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Hemoglobin profiles of siblings of thalassemia patients

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Abstract

Background Thalassemia and hemoglobinopathies are the most common inherited disorders in many areas of the world, including South East Asia. The siblings of thalassemia major is a group of high risk to carry the gene of thalassemia. Determining the carrier is useful for early treatment planning and prevention to the next child.

Objective To determine carrier status among siblings of thalassemia patients using a capillary electrophoresis system.

Methods A cross-sectional study on the siblings of thalassemia major patients was performed from January 2011 to February 2012 at Dr. Moewardi Hospital. Complete blood counts were performed in the siblings. Subjects with mean corpuscular volume (MCV) <80 fl and mean corpuscular hemoglobin (MCH) <27 pg were subjected to analize hemoglobin fraction by capillary electrophoresis.

Results Of the 26 subjects, there were 12 males and 14 females. The mean age was 9.38 (SD 6.8) years (range 1 to 29 years). From the siblings, 10 were identified as normal, 5 were identified as ß thalassemia carriers and 5 were hemoglobin E (HbE) carriers. Six siblings were diagnosed with ß thalassemia/ HbE.

Conclusion There are high occurrence of the two common types of thalassemia carriers (ß and HbE) in our small group of subjects who had a family history of thalassemia. Most of the siblings of thalassemia had low MCV and MCH. **[Paediatr Indones. 2015;55:70-3.]**.

Keywords: *thalassemia, screening, sibling, capillary electrophoresis*

Thalassemia and hemoglobinopathies are the most common inherited disorders among humans and represent a major public health problem in many areas of the world, including South East Asia. *The World Health Organization* (WHO) reported that 250 million people worldwide (4.5%) carrying thalassemia genes and that 300,000 – 400,000 babies with severe forms of these diseases are born each year. In South East Asia carrier of hemoglobinopathies rates may exceed 60% of the population.[1] Among the structural hemoglobin varians, HbE is the most common type. A high incidence of HbE (more than 50%) has been reported.[2] In Thailand and other South East Asian countries, the HbE is very common, with 20–30% of the population being α-thalassemia carriers, 39% β-thalassemia carriers, and 20–30% HbE carriers.[3]

The incidence of thalassemia is still high due to associated gen factors and inadequate screening. The

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screening of thalassemia siblings are crucial because they are usually asimptomatic and unless diagnosed by laboratory testing they might be unaware of the carrier status.[4,5] Most subject with HbE thalassemia carrier usually have low MCV and MCH and should not be missed screen especially in South East Asia region where the prevalence is high.[6,7]

A previous study measured hemoglobin fractions with a capillary electrophoresis system as it is fast, accurate, precise, and do not require a large volume of blood. The advantages of this method are the ability to separate hemoglobin fractions quantitatively into HbA2, HbE, and HbF. This is important to diagnose thalassemia and hemoglobinopathies.[8]

Until 2012, there were fifty thalassemia patients in the Department of Child Health, Faculty of Medicine Sebelas Maret University, Surakarta and a study on the profiles of hemoglobin on the siblings of thalassemia patients has never been done. The aim of screening for thalassemia and Hb disorders is to offer carrier testing to the siblings of thalassemia, ideally before they have children, in order to identify carrier couples and inform them of the risk and their options. This study was aimed to reveal the hemoglobin analysis on the siblings of thalassemia patients using capillary electrophoresis system.

Methods

This cross-sectional study was conducted at Dr. Moewardi Hospital from January 2011 to February 2012 on 26 siblings of thalassemia patients. Informed consent was obtained and each participants received a written report of the test results of the screening test. Exclusion criteria were congenital heart abnormalities, hypertension, and diabetes mellitus.

The primary screening was samples with mean corpuscular volume (MCV) <80 fl and mean corpuscular hemoglobin (MCH) <27 pg and those samples were further studied in the secondary screening as capillary electrophoresis using capillary electrophoresis *Sebia Minicap*. The handling and storage of blood samples were complete blood count (CBC) and red blood cell (RBC) indices using an *Advia 120* automatic cell counter.

Subject categorized into four groups: β-thalassaemia/HbE (subject who had HbE + HbA2 = 25-80%, HbF = 6-50%, HbA = 5-60%), β-thalassaemia carriers (subject who had HbA2 >3.2%, HbF 0.5- 6%), Hb E carriers (subject who had HbA2 <3.2, HbF <0.5, HbE >20%), and normal (Hb and MCH normal).

The principle of capillary electrophoresis is using buffer as liquid-flow electrophoresis. The separation of the fractions of hemoglobin using the principle of electroosmotic flow on alkaline pH (9.5). The hemoglobins were measured at 415-nm wavelength. The separation can be done in multiple samples for eight minutes. The results with an electropherogram divided into 15 zones based on standardizing the location of HbA. Hemoglobins normal and variant are displayed as peaks and the zone to which a variant belongs is identified automatically.

Results

The data that were collected from the siblings of thalassemia major patients showed that of the 26 subjects who responded for thalassemia screening, the majority were female. The youngest age was 1 year old and 29 years old as the eldest.

Twenty two out of 26 subjects had MCV < 80 fl and MCH < 27 pg. There were 6 with ß thalassaemia/ HbE, 5 with ß thalassaemia carriers, and 5 with Hb E carriers (subject who had HbA2 <3.2, HbF< 0.5, HbE> 20%) (**Table 1**). Ten subjects with hypochromic microcytic but with normal hemoglobin

Table 1. Characteristics and hematological features of subjects

Characteristics	N
Age	
<10 years	15
10-20 years	9
>20 years	2
Gender	
Male	12
Female	14
Primary screening	
MCV< 80 fl, MCH< 27 pg	22
Normal	4
Secondary screening	
β thalassemia/ HbE	6
β thalassemia carrier	5
HbE carrier	5
Normal	10

analysis finding. These 10 children received a trial of iron therapy with the laboratory tests repeated after a month. The mean Hb, MCV, MCH and Hb A2 values in the β-thalassemia/ HbE subjects were 7.3 (SD 1.2) g/dL, 60.5 (SD 10) fl, 18.2 (SD 1.4) pg, 52 (SD 11) %, respectively; whereas in normal subjects they were 12.9 (SD 0.7) g/dL, 79.3 (SD 4.0) fl, 27.1 (SD 1.4) pg, 2.7 (SD 0.2) %, respectively (**Table 2**). Six siblings were identified as β thalassemia/ HbE (**Table 3**).

Discussion

The screening protocol for thalassemia usually relies on using a complete cell blood count. Subjects with low MCV (<80 fl) and MCH (<27 pg) usually have further investigation using electrophoresis.[9] Carriers of HbE, however, should not be missed by screening especially in South East Asian communities where the prevalence is high.[7] The people targeted by screening are the sibllings of thalassemia because they are one of the highest risk group of thalassemia. Population screening for carrier detection has been advocated to reduce its occurrence and is being practiced in many countries, especially in Indonesia.

Table 2. Mean hematological features of β-thalassemia/ HbE and normal subjects

Hematological parameter	β-thalassemia/ HbE	Normal
Hb (SD), g/dL	7.3 (1.2)	12.9 (0.7)
MCV (SD), fl	60.5 (10)	79.3 (4.0)
MCH (SD), pg	18.2 (1.4)	27.1 (1.4)
HbA2+ HbE (SD), %	52 (11)	2.7 (0.2)

Table 3. Hematological data of six cases of β-thalassemia/ HbE

Cases number	Hb (g/dL)	MCV (fl)	MCH (pg)	HbA2+HbE (%)
2	8.0	52.1	16.5	61.3
10	8.6	50.8	16.4	47.6
16	7.7	58.1	18.5	50.4
20	7.6	59.0	18.9	67.6
23	5.1	64.8	18.7	36.2
26	7.0	78.0	20.0	48.7

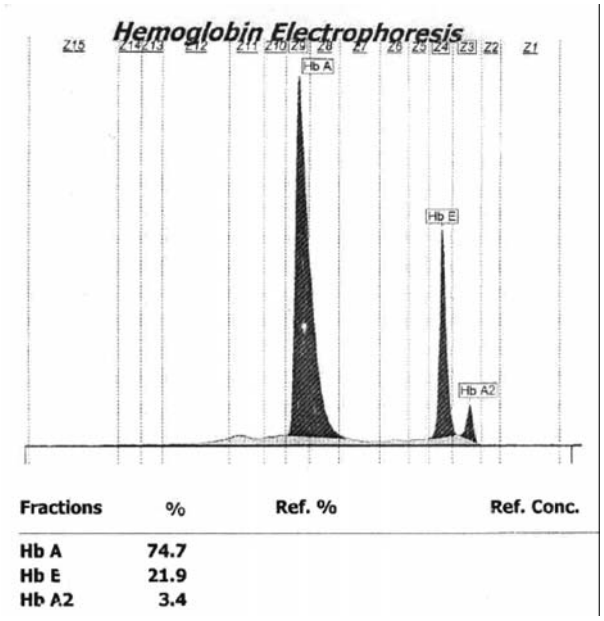

Figure 1. Results of hemoglobin electrophoresis in a blood sample of HbE carrier

The identification of ß thalassemia minor is essential for two reasons. Firstly, to differentiate it from iron deficiency since both present as microcytosis and hypochromia. Secondly for prevention of ß thalassemia major by genetic counseling. Through genetic counseling birth rate of ß thalassemia major can be reduced by as much as 90%.[1] Hemoglobin electrophoresis is essential for definitive diagnoses of ß thalassemia carriers. The HbA2 is the important finding to diagnose ß thalassemia carriers.[10] Carriers of ß thalassemia usually have an elevated concentration of HbA2 (>3.5%) with or without an elevated concentration of HbF (>1.5%), as determined by hemoglobin electrophoresis. By contrast, α thalassemia carriers have normal hemoglobin electrophoresis.[11]

Some studies using the capillary electrophoresis system *SEBIA* obtained result of measuring the frac-

tions of hemoglobin and variant hemoglobin as easy and accurate, with high resolution performance.[12,13] The screening strategy is therefore simple, reliable, cost-effective, and practical. Using this strategy for a population-based screening program in primary health care settings should facilitate prevention and control of thalassemia and hemoglobinopathies in most South East Asian communities.[14]

Out of 26 subjects studied, 10 had presumptive ß thalassemia/HbE carriers and 6 siblings were identified as asymptomatic ß thalassemia major/intermedia on the siblings of thalassemia found presumptive β thalassemia carrier and HbE carrier. One way of achieving this goal is to screen the population at risk and instruct the identified carriers of the genetic implications.

We concluded that there are high occurrence of the two common types of thalassemia carriers (ß and HbE) in our small group of subjects who had a family history of thalassemia. Most of the siblings of thalassemia had low MCV and MCH.

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Conflict of interest

None declared

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