI Reference Seq: NG_059281 to determine the genotype (Applied Biosystems, 3500 Genetic Analyzer, Hitachi Corp., Tokyo, Japan).

Result

There were ninety-one study participants (52 females, 39 males) with median ages of 22.25 ± 7.56 years old. Participants were asked to identify their ethnic background according to commonly recognized ethnic categories. Of the 91 subjects included in the study, 77 were Javanese, 5 Sundanese, and 2 each were of Chinese, Madurese, Arab, and Batak ethnicity. Most of the subjects have several symptoms of β-thalassemia, such as malnutrition and short stature, severe anemia, and splenomegaly. RBC parameters of all subjects provided in Table 3, with mean value of RBC, Hb, hematocrite, MCV, MCH, and MCHS are 3.93 × 10^6/Ul, 7.0 g/dL, 28%, 82 fL, 24 pg, and 33 g/dL, respectively.

The result of the Sanger sequencing showed the following genotype variation: 36 (39.5%) with IVS-1-5/CD 26, 19 (20.8%) with CD 35/CD 26, 3 (3.2%) subjects with each CD 26/CD 41–42 and CD 26/IVS-1-1, 2 (2.1%) with CD 26/CD 15 and IVS-1-5/CapM+1, and one subject (1%) with each CD 26/CD 30, CD 26/CD 6–10, CD 26/IVS-1-2, CD 26/IVS-2-16, CD 8/CD 26, CD 92/IVS-2-16, CD 26, CD 26/IVS-1-2, CD 26/CD 41, CD 26/IVS-1, CD 35/IVS-2-16, IVS-1-5/CD 35, IVS-1-5/IVS-1-1, IVS-1-5/−42, IVS-5 Homozygot, HBB eks 1–2, and dan HbE Homozygot. While 9 subjects (9.8%) had no mutation detected.

Table 4 shows variability of genetic mutation pattern among β-thalassemia subjects. There were 22 mutations detected, with IVS-1-5/CD26 (40.4%) being the most common identified, followed by CD 35/CD 26 (21.3%). Distribution of blood type also varied among all the mutation types. In the most common mutation, IVS-1-5/CD26, the most common blood type

Table 2 Sequence and Size of the Primers Used for DNA Amplification

Primers	Sequence	Size (bp)
Primer 1	5′ CCA AGG ACA GGT ACG GCT GTC ATC 3′	704 bp
Primer 5	5′ CCT TCC TAT GAC ATG AAC TTA ACA TT 3′	
Primer 6	5′ CTT TCC CTA ATC TCT TTC TTT CAG G 3′	470 bp
Primer 9	5′ GGA ACA AAG GAA CCT TTA ATA G 3	

Abbreviations DNA, Deoxyribonucleic Acid; bp, base pair.

Table 3 RBC Parameters of Subjects

Subject No.	RBC (10^6/Ul)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
1	3.07	6.4	20.7	67.4	20.8	30.9
2	4.04	9.6	29.9	74.0	23.8	32.1
3	2.75	7.6	22.4	81.5	27.6	33.9
4	4.40	8.2	25.4	57.7	18.6	32.3
5	3.07	7.9	23.0	74.9	25.7	34.3
6	3.88	10.2	29.9	77.1	26.3	34.1
7	3.17	8.2	24.9	78.5	25.9	32.9
8	3.29	8.6	25.9	78.7	26.1	33.2
9	3.44	8.4	25.4	73.8	24.4	33.1
10	4.26	8.4	26.3	61.7	19.7	31.9
11	3.04	7.8	22.9	75.3	25.7	34.1
12	4.40	8.9	27.4	62.3	20.2	32.5
13	3.97	8.7	27.0	68.0	21.9	32.2
14	4.01	8.9	27.4	68.3	22.2	32.5
15	3.79	8.7	25.6	67.5	23.0	34.0
16	4.19	8.9	27.3	65.2	21.2	32.6
17	6.31	11.6	34.5	54.7	18.4	33.6
18	6.96	11.2	37.2	53.4	16.1	30.1-
19	5.95	10.1	32.5	54.6	17.0	31.1
20	3.22	7.3	22.3	69.3	22.7	32.7
21	4.30	8.7	26.5	61.6	20.2	32.8
22	3.54	6.4	20.8	58.8	18.1	30.8
23	2.79	6.3	19.3	69.2	22.6	32.6
24	3.19	5.9	19.1	59.9	18.5	30.9
25	2.50	6.4	19.1	76.4	25.6	33.5
26	3.40	9.1	26.1	76.8	26.8	34.9
27	2.97	7.6	22.6	76.1	25.6	33.6
28	4.25	9.6	31.3	73.6	22.6	30.7-
29	3.46	7.1	22.1	63.9	20.5	32.1
30	3.84	9.2	28.0	72.9	24.0	32.9
31	4.56	10.8	32.3	70.8	23.7	33.4
32	3.59	9.8	28.6	79.7	27.3	34.3
33	3.88	10.7	33.3	85.8	27.6	32.1
34	4.82	8.1	27.3	56.6	16.8	29.7-
35	3.14	7.6	23.5	74.8	24.2	32.3
36	4.13	10.4	30.7	74.3	25.2	33.9
37	3.08	6.9	21.8	70.8	22.4	31.7
38	4.40	10.4	30.7	69.8	23.6	33.9
39	3.66	8.5	25.3	69.1	23.2	33.6
40	4.24	10.1	30.8	72.6	23.8	32.8
41	3.56	9.1	26.5	74.4	25.6	34.3
42	3.54	9.7	29.2	82.5	27.4	33.2
43	3.86	10.4	31.7	82.1	26.9	32.8
44	3.80	9.8	29.3	77.1	25.8	33.4
45	4.32	10.5	31.6	73.1	24.3	33.2
46	4.37	11.0	33.3	76.2	25.2	33
47	3.51	8.5	25.5	72.6	24.2	33.3
48	4.91	10.4	29.7	60.5	21.2	35.0
49	4.88	11.5	34.3	70.3	23.6	33.5
50	3.28	11.1	33.5	102.1	33.8	33.1

(*Continued*)

Table 3 (Continued).

Subject No.	RBC (10^6/Ul)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
51	4.09	10.1	31.6	77.3	24.7	32.0
52	4.82	11.8	35.6	73.9	24.5	33.1
53	3.59	7.7	23.2	64.6	21.4	33.2
54	2.85	7.7	22.8	80.0	27.0	33.8
55	4.52	9.0	28.5	63.1	19.9	31.6
56	5.31	12.3	37.6	70.8	23.2	32.7
57	5.41	16.4	47.0	86.9	30.3	34.9
58	3.85	9.5	28.1	73.0	24.7	33.8
59	4.09	8.1	25.4	62.1	19.8	31.9
60	3.55	6.5	21.8	61.4	18.3	29.8
61	4.75	11.1	33.2	69.9	23.4	33.4
62	3.09	7.9	22.9	74.1	25.6	34.5
63	3.27	9.0	26.3	80.4	27.5	34.2
64	4.91	12.8	37.1	75.6	26.1	34.5
65	3.89	7.8	24.3	62.5	20.1	32.1
66	4.31	10.4	30.7	71.2	24.1	33.9
67	5.99+	11.7	36.9	61.6	19.5	31.7
68	3.49	8.6	25.8	73.9	24.6	33.3
69	3.53	9.7	28.7	81.3	27.5	33.8
70	3.51	8.7	25.8	73.5	24.8	33.7
71	3.75	9.8	29.8	79.5	26.1	32.9
72	3.40	9.3	27.1	79.7	27.4	34.3
73	4.47	9.7	29.0	64.9	21.7	33.4
74	3.83	8.5	25.5	66.6	22.2	33.3
75	3.53	8.3	25.8	73.1	23.5	32.2
76	4.59	8.8	27.4	59.7	19.2	32.1
77	4.73	11.2	34.4	72.7	23.7	32.6
78	4.43	7.7	26.8	60.5	17.4	28.7
79	4.42	10.1	31.7	71.7	22.9	31.9
80	3.86	10.3	29.5	76.4	26.7	34.9
81	2.46	5.5	17.0	69.1	22.4	32.4
82	3.92	9.4	28.3	72.2	24.0	33.2
83	3.25	8.2	24.6	75.7	25.2	33.3
84	2.65	6.9	19.9	75.1	26.0	34.7
85	4.69	11.8	35.7	76.1	25.2	33.1
86	4.11	11.0	34.2	83.2	26.8	32.2
87	3.02	8.6	24.6	81.5	28.5	35.0
88	4.19	8.9	26.6	63.5	21.2	33.5
89	3.57	8.8	25.7	72.0	24.6	34.2
90	3.54	8.1	24.8	70.1	22.9	32.7
91	3.73	8.5	25.1	67.3	22.8	33.9

was B (38.8%), followed by O and A (33.3% and 22.2%), respectively. Although, in the CD 35/CD 26 mutation, the most common blood type was O, followed by B and A (47.3%, 31.5%, and 21%), respectively. Iron overload conditions occur in all mutation types, although most subjects with the IVS-1-5/CD26 mutation had no sign of iron overload.

The distribution of ethnicity in this study is Javanese, Sundanese, Chinese, Arabians, Maduranese, and Batak for 82 (90.1%), 3 (3.3%), 2 (2.2%), 2 (2.2%), 1 (1.1%), and 1 (1.1%), respectively. In Sundanese, genetic mutations occur in CD 26/CD 15, CD 26/IVS-2-16, and with unknown mutations. In Chinese subjects, mutations occur in IVS-1-5/CD 26 and unknown mutations. In Arabian subjects, mutation occurs in CD 35/CD 26, which is the majority pattern. The

Table 4 Genetic Pattern of Gender, Blood Type, and Iron Overload Phenotype

Genetic Pattern	Sex		Blood Type				Iron overload	
	Male	Female	A	AB	B	O	No	Yes
CD 26/CD 15	2	0	0	0	2	0	1	2
CD 26/CD 30	1	0	1	0	0	0	0	1
CD 26/CD 41–42	1	2	1	0	2	0	1	3
CD 26/CD 6–10	1	0	1	0	0	0	1	1
CD 26/IVS 1-II	0	2	0	0	0	2	0	2
CD 26/IVS-1-1	2	1	2	0	0	1	1	2
CD 26/IVS-II-16	0	1	0	0	1	0	1	1
CD 35/CD 26	11	8	4	0	6	9	8	2
CD 8/CD 26	0	1	1	0	0	0	0	1
CD 92 C > A/IVSII-16	0	1	0	0	1	0	1	1
CD 26	1	0	0	1	0	0	1	1
CD 26/CD 41	0	1	0	0	0	1	0	1
CD 26/IVS-1 G > C	0	1	0	0	1	0	1	1
CD 35/IVS-II-16	0	1	0	0	0	1	0	1
HBE Homozygous	1	0	0	0	1	0	1	1
IVS-1-5/CD 26	13	23	8	2	14	12	18	2
IVS-1-5/CD 35	1	0	0	0	0	1	1	1
IVS-1-5/IVS-1-1	0	1	0	0	1	0	1	1
IVS-1-5/Cap M+1	2	0	0	0	0	2	0	1
IVS-1-5/-42	0	1	0	0	0	1	0	1
IVS-5 Homozygous	0	1	0	0	0	1	1	1
Normal HBB 1-2	1	0	1	0	0	0	1	1
Null	2	7	4	1	0	4	5	1

Maduranese subjects have a mutation occurring in IVS-1-5/CD 35, and the Batak ethnic group has an unknown mutation locus. Therefore, Javanese, as the majority ethnicity, have variability of genetic pattern. Three out of 9 subjects with unknown mutations were from minority ethnic groups (Sundanese, Batak, and Chinese).

As shown in Table 5, malnutrition identified by short stature mostly occurs in subjects with the IVS 1–5/CD 26 mutation (39.5%), followed by the genotype CD 35/CD 26 mutation (20.8%). Sixteen out of 91 (17.5%) subjects had short stature, even though short stature was identified in only 8 out of 22 types of mutation (36.3%). Short stature was only identified in subjects with the mutation detected. Among 91 subjects involved, 75 subjects (82.4%) undergo routine transfusion monthly. Severe anemia marked by decreasing Hb <8 mg/dL mostly occurs in subjects with IVS 1–5/CD26 mutation (12%), followed by CD 35/CD 26 mutation (2.1%). Splenomegaly was detected in 54 out of 91 subjects (59.3%) with Schuffner 1–8, 4 subjects (4.3%) have undergone splenectomy, and 33 subjects (36.3%) have no splenomegaly. Splenomegaly was found mostly in IVS 1–5/CD26 mutation subjects (31.8%), followed by CD 35/CD 26 (10.9%).

Table 5 Genetic Pattern of Subjects Condition: Malnutrition, Severe Anemia, and Splenomegaly

Genetic Pattern	Malnutrition and Short Stature		Severe Anemia Less Than 8 g/dl		Splenomegali		
	No	Yes	No	Yes	Splenectomy	No	Yes
CD 26/CD 15	1	1	1	1	0	1	1
CD 26/CD 30	0	1	0	1	0	0	1
CD 26/CD 41–42	3	0	2	1	1	0	2
CD 26/CD 6–10	1	0	1	0	0	0	1
CD 26/IVS 1-II	1	1	2	0	0	1	1
CD 26/IVS-1-1	3	0	3	0	0	1	2
CD 26/IVS-II-16	1	0	1	0	0	1	0
CD 35/CD 26	17	2	17	2	0	9	10
CD 8/CD 26	1	0	1	0	0	1	0
CD 92 C > A/IVSII-16	1	0	1	0	0	1	0
CD26 (T/A)	1	0	1	0	0	0	1
CD26/Cd41	1	0	1	0	0	0	1
CD26/IVS-1 G > C	1	0	1	0	0	0	1
CD35/IVS-II-16	1	0	1	0	1	0	0
HBE Homozygous	1	0	0	1	0	0	1
IVS-1-5/Cd 26	28	8	25	11	1	7	28
IVS-1-5/Cd 35	0	1	0	1	0	1	0
IVS-1-5/IVS-1-1	1	0	1	0	0	1	0
IVS-1-5/Cap M+1	1	1	1	1	0	1	1
IVS-1-5/-42	1	0	1	0	0	1	0
IVS-1-5 Homozygous	1	0	1	0	0	0	1
Normal HBB 1-2	0	1	1	0	0	1	0
Null	9	0	9	0	1	6	2

Discussion

Our study reported genotype variation in a total of 91 β-thalassemia subjects in East Java. The DNA sequencing and amplification revealed that 89 out of 91 subjects have genetic mutations. There were 22 variations in genotype mutation we found, with IVS-1-5/Cd 26 being the most frequent genotype (40.4%). This result is similar to a previous study in the same region with younger subjects, in which the most common genotype is IVS-1-5/Cd 26.[10] This genotype similarity is due to the similarity of the study region and distribution of the subjects of our study. An early study about the genotype of b-thalassemia subjects in the same city as us revealed that mutation in IVS-1-5 was the most common.[14] The first study of genotype mutation of β-thalassemia in Indonesia by Injo et al also revealed that IVS-1-5 was the most frequent genotype, followed by CD 26.[11] Those results were comparable to our study. Besides, those findings show there was no change of genotype mutation in the same region and ethnic group over a long period. Another study in Central Java by Rujito et al, including more subjects, concluded that the most frequent genotype mutation was CD 26/IVS-1-5 (40.67%), almost the same as our result.[15]

A slight difference with our study was found in a study from the special region of Yogyakarta, which revealed that the splice-site mutation in IVS-1-5 was the most frequent in b-thalassemia subjects (71.4%), while the most frequent frameshift mutation is CD 35 (10.7%),[13,15] while the most frequent frameshift mutation in our study was in CD 26. Another study in Bandung, including 291 subjects with eight types of mutations, has homozygous IVS-1-5 as the most common genotype mutation in β-thalassemia subjects.[16] These differences can be due to different geographical locations, ethnicities, and numbers of subjects. In our study, the distribution of ethnicity was variable; the major ethnicity was Javanese (82%), which has variability of genetic pattern. From the other ethnics, only Chinese and Arabians have the most common mutation pattern; therefore, Sundanese, Maduranese, and Batak have the other pattern, and three of them still have an unknown pattern of genetics. Multiethnics found in our study are comparable with the result from Hernaningsih et al.[10] This conclusion is also linear with a study by Adhiyanto et al; they were doing screening of b-globin gene mutation in 180 adolescent schoolgirls in Malang of East Java and Sukabumi of West Java. Their study shows different dominant polymorphisms in genotype mutation; in Malang the most common polymorphism is CD 26, while in Sukabumi the most common polymorphism is IVS-1-5.[12]

Genetic polymorphism is associated with bone density and growth in β-thalassemia subjects.[17] In our study, short stature was found only in subjects with genetic mutation, but there is no clinical short stature in subjects with no mutation detected. In this study, there is more short stature in heterozygous genotypes than homozygous. This finding is similar with another study which compound heterozygous IVS-1-5 subjects have severe phenotypes with the highest number of pathological short stature (63%).[18] Bone disease in β-thalassemia was caused by bone marrow expansion due to ineffective erythropoiesis, which resulted in a decrease in bone tissue. Moreover, lower bone density is worsened by many endocrine problems, such as hypogonadism and hypothyroidism following iron overload in b-thalassemia subjects.[17]

Serum iron overload was found in both mutation-detected and undetected subjects, but higher genetic polymorphism CD 26/CD 41–42 has a higher prevalence of iron overload. This result is comparable with a previous study by Saad et al, which found severe iron overload in mutation CD 26, followed by milder iron overload in CD 41–42 and IVS-1-5.[19] Similar results were obtained for anemia. In CD 26/IVS-1-5 mutation, there is a higher prevalence of severe anemia (Hb < 8 g/dL), which is linear with a previous study that said that CD 26 polymorphism is associated with severe hypochromic microcytic anemia. In addition, the morphology of red blood cells changes in more than 85.7%.[20] Another study from Central Java revealed that the higher prevalence of anemia (46.16%) was in genotype CD 26/CD 35, followed by CD 26/IVS-1-5 (30.7%), contrary to our study, in which the highest prevalence of anemia was in CD 26/IVS-1-5 (57.8%), followed by CD 26/CD 35 (10.5%). This difference can be due to geographical location and the number of subjects studied.[20] Severe anemia was predominantly observed in patients with the codon 26 mutation, which was also the most frequently identified frameshift mutation in this study. This mutation leads to an amino acid substitution from glutamic acid to lysine, resulting in hemoglobin E formation,[21] impaired β-globin mRNA processing, and subsequent α/β-globin chain imbalance that causes ineffective erythropoiesis and more severe anemia [22].

In this study, the genetic mutation IVS-1-5/CD 26 was the most frequent polymorphism in the splenomegaly phenotype (51.8%), followed by CD 35/CD 26 (18.5%). Unfortunately, there was very limited study about polymorphism and splenomegaly in β-thalassemia subjects.

Conclusion

In conclusion, β-thalassemia major shows marked phenotypic variability related to genotype mutations. In East Java, the IVS-1-5/CD 26 and CD 35/CD 26 genotypes were most prevalent and were associated with more severe clinical manifestations, with the codon 26 mutation emerging as a key contributor to anemia severity and growth impairment.

Ethical Clearance

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of Airlangga University Hospital with Ethical Clearance No. 214/KEP/2025.

Informed Consent

Informed consent was obtained from all subjects involved in the study prior to the study commencement.

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Disclosure

The authors report no conflicts of interest in this work.

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