The South Asian Journal *of* Medicine

Original Article

B-Thalassaemia/Hb E Compound Heterozygosity For 41/42 (-TTCT) Mutation in A Javanese Sibling

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Volume: 4-Issue: 1 **February 1, 2018** **ESTABLISHED IN: 2015**

ABSTRACT

Background:

Thalassaemia an inherited gene disorder is caused by the decrease or absence of one or two types of globin chains of the adult haemoglobin. The aim of the study was to report compound heterozygosity for 41/42 (-TTCT) and codon 26 (G→A) mutation in a Javanese sibling and the mutations inherited from parents with either β-thalassaemia or Hb E traits.

Methodology:

Two families whose parents were β-thalassaemia carriers were recruited. The first family have five siblings and the second family two siblings. Full Blood Count, MCV, MCH, peripheral blood morphology and haemoglobin electrophoresis was done together with DNA analysis. Mahidol scoring for clinical severity was adopted.

Results:

Three siblings from two families were deemed normal as they did not inherit the mutations (incidence 40% to 50%). Four siblings inherited mutations (incidence 50% to 60%). Moreover, two siblings inherited mutations from both parents and deemed compound heterozygosity (incidence 20% to 50%). Mahidol scoring showed that the sibling with IVS-1-nt5 (G→C) and codon 26 (G→A) mutations had moderate disease and codon 26 (G→A) with codon 41/42 (-TTCT) mutations was severe. Codon 41/42 (-TTCT) compound heterozygosity to our knowledge was the first report found in the Javanese sibling. Both siblings require regular blood transfusions. The peripheral blood morphology displayed hypochromic microcytosis and variation of red blood cell shapes and lower MCH and MCV levels in the affected families.

Conclusion:

The incidence of inheritance from parents with β-thalassaemia traits from our study may form a probable prediction in family inheritance.

Key Words:

β-thalassaemia/Hb E compound heterozygosity, mutations.

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SAJMED.2019; 4(1):102-108.

Received at: November 19, 2018
Revised at: January 09, 2019
Published at: February 1, 2019

Conflict of Interest:

The authors declared that there is no Conflict of Interest.

INTRODUCTION

Thalassaemia, an inherited autosomal recessive gene disorder is the commonest monogenic disorder worldwide. It is a group of anaemias that caused by the decrease or absence of one or two types of globin chains of adult haemoglobin. Thalassaemia is prevalent in the regions where malaria is or was endemic in the Mediterranean Basin, Africa, Middle-East, India. Southeast Asia and Southern China where migration flows resulted in thalassaemia being spread to Northern Europe, North and South America (1) and throughout the world (2). Haemoglobinopathies are the most common genetic disorders and prevalent in Southeast Asia. Haemoglobin E (Hb E) in Southeast Asia attaining a frequency of between 50-60% at the junction of countries bordering Thailand, Laos and Cambodia (3-6). Thalassaemia and haemoglobin (Hb) variant are the most common genetic disorders in Southeast Asia (5) which accounts for 50% of the world's thalassaemia carrier (6). The prevalence of α- and β-thalassaemia in Medan, Indonesia population was 3.35% and 4.07% respectively (7) with Hb E being the most common Hb variant found in Indonesia (5) and Southeast Asia (8). The thalassaemia trait which inherits an individual only has one mutation from one of the parents (9), mutation on chromosome 11 (α-thalassaemia) or on chromosome 16 (β-thalassaemia). α-thalassaemia is most often due to gene deletion whereas β-thalassaemia is very heterogenous at the molecular level. β-thalassaemia mutations are relatively population specific (10-13). If thalassaemia trait patient marries another trait there is a chance to have thalassaemia major disorder or severe anaemia (14). Compound heterozygosity between β-thalassaemia and Hb E leading to β-thalassaemia/Hb E is common in patients displaying variable severity of anaemia has a wide spectrum of severity causing a major public health problem in Southeast Asia (15, 16). Screening for thalassaemia or thalassaemia trait is based on conventional low mean cell volume (MCV) and mean cell haemoglobin (MCH) and haemoglobin -typing and diagnosis is further confirmed by raised HbA_2 (5, 17). Blood smear showed microcytosis, hypochromia and marked variation in size and shape of red blood cells.

Alpha-thalassaemia is associated with variable degree of α-globin chain deficit molecular defects whilst β-thalassaemia mutations are very heterogenous both in molecular defects and clinical maintenance are population specific (10, 12). In Indonesia the most common beta-globin gene mutations are IVS1-nt5 (G→C) (18) and IVS-nt1 (G→T), codon 15/Cd15 (TGG $^{\text{Triptophan}}\rightarrow$TAG$^{\text{stop}}$), codon 26 Hb E (GAG$^{\text{Glutamate}}\rightarrow$AAG$^{\text{Lysine}}$) and Hb Malay/Codon 19 (AAC$^{\text{asparagine}}\rightarrow$AGC $^{\text{serine}}$) (19). Different types of mutation can give different clinical features. Each ethnic group had its own particular β-thalassaemia alleles (11-14). The frame shift mutation at codon 41/42(-TTCT) is a common allele in Southern China and East Asian populations have been described in a Spanish patient in Canary Islands (20) and in an Indonesian patient from Jakarta (18) This mutation was first described in a Taiwanese patient and was also found in Koreans (5.3% of thalassaemia cases) suggesting this mutation was introduced by a gene-flow from Southern China (21). A scoring system that reflects the clinical severity of β-thalassaemia/Hb E has been adopted (22).

The aim of the study was to report compound heterozygosity for 41/42 (-TTCT) with codon 26 (G→A) mutation in a Javanese sibling and the mutations inherited from parents with either β-thalassaemia or Hb E traits.

METHODS

The study received ethical approval from the Health Research Ethical Committee (No. 226/TGL/KEPK FK USU-RSUP HAM/2017) Faculty of Medicine, University of Sumatera Utara/Haj Adam Malik Hospital, Medan, Indonesia. Patient Informed Consent was obtained and siblings below 18 years old, approval was given by their parents'.

Subjects. Two families and their siblings were recruited from the Thalassaemia Ward of the Adam Malik Hospital before blood transfusion of the affected siblings. The first family (Bataks) had five siblings and the second family (Javanese) had two siblings making a total of eleven patients. The parents of the families were carriers of either β-thalassaemia or Hb E (see Table 1).

Blood collection and Laboratory investigation. From a clean venepuncture about 3 mL of blood each was drawn into two vacutainer tubes containing EDTA anticoagulant. Each tube of EDTA blood was used for Full Blood Count, mean cell volume (MCV), mean cell haemoglobin (MCH) determined in the Sysmex XN-1000 analyser and peripheral blood morphology performed. Haemoglobin analysis using capillary electrophoresis kits (Minicap, Sebia, France) were also determined. Detection of beta globin chain mutation by DNA Sequencing and amplification refractory mutation systems-polymerase chain reaction (ARMS-PCR) performed by Eijkman Institute of Molecular Biology, Jakarta, Indonesia.

Mahidol scoring system (22). A scoring system that reflects the clinical severity of β-thalassaemia/Hb E consisting of 6 clinical criteria of total score of 0-3.5 (mild), 4-7 (moderate and 7.5-10 as severe was performed. The type of beta-globin gene mutations of the two affected siblings with β-thalassemia/Hb E from the two families were compared.

RESULTS

Characteristics of the two families and their siblings with β-thalassaemia and Hb E gene mutations

The characteristics of the parents and siblings with inherited gene mutations are shown in Table 1. The fifth child age 9 years from the first family and first child 10 years old from the second family inherited both mutations from their parents and diagnosed to have compound heterozygosity. Two siblings from the first family did not inherit mutations from the parents were considered normal (40%) and one from the second family (50%).

Table 1: Characteristics of the two families and their siblings with beta-thalassaemia and haemoglobin E traits and gene mutations.

	Diagnosis	Sex	Age (years)	Gene mutation
Family-1 (Bataks)				
Father	β-Thalassemia trait	Male	50	IVS-1-nt5 (G → C)
Mother	Hb-E trait	Female	46	Codon 26 (G → A)
1st Child	Hb-E trait	Male	26	Codon 26 (G → A)

2nd Child	Normal	Male	24	normal
3rd Child	β-Thalassemia trait	Male	23	IVS-1-nt5 (G → C)
4th Child	Normal	Female	12	normal
5th Child	β-Thalassemia/Hb-E	Female	9	IVS-1-nt5 (G → C) Codon 26 (G → A)
Family-2 (Javanese)				
Father	Hb-E trait	Male	32	Codon 26 (G → A)
Mother	β-Thalassemia trait	Female	32	Codon 41/42 (-TTCT)
1st Child	β-Thalassemia/Hb-E	Female	10	Codon 26 (G → A) Codon 41/42 (-TTCT)
2nd Child	Normal	Male	8	Normal

However, three siblings inherited mutations from the first family (60%) and one from second family (50%). One sibling from both first and second family inherited both mutations β-thalassaemia/Hb E (compound heterozygosity) from their parents (20% and 50%) respectively. The mutations from these two siblings were different IVS-1-nt5(G→C) with codon 26 (G→A) first family and the first child from the second family, Codon 41/42 (-TTCT) with codon 26 (G→C). Codon 41/42 (-TTCT) results in the absence of beta-globin chain synthesis (β^0) while IVS1nt5 (G→C) mutation result in reduced beta-globin chain synthesis (β^+). Both the siblings require regular blood transfusion. The severity of the disease in these two siblings are shown in Table 3.

Table 2: The haematological levels and peripheral blood morphology of the two families with thalassemia.

Hb	MCV g/dL	MCH fL	Hb-A pg	Hb-A2 %	Hb-E %	Hb-F %	Peripheral Blood morphology %	
Family-1 (Bataks)								
Father	13.2	68	21.0	95.1	4.9	-	-	hypochromic microcytic, target cells, ovalocytes
Mother	11.9	72	24.5	73.6	3.5	22.9	-	hypochromic microcytic
1st Child	13.4	75	24.1	72.6	3.9	23.5	-	hypochromic microcytic, target cells
2nd Child	17.4	89	31.0	96.8	3.2	-	-	normochromic normocytic
3rd Child	12.2	65	20.0	94.8	4.9	-	0.3	hypochromic microcytic, target cells, ovalocytes
4th Child	12.7	83	28.6	97.1	2.9	-	-	normochromic normocytic
5th Child	7.3	70	23.1	75.3	3.5	15.6	5.6	hypochromic microcytic, target cells, schistocytes, tear drop cell
Family-2 (Javanese)								
Father	14.8	76.3	25.3	70.7	3.2	26.1	-	hypochromic microcytic, target cells, ovalocytes
Mother	11.4	60.2	18.8	93.5	6.2	-	0.3	hypochromic microcytic
!st child	7.3	75.5	25.2	72.4	3.8	16.7	7.1	hypochromic microcytic, target cells, schistocytes, tear drop cell,
2nd Child	12.6	81.5	27.4	97.0	3.0	-	-	normochromic normocytic

The haematological levels and peripheral blood morphology of the two families with thalassaemia

The haematological analysis showed that the two siblings with β-thalassaemia/Hb E had low haemoglobin levels before blood transfusion. The mean haemoglobin levels for the fifth child from the first family before blood transfusion was 7.3 g/dL (range 5.5 – 10.1 g/dL) and the first child from the second family 5.4 g/dL (range 3.2 -8.3 g/dL). The families affected by the disease did not require any blood transfusion as recorded. Lower levels of MCV, MCH were seen in the affected patients and peripheral blood morphology showed the hypochromic microcytosis and variable red blood cells shapes. (Table 2).

Mahidol Score between the two siblings with compound heterozygosity β-thalassaemia/Hb E

The scoring system reflecting the clinical severity of β-thalassaemia/Hb E showed that the sibling from the first family had a score of 5.5 which is moderate in clinical severity compared to the sibling from the second family with 7.5 score reflecting the severity of the disease (Table 3). This could be explained that within seven months of the disease the sibling from the first family received a total of 1300 cc packed red cells compared to 3400 cc for the other sibling from the second family. This would suggest that codon 41/42 (-TCTT) mutation compound heterozygosity had a more severe effect in the Javanese sibling. However, the gene flow of this patient family was not determined.

Table 3: Mahidol Score between the two siblings from families 1/2 with compound heterozygosity β-thalassaemia/Hb E.

Criteria	Family-1	Score	Family-2	Score
Steady state haemoglobin g/dL	7.53	0	5.7	2.0
Age of Onset (years)	4.0	0.5	6.0	0.5
Age (years) at first transfusion	4.0	1.0	4.0	1.0
Need for transfusion (weeks)	4 - 5	2.0	1 - 2	2.0
Spleen size (cm)	6.0	1.0	10.0	1.0
Growth retardation	+	1.0	+	1.0
Total score:		5.5		7.5
Interpretation:	Moderate		Severe	

Discussion

Thalassaemia, an inherited autosomal recessive gene disorder is the commonest monogenic disorder worldwide. It is a group of anaemias that caused by decrease or absence of one or two types of globin chains of adult haemoglobin. β-thalassaemia is very heterogenous at the molecular level and β-thalassaemia mutation are relatively population specific (10-13). Haemoglobinopathies are the most common genetic disorders in Southeast Asia. Compound heterozygosity of β-thalassaemia/Hb E is common in patients

displaying a wide spectrum of severity causing a major public health problem in Southeast Asia (15,16).

Our study on two families of ethnic origin Bataks and Javanese whose parents were carriers for either β-thalassaemia or Hb E, three siblings (two from first family) showed that they did not inherit the mutation suggesting 40% to 50% of the siblings are normal. Three siblings from the first family and one from the second family inherited the mutation suggesting 50% to 60% of siblings will inherit the mutation. One sibling from each family inherited both mutations from their parents suggesting that 20% to 50% of the siblings may develop the more severe form of the disease. This incidence of inheritance from parents with β-thalassaemia traits from our study may form a probable prediction in family inheritance while a bigger study need to confirm this. β-thalassaemia/Hb E is very heterogenous was seen in the fifth child of the first family had gene mutation IVS-1-nt5 (G→C) with codon 26 (G→A) and the first child from the second family codon 26 (G→A) with codon 41/42 (-TTCT) both were compound heterozygosity. Mahidol score was applied to reflect the severity of the disease. The sibling with gene mutations IVS-1-nt5 (G→C) with codon 26 (G→A) scored 5.5 suggesting moderate thalassaemia/Hb E and gene mutations codon 26 (G→A) with codon 41/42 (-TTCT) scored 7.5 suggesting a severe disease. This was further confirmed by blood transfusions where the sibling with the later mutations (severe) require a larger red blood cell volume transfusion than the former (moderate) within the same period. Both siblings require regular blood transfusions as treatment with the moderate severity sibling receiving transfusions between 4 to 5 weeks whilst the severe form needs 1 to 2 weeks. This suggest that codon 41/42 (-TTCT) mutation causes clinical severity in β-thalassaemia/Hb E. The frameshift codon 41/42(-TTCT) mutation is commonly seen in patients from South China or East Asia and in a patient from the Spanish Canary Islands (20) and was reported in a patient with β-thalassaemia from Jakarta, Indonesia previously but ethnicity was not described (18). The presence of frameshift codon 41/42 (-TTCT) in a Javanese family with compound heterozygosity in our study add to the rare find or first report in Indonesian patient with β-thalassaemia/Hb E. The gene flow of this family was not determined.

The prevention and control of thalassaemia requires well-planned programme to educate and establish the epidemiology of the disorders and raise the awareness of genetic risk or genetic counselling among the medical professionals and the population at large.

Acknowledgement

We wish to express our sincere gratitude to Mrs Julida Sembiring for her technical assistance and to Eijkman Institute for Molecular Biology, Jakarta, Indonesia for performing the DNA analysis.

REFERENCES

1. Angastiniotis M, Modell B, Englezos P, Boulyjenkov V. Prevention and control of haemoglobinopathies. Bull World Health Organ 1995; 73 (30;375-86.
2. Ikehara K. Advances in the study of genetic disorders, Europe. In Tech 2011; p101
3. Wasi P. Hemoglobinopathies in Southeast Asia. In: Brown JE ed. Distribution and evolution of haemoglobin and globin Loci. New York, Elsevier1983:179-203.
4. Fucharoen S, Winichagoon P. Hemoglobinopathies in Southeast Asia. Hemoglobin 1987; 11:65-8.
5. Fucharoen S, Winichgoon P. Hemoglobinopathies in Southeast Asia. Indian J Med Res 2011; 134(4):498-506.
6. Rathod DA, Kaur a, Patel V et al. Usefulness of cell counter-based and formulas in detection of β-thalassaemia trait in areas oh high prevalence. Am J Clin Pathol 2007; 128 (4):585-9.
7. Ganie RA. Distribution of (alpha and beta) thalassaemia trait and haemoglobin E in Medan. Majalah

Kedokteran Nusantara 2008; 41 (2):117-22.

8. Clarke GM, Higgins TN. Laboratory investigation of hemoglobinopathies and thalassaemia: reviews and update. Clin Chem 2000; 46 (8 Pt 2):1284-90.

9. Weatherall DJ, Clegg J. The thalassaemia syndromes. Oxford: Blackwell Scientific, 2001; 289:422-39.

10. Furuumi H, Firdous N, Inoue T et al. Molecular basis of α-thalassaemia in the Maldives. Hemoglobin 1998; 22:141-51.

11. Setiangsih I, Williamson R, Dand D, Harahap A, Marzuki S, Forrest S. Phenotypic variability of Filipino α(0)-thalassaemia/Hb E patients in Indonesia. Am J Hematol 1999; 62:7-12.

12. Colah R, Gorashakar A, Nadkarni A et al. Regional heterogeneity of α-thalassaemia mutations in the multi ethnic Indian ao. Blood Cells Molecules Dis 2009; 42:241-8.

13. Colah R, Gorashakar A, Phanasgaonkar S et al. Epidemiology of α-thalassaemia in Western India mapping the frequencies and mutations in sub-regions of Maharashtra and Gujarat. Br J Haematol 2010; 149:739=47. p431-69.

14. Steierg M. Disorders of haemoglobin. Camridge: Cambrige Uiversity Press 2001; p43-69.

15. Olivieri NF, Thayalsulthan V, O'Donnell A et al. Emerging insights in the management of haemoglobin E beta-thalassaemia. Ann NY Acad Sci 2010; 1202:155-7.

16. Premawardhena A, Fisher CA, Olivieri NF et al. Hemoglobin E β-thalassaemia in Sri Lanka. Lancet 2002; 366:1467-70.

17. Cao A, Galanello R. Beta-thalassaemia. Genet Med 2010; 12 (2):61-76

18. Lie-Injo LE, Cai SP, Wahidijat I et al. β-thalassaemia mutations in Indonesia and their linkagte yo β-haplotypes. Am J Hum Genet 1989; 45:971-.5.

19. Wahidiyat P, Gatot D, Tjitrasari T. Phenotypic diversity in beta-Hb E thalassaemia patients. Pediatrics Indonesia (Internet) 2006 March cited 11 June 2017 46 (3-4):82-86. www.paediatricaindonesiana.org/ijdex.php/paediatrica-indonesiaana/article/view/906/749.

20. Ropero P, de la Iglesia S, Jose M et al. Origin of the frameshift codons 41/42 (-TCTT) mutation in the first cases described in the Spanish population. Hemoglobin 2008; 32 (5):515-9.

21. Park SS, Lee VJ, Kim JV et al. β-thalassaemia in the Korean population. Hemoglobin 2002; 26 (2):135-43.

22. Sripichai O, Makrasara W, Munkongdee T et al. A scoring system for the classification of β-thalassaemia/Hb E disease severity. Am J Hematol 2008; 83:4832-4. Med 2000; 28:2773-8.

23. Warren BL, Eid A, Siager P et al. Caring for the critically ill patient: high-dose antithrombin III in severe sepsis: a randomized controlled trial. JAMA 2001; 286:1869-78.

24. Clet'h C, Fesriene F, Karoubi P, Fosse JP, Cupa M et al. Diagnostic and prognostic value of procalcitonin in patients with septic shock. Crit Care Med 2004; 32:1166-9.

25. Otto GP, Soselorf M, Claus RA, Rodel J, Menge K et al. The late phase of sepsis is characterized by an increased microbiological burden and death rate. Crit Care 2011; 15:IR 183.