Cytogenetic mutation in a family with sickle-cell beta-thalassemia in North Sumatera, Medan, Indonesia: A preliminary study

Christie Nur Andani1*, Adi Koesoema Aman1, Herman Hariman1, Bidasari Lubis2

ABSTRACT

Background: The prevalence of Hb S/β-Thalassemia has not been widely reported, and until now, there is a lack of statistical data on the prevalence of the disease in Indonesia. In 1974, cases of β-thalassemia with Hb S were found in one family in Jakarta. The aim of this study was to describe the cytogenetic mutation in a family with Hb S/β-thalassemia in North Sumatera.

Methods: This was a descriptive study with a cross-sectional design conducted in one family of HbS patients with thalassemia trait, with a total of five samples. Complete blood count, hemoglobin electrophoresis examination, peripheral blood smear, PCR, and amplicon were performed to describe the hematology and cytogenetic profile of samples. Data were analyzed descriptively.

Results: The father (64 years old) suffered from HbS (HbA = 58.3%, HbS = 38.3%, HbA2 = 2.8%) with heterozygote HbS mutation type. The mother (36 years old) suffered from β-thalassemia trait (HbA = 93.8, Hbf = 1.2%, HbA2 = 5%) with β-thalassemia heterozygote IVS1-nt5 mutation type. The first child (male, 18 years old) suffered from HbS/β-thalassemia (HbA = 25.9%, HbF = 22.4%, HbS = 48.2%, Hbf = HbA2 = 3.5%), the second child was normal (HbA = 97.4%, HbA2 = 2.6%) and the third child (female, 10 years old) suffered from HbS β-thalassemia (HbA = 3.3%, Hbf = 25.5%, HbS = 67.6%, HbA2 = 3.6%). The first and third child had β-thalassemia with double heterozygote HbS and IVS1-nt5 mutation type.

Conclusion: Population migration between nations followed by marriage could lead to cytogenetic mutations and cause sickle-cell beta-thalassemia. The finding of double heterozygous HbS mutation and beta thalassemia need to be explored further about the patient's fathers family.

Keywords: Beta-thalassemia, HbS mutation, Indonesia


BACKGROUND

Sickle cell disease is a genetic disease with hemoglobin abnormality that causes vascular occlusion, which ultimately damages the multi-system of the human body. This genetic abnormality results from a single amino acid substitution in the gene encoding the β-globin subunit (6 Glu > Val) that produces abnormal haemoglobin (Hb S). The substitution of hydrophilic glutamic acid in the sixth position of β-globin by hydrophobic valine residual forms a hydrophobic interaction in β-chain of another deoxy Hb S molecule. Polymere shape and longways in the helix fiber are classified together, stiffness, and induct red blood cell change.

Hb S was found in the Mediterranean region, African Saharan desert region, the Middle East and India. Every year, around 300,000 babies are born with homozygous sickle cell disease, and the number is estimated to rise to 400,000 cases in 2050. More than 75% of sickle cell disease was found in Sub-saharan Africa. The United Nations has announced sickle cell disease as a global health problem and the WHO recommends 50% of their members to create sickle cell disease control in 2020.

Sickle cell disease was sporadically found in Indonesia by Lie Injo Luan Eng. He explained that in the nineteenth century (1835 to 1890), a few thousand African soldiers became a part of the Dutch colony in the previous Hindia Holland. The selection of African soldiers was possibly the main reason for the sporadic discovery of sickle cell disease in Indonesia. Following the discovery of sickle cell disease, cases of β-thalassemia with Hb S were found in one family in the Pediatric Department of the University of Indonesia, Jakarta in 1974. Thalassemia is one of the eight most catastrophic diseases. According to the Indonesian Thalassemia Foundation and Parents of Thalassemic Patient Association, the prevalence of this disease had
increased from 2011 to 2015. In 2015, the number of thalassemia patients reached 7,029. Screening results in the community from 2008 to 2014 found 93 (5.41%) thalassemia carriers in 1,718 people. Screening results in thalassemia family from 2009 to 2014 found 93 (28%) thalassemia patients in 332 people.  

The combination of sickle cell mutation and β-thalassemia mutation generates a compound heterozygous condition known as Hb S/β-thalassemia (Hb S/β-Thal), which was described at first by Silverstroni and Bianco in 1944. However, the prevalence of Hb S/β-Thalassemia has not been widely reported. To date, there is a lack of statistical data on the prevalence of the disease in Indonesia.

**METHOD**

This was a descriptive study with a cross-sectional design conducted by the Department of Clinical Pathology, Faculty of Medicine, University of North Sumatera, in collaboration with the Department of Pediatrics, Faculty of Medicine, University of North Sumatera in H. Adam Malik General Hospital Medan and Eijkman Institute for Molecular Biology Jakarta for the detection of mutations in the β-globin gene. The study began in April 2018 and ended in June 2018. The target population was patients with Hb S who sought treatment at H. Adam Malik General Hospital together with their parents and siblings. This study was conducted in a family consisting of 5 people who had Hb S with thalassemia trait.

The measured profiles were complete blood count, peripheral blood smear, hemoglobin electrophoresis, PCR and amplicon examination. Complete blood count consisted of hemoglobin levels, platelets, erythrocyte, and leukocyte count using the Sysmex XN-1000 machine. Hemoglobin electrophoresis examination was performed to determine the type of hemoglobin by capillary electrophoresis method using the Minicap Sebia machine. PCR 293 bp was performed as a technique of multiplication (amplification) of DNA pieces in vitro in a specific area that was limited by two oligonucleotide primers in the range of 293 bp. Amplicon (293 bp) was used to detect IVS-1 nt5 (G > C), IVS-1 nt1 (G > T), codon26 / HbE (G > A) and codon 15 (G > A).

**RESULTS**

A total of 5 people, who were family members, were included in this study. Characteristic of samples can be seen in Table 1.

As shown in Table 2, hemoglobin levels were low in the third child (7.5 g/dL) and first child (9.3 g/dL). The erythrocyte index showed that low MCV levels were found in the mother (63.1 fL), first child (66 fL) and third child (59.5 fL). Low MCH levels were found in the mother (20.4 pg), first child (22.3 pg) and third child (20.5 pg). In contrast, both MCV and MCH levels in the father and second child were normal. Leukocyte and platelet counts were normal in all family members.

A peripheral blood smear of each member of the family was examined. The results can be seen in Figure 1. The second child was not examined for peripheral blood smear because she had a normal hematology profile (Table 2) and hemoglobin electrophoresis (Table 3).

As shown in Table 3, there were decreased levels of HbA in the father (58.3%), mother (93.8%), first child (25.9%) and third child (3.3%). However, the HbA level in the second child was normal. The HbF level obtained in the mother was 1.2%, first child was 22.4%, and third child was 25.5%. The HbS level found in the father was 38.9%, first child was 48.2%, and third child was 67.6%. There was an increase in HbA2 levels in the mother (5%), first child (3.5%) and third child (3.6%).

The results of genetic mutation examination using the PCR 293 bp method and DNA sequencing can be seen in Table 4.

As shown in Table 4, it can be ascertained that the father was the carrier of nature/beta-thalassemia trait with the type of Hb S mutation (Codon 6, GAG[Glu]→ GTG[Val] heterozygote). It can be ascertained that the mother was the carrier of character/beta-thalassemia trait with the type of heterozygous IVS1-nt5 (G > C) mutation. It can be determined that the first and third child were

**Table 1.** Baseline characteristic of samples

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gender</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td>Male</td>
<td>65 years old</td>
</tr>
<tr>
<td>Mother</td>
<td>Female</td>
<td>40 years old</td>
</tr>
<tr>
<td>1st Child</td>
<td>Male</td>
<td>18 years old</td>
</tr>
<tr>
<td>2nd Child</td>
<td>Female</td>
<td>16 years old</td>
</tr>
<tr>
<td>3rd Child</td>
<td>Female</td>
<td>11 years old</td>
</tr>
</tbody>
</table>

**Table 2.** Hematology profile of samples

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Father</th>
<th>Mother</th>
<th>Child-1</th>
<th>Child-2</th>
<th>Child 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>15.3</td>
<td>11.3</td>
<td>9.3</td>
<td>14.0</td>
<td>7.5</td>
</tr>
<tr>
<td>RBC (10^6/µL)</td>
<td>5.15</td>
<td>5.53</td>
<td>3.80</td>
<td>4.83</td>
<td>3.65</td>
</tr>
<tr>
<td>WBC (10^3/µL)</td>
<td>10.43</td>
<td>7.44</td>
<td>10.97</td>
<td>8.54</td>
<td>10.26</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>43.4</td>
<td>34.9</td>
<td>32.3</td>
<td>42</td>
<td>21.7</td>
</tr>
<tr>
<td>Platelets (10^3/µL)</td>
<td>206</td>
<td>250</td>
<td>430</td>
<td>385</td>
<td>156</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>84.3</td>
<td>63.1</td>
<td>66</td>
<td>87</td>
<td>59.5</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>35.3</td>
<td>20.4</td>
<td>22.3</td>
<td>29.0</td>
<td>20.5</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>35.3</td>
<td>32.4</td>
<td>34.4</td>
<td>33.4</td>
<td>34.6</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>13.2</td>
<td>16.6</td>
<td>16.1</td>
<td>12.1</td>
<td>19.0</td>
</tr>
</tbody>
</table>
channel) causes potassium missing and cellular dehydration. As cellular dehydration increases, cellular Hb concentration will lead to HbS deoxypolimerasation.

Hb becomes denaturated, and the hemichrome is concentrated in the internal side of the membrane with cytoskeleton protein especially protein band 3. This process is happening simultaneously with missing of heme and Fe release that provoke oxidation in the microenvironment. IgG anti-band 3 is accumulated at protein 3 aggregate, inducing erythrophagocytosis by macrophages.

Endothelial damage which is from sub endothel structure can activate adhesion process. Some of the adhesion protein is activated from the extracellular stimulus. Vascular regulation depends on endothelium mediator like endothelin 1 (ET1) as a vasoconstrictor and nitric oxide (NO) as a vasodilator. In sickle cell disease, NO plasma is low and ET1 rise when VOC happen. Free Hb will obliterate NO, and free radical will be formed. Hemolysis also releases erythrocyte arginase-1 into plasma. Arginase metabolize plasma arginine into ornithine, decreasing required substrate which is needed to synthesize nitric oxide and combining bioavailability reduce nitric oxide in sickle cell disease. Hemolysis will degrade L-arginine, enzymatic substrate producing NO which is endothelial synthase.

The character of sickle cell trait is marked with a heritage of one normal genetic hemoglobin from the father/mother and one mutated globin gene from the father/mother. Sickle cell terminology show heterozygosity for sickle cell gene (ββS). This trait has no clinical symptom in the majority but has a majority in genetical concern. This will provide partial protection against deaths from malaria plasmodium falciparum.

Table 3. Hemoglobin electrophoresis of samples

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Father</th>
<th>Mother</th>
<th>Child-1</th>
<th>Child-2</th>
<th>Child-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb A</td>
<td>58.3%</td>
<td>93.8%</td>
<td>25.9%</td>
<td>97.4%</td>
<td>3.3%</td>
</tr>
<tr>
<td>Hb F</td>
<td>-</td>
<td>1.2%</td>
<td>22.4%</td>
<td>-</td>
<td>25.5%</td>
</tr>
<tr>
<td>Hb S</td>
<td>38.9%</td>
<td>-</td>
<td>48.2%</td>
<td>-</td>
<td>67.6%</td>
</tr>
<tr>
<td>Hb A2</td>
<td>2.8%</td>
<td>5.0%</td>
<td>3.5%</td>
<td>2.6%</td>
<td>3.6%</td>
</tr>
</tbody>
</table>

Table 4. Mutation type of samples

<table>
<thead>
<tr>
<th>Subject</th>
<th>Type of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td>HbS (Codon 6, GAG\textsuperscript{Glu\textsubscript{6}} \succ GTG\textsuperscript{Val\textsubscript{6}})</td>
</tr>
<tr>
<td>Mother</td>
<td>IVS1-nt5(G &gt; C)</td>
</tr>
<tr>
<td>Child-1</td>
<td>HbS (Codon 6, GAG\textsuperscript{Glu\textsubscript{6}} \succ GTG\textsuperscript{Val\textsubscript{6}}) IVS1-nt5(G &gt; C)</td>
</tr>
<tr>
<td>Child-2</td>
<td>Not checked</td>
</tr>
<tr>
<td>Child-3</td>
<td>HbS (Codon 6, GAG\textsuperscript{Glu\textsubscript{6}} \succ GTG\textsuperscript{Val\textsubscript{6}}) IVS1-nt5(G &gt; C)</td>
</tr>
</tbody>
</table>

Figure 1. (A) Father’s peripheral blood smear was normal; (B) Mother’s peripheral blood smear, target cell was found; (C) Second child’s peripheral blood smear was normal; (D) Third child’s peripheral blood smear, target cell, and sickle cell were found

patients with beta-thalassemia with the type of double heterozygous HbS mutation (Codon 6, GAG\textsuperscript{Glu\textsubscript{6}} \succ GTG\textsuperscript{Val\textsubscript{6}}) and IVS1-nt5 (G > C).

In the PCR examination using forward com C : ACCTCACCCCTGAGGAGC and using reverse : TLR 62320 : TAGCAAGGTATACA AGACAGGTATAGAGCACAAATAG

DISCUSSION

The long polymer creation induces several cellular abnormalities, which is part of its pathophysiology mechanism. Dysregulation of cation hemostasis as a consequence of several ions activation like co-transport K-Cl and K-Ca channel (Gardos

The differential count is normal at birth, but in the early neonatal period, hemoglobin S is relatively low. Since the first year, hemoglobin F is replaced by hemoglobin S. There is reduced hemoglobin concentration and increased in total reticulocyte. Reticulocyte distribution width (RDW) is generally increased and correlate with the severity of the disease. Neutrophil counts may increase between and during the crisis. Anemia sickle cell disease with high hemoglobin F presentation tend to get higher hemoglobin concentration and MCV.

A basic pathophysiologic defect in β-thalassemia means reduced or no β-globin chain production with relatively abundant α chain. The direct consequence is reduced hemoglobin production and imbalance globin chain synthesis. This process is termed as “not effective erythropoiesis” and become the characteristic of β-thalassemia.
Peripheral hemolysis which contributes to anemia is less prominent in thalassemia major than in thalassemia intermedia, and globin chains occur which cannot induce membrane damage in peripheral erythrocytes. The level of globin chain imbalance is determined by the nature of the mutation β gene.

Heterozygous β-thalassemia carrier usually shows low mean cellular hemoglobin (MCH), low average cell volume (MCV), and elevated HbA2 levels, which may be related to normal or slightly low hemoglobin levels. Peripheral blood images show morphological erythrocytes changes that are less severe than those affected. Major β-thalassemia is characterized by a decrease in hemoglobin level (< 7 g / dl); > 50 and < 70 fl of MCV; and > 12 and < 20 pg of MCH. Thalassemia intermedia is characterized by Hb level between 7 and 10 g / dl, MCV between 50 and 80 fl and MCH between 16 and 24 pg. Affected individuals show microcytosis, hypochromia, anisocytosis, poikilocytosis (teardrop and ovalocytes), target cells and erythroblasts. The number of erythroblasts is related to the level of anemia and markedly increases after splenectomy. Clinically, HbS-beta-thalassemia causes symptoms of moderate anemia and signs of sickle cell disease, which is usually less severe than sickle cell disease. Mild to moderate microcytic anemia usually appears.

Hb S / β 0-Thal, where the production of Hb A is insoluble, is often clinically indistinguishable from sickle cell disease, which causes microcytosis, hypochromia and sometimes Hb F increases. The HbS-β0 form in the hemoglobin analysis has no HbA fraction, whereas in HbS-β + there is an HbA fraction.

The distribution of hemoglobin concentrations is bimodal, which is higher in those with sickle / β + thalassemia cells than in sickle / β-thalassemia cells. Classic sickle cells are very rare, especially in sickle cells / b + thalassemia. There are several boat-shaped cells, hypochromia, and microcytosis. The target cell is prominent and basophilic stippling may be seen

In Hb S / β + -Thal, the variable number Hb A dilutes Hb S and effectively inhibits the age of cell damage due to polymerization. Hb A levels vary from < 5% to 45%. Levels of hemolysis and higher Hb A levels are usually associated with a mild phenotype.

In this case, the father can be ascertained as the carrier of beta-thalassemia with heterozygous HbS mutations with hematological results within normal limits, accompanied by decreased HbA (58.3%) and the presence of 38.9% of HbS on hemoglobin electrophoresis. In the mother, it can be ascertained that she was the carrier of beta-thalassemia with heterozygous type IVS1-nt5 mutations with decreased MCV and MCH levels and there was increased HbA2 levels and HbF in hemoglobin electrophoresis. Reduction mutation of globin beta triggers the synthesis of globin γ in the promoter of the globin gene and forms HbF. In the first and third children, it can be ascertained that they were beta-thalassemia patients with HbS and IVS1-nt5 double heterozygous mutations. Hematological examination found low Hb levels, low MCV and MCH with decreased HbA levels, increased HbA2, HbF, and HbS in hemoglobin electrophoresis. Decreased amount of beta from the beta globin chain produces alpha globin chains that do not bind and settle on erythroid precursors in the bone marrow and cause ineffective erythropoiesis.

In HbS / β + -Thalassemia, the variable amount of Hb A dilutes Hb S and effectively inhibits the age of cell damage due to polymerization. Hb A levels vary from <5% to 45%. The level of hemolysis and higher Hb A levels are associated with a lighter phenotype. Mutation of IVS1 nt5 (G>C) disrupts the splicing process in mRNA. Beta-thalassemia IVS1-nt5 mutations occur in the RNA consensus splice sites. Mutations in the sixth codons of β globin eliminate the recognition enzyme site for several recirculating enzymes that cut DNA in the CCTNAGG sequence. Where N represents nucleotides, replaces adenine with thymine, removing the side assessed by the enzyme restrictase.

Lie Injo Luan Eng predicted that the transport of African Soldiers was the cause of sporadic sickling genes in the 19th century in Indonesia. This was similar to the pattern of the spreading of HBs in Uruguay, which was thought to be due to the entry of African slaves from South Brazil to Northern Uruguay in the 19th century. High HbF levels (20%) describe the Arabic-Indian haplotype with mild clinical symptoms. According to a case report by Mohanvir in 2017, the clinical picture of HbS beta-thalassemia resembles thalassemia intermedia. The prognosis is better than major beta-thalassemia and sickle cell disease.

The HbS β-thalassemia case report, which was first reported in Jakarta in 1974, was found in one Chinese family. The father had HbS trait, the mother had beta-thalassemia trait, the first child was
normal, the second child had beta-thalassemia, and the third child had HbS beta-thalassemia. However, the study was limited and was not followed by an examination of DNA mutations in the patients.

The possibility of population migration between countries followed by marriage and the discovery of heterozygous double mutations of HbS and beta-thalassemia are needed to be examined further about the patient’s family.

CONCLUSION
Population migration between nations followed by marriage could lead to cytogenetic mutation and cause sickle-cell beta-thalassemia. The finding of double heterozygous HbS mutation and beta-thalassemia need to be explored further about the patient’s father’s family.

ETHICAL CLEARANCE
This study obtained ethical clearance from the Health Research Ethics Committee of the Faculty of Medicine, University of North Sumatera/H. Adam Malik General Hospital (No. 553/TGL/KEPK FUSU-RSUP HAM/2018).

CONFLICT OF INTEREST
No conflict of interest to disclose.

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AUTHOR’S CONTRIBUTION
Christie Nur Andani was the owner of the idea, wrote the manuscript, revised the final manuscript and performed the laboratory tests. Christie Nur Andani, Adi Koesoema Aman and Bidasari Lubis joined together to establish the study design, collected the clinical and laboratory data, analyzed and interpreted all of the results. Adi Koesoema Aman with Bidasari Lubis was in charge of the sampling process.

REFERENCES:

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