Original Article

Clinical features of patients with hemolytic anemia due to red blood cells membrane defect

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Abstract

Background Hemolytic anemia may result from corpuscular or extracorpuscular abnormalities. One of the types of corpuscular abnormalities is membrane defect. The diagnosis is sometimes difficult and it may need special hematologic investigations. There are no data yet on the clinical features of red blood cell membrane defect in Cipto Mangunkusumo Hospital.

Objective To evaluate the clinical features and laboratory findings of patients with hemolytic anemia due to red blood cells membrane defect in Cipto Mangunkusumo Hospital.

Methods This was a descriptive study on patients with red blood cells membrane defect who came to the Thalassemia Center at Cipto Mangunkusumo Hospital during 2002-2004.

Results In 2002-2004, there were 241 new cases of hemolytic anemia consisted of 116 patients with beta-thalassemia, 109 with HbE-beta thalassemia, 3 with alpha-thalassemia, and 13 with red blood cells membrane defect. The red cells membrane defect patients consisted of 4 males and 9 females, ranging in age from 1 months to 14 years. All subjects came to the hospital due to paleness as a chief compaint. Hepato-splenomegaly was found in 5 of 13 cases. Laboratory findings revealed hemoglobin level 6.4-13.1 g/dl (mean 9.4 ± 2.1 g/dl), MCV 58.4-94.5 fl (mean 81.2 ± 10.2 fl), MCHC 31.7-35.8 g/dl (mean 33.9 ± 1.1 g/dl), RDW 15.8-28.4% (mean $20.1\pm3.6\%$) and normal hemoglobin electrophoresis. Peripheral blood smear showed anisocytosis, poikilocytosis, spherocytes, ovalocytes, stomatocytes, target cells, and fragmented cells. The most common diagnosis in this group was Southeast Asian Ovalocytosis (5/13).

Conclusions In facing hemolytic anemia with normal Hb electrophoresis or normal RBC enzyme level, the possibility of red cells membrane defect should be taken into consideration as a cause of this disorder. The clinical features and laboratory findings of red blood cells membrane defect patients are highly variable. Occasionally, hematologic investigations are necessary **[Pediatr Indones 2006;46:41-45].**

Keywords: Hemolytic anemia, red blood cells membrane defect, thalassemia, DNA analysis

Hemolytic anemia may result from corpuscular or extracorpuscular abnormalities. One of the types of corpuscular abnormalities is membrane defect.[1] Clinical features of patients with red blood cells membrane defect are highly variable.[2,3] The diagnosis is sometimes difficult and it may need special hematologic investigations.[1] There is no data yet on the clinical features of red blood cells membrane defect in Cipto Mangunkusumo Hospital.

The aim of this study was to evaluate the clinical features and laboratory findings of patients with hemolytic anemia due to red blood cells membrane defect in Cipto Mangunkusumo Hospital.

Methods

This was a descriptive study on 13 patients with hemolytic anemia with normal Hb electrophoresis as well as red blood cells enzyme level which on

further examination showed red cells membrane defects who came to the Thalassemia Center at Cipto Mangunkusumo Hospital during 2002 until 2004. Complete blood cells count was done using Particle Counter PCE 210 (Erma Inc, Tokyo). Blood smears were stained by Giemsa and evaluated by light microscope. Hemoglobin electrophoresis was performed by HPLC programme. Red cells membrane defect was detected by DNA analysis. The study was approved by the Committee of Medical Research Ethics, Medical School, University of Indonesia.

Results

During the period of 2002-2004, there were 241 new cases of hemolytic anemia which consisted of 116 beta-thalassemia, 109 HbE-beta thalassemia, 3 alpha-thalassemia, and 13 patients with red blood cells membrane defect. The age of the patients with red cells membrane defect ranged from 1 month to 14 years old. Other clinical characteristics are shown on **Table 1**.

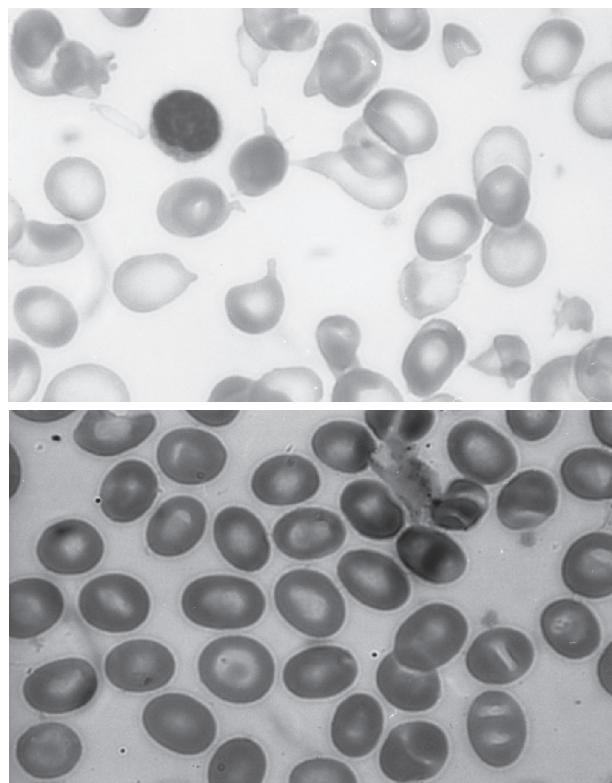Most of them were female (9 of 13 patients). All of the subjects came to the hospital due to paleness as a chief complaint. Hepato-splenomegaly was found in 5 of 13 cases. Laboratory findings revealed mild anemia, normal mean corpuscular volume (MCV), normal mean corpuscular hemoglobin concentration (MCHC), high red blood cell distribution width (RDW) and normal hemoglobin electrophoresis (**Table 2**). Peripheral blood smear showed anisocytosis, poikilocytosis, spherocytes, ovalocytes, stomatocytes, target cell, and fragmented cell (**Figures 1A** and **1B**).

FIGURE 1 A & 1 B. BLOOD SMEAR OF PATIENTS WITH HEMOLYTIC ANEMIA

TABLE 1. CHARACTERISTICS OF SUBJECTS

Variable	Total (n=13)
Gender	
Male	4
Female	9
Clinical features*	
Pale	13
Hepatomegaly	5
Splenomegaly	5
Hyperbilirubinemia	4
Repeated PRC transfusions	5
History of family	2

* Could be more than one

TABLE 2. LABORATORY FINDINGS

Variable	Range	Mean+SD
Hemoglobin (g/dl)	6.4-13.1	9.4±2.1
MCV (fl)	58.4-94.5	81.2±10.2
MCH (pg)	18.5-33.8	27.6±4.2
MCHC (%)	31.7-35.8	33.9±1.1
RDW (%)	15.8-28.4	20.1±3.6
Hemoglobin electrophoresis	Normal	

Discussion

The clinical features and laboratory findings of these 13 cases were consistent with hemolytic anemia. Other causes of hemolysis, such as hemoglobinopathy and enzyme deficiencies had been ruled out. Further examinations using DNA analysis showed RBC membrane defect. The most common diagnosis established in these cases was Southeast Asian ovalocytosis (5/13).

Southeast Asian ovalocytosis (SAO) is an asymptomatic trait characterized by oval red blood cells (RBCs) that are rigid and resistant to invasion by several malarial parasite strains.[4] Molecular characterization of band 3 protein is due to deletion of codons 400-408, resulting in the absence of 9 amino acids located at the boundary between cytosol and first transmembrane segment in band 3, the erythrocyte anion transport protein without changes in the secondary and quaternary structure of the protein.[5-9] Band 3 protein, the major integral protein of RBC membrane, consists of two domains with distinct structure and function.[4,8] The N-terminal cytoplasmic domain provides a binding site for ankyrin that anchors the spectrin-based membrane skeleton to the membrane. At the functional levels, SAO band 3 mutation is characterized by a decreased transport anions, a propensity to form linear aggregates in the membrane, an increased oligomerization of band 3, and an increased retention of SAO band 3 by the membrane skeleton.[4,10]

SAO band 3 mutation has an increased propensity to form oligomers, which appears as longitudinal strands of intramembrane particles and exhibits increased association with membrane skeleton. This band 3 oligomerization underlies the increase of membrane rigidity by precluding membrane skeletal extension, which is necessary for membrane deformation.[6] The membrane is 10 to 20 times more rigid than normal. SAO band 3 tightly binds to ankyrin and thus to the underlying skeleton.[10] Band 3 also has a common polymorphism, the Memphis-1 variant, in which the point mutation Lys $56\rightarrow$glu produces a protein with slower electrophoretic mobility and an abnormal chymotryptic fragment.[10,11] The Lys-$56\rightarrow$Glu-56 substitution, suggesting linked polymorphism.[9]

This abnormality is very common in Melanesia, particularly in lowland tribes, where malaria is endemic. In these tribes, 5 to 25% of the natives are affected.[4] Allen *et al*[12] showed that the prevalence of SAO in Papua New Guinea is 6%, Meck *et al*[2] found 15% in Malaysian population and Eijkman studies found 27.2% in Irian Jaya with the highest prevalence in Sumba Island (33%).[13] Southeast Asian Ovalocytosis is also found in an African-American family and in a South African.[11,14] The prevalence of SAO in our study was different than that of other studies due to different methodologies.

Affected heterozygous individuals are asymptomatic although one subject has been described to have compensated hemolysis.[10,14] Our patients showed different clinical features with more severe symptoms. Coetzer *et al*[14] reported SAO in South African children. All affected subjects exhibited evidence of hemolysis, ranging from severe transfusion-dependent to compensated hemolysis. One patient continued to show signs of hemolysis after splenectomy.[14] In our study, it is probable that it was in combination with another disorder. Further examination, including DNA analysis should be done.[15]

The absence of homozygosity in SAO band 3 mutation suggests a possibility that the homozygous state is incompatible with life. Although, the mechanism remains unclear. There was a 50% decrease in anion transport of SAO RBC suggesting that the mutant allele is not functional in terms of anion transport, hence may not be capable of chloride-bicarbonate exchange.[4]
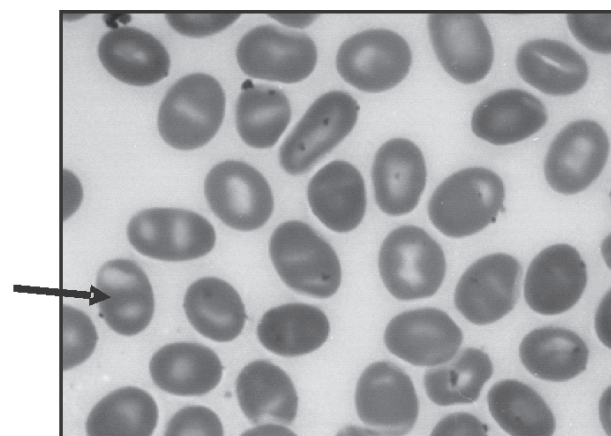

FIGURE 2. SOUTHEAST ASIAN OVALOCYTOSIS

Our subjects' blood smears showed that most red cells were rounded elliptocytes, yet a few were transversed by one or two transverse bars which divided the central clear space (**Figure 2**). These "elliptical knizocytes" or "stomatocytic elliptocytes" are not found in any other condition.[10]

Other diagnosis of red cells membrane defect in our study was spherocytosis in 2 of 13 patients. Hereditary spherocytosis (HS) is a common hemolytic anemia characterized by chronic hemolysis with broad spectrum clinical severity ranging from asymptomatic condition to life-threatening anemia requiring splenectomy.[16] Hassoun *et al*[16] described a

new truncated B spectrin mutant, caused by a point mutation involving the donor splicing site of intron 17, and resulting in a double exon skipping. They showed evidence of instability of the transcriptional message, the susceptibility of the truncated spectrin to proteolytic degradation that leads to the scarcity of the aberrant protein and ultimately to spectrin deficiency on the membrane.[16] Most of the mutations described are responsible for a phenotype of mild to moderate autosomal dominant form of HS associated with a conspicuous spherocytosis with frequent speculated cells (8% to 15% acanthocytes). One missense mutation appears to be associated with a recessive form of the disease.[17]

Spherocytic red cells result from loss of membrane surface area and, consequently, exhibit increased cell sphericity and reduced cellular deformability. Increased splenic sequestration of these indeformable spherocytic red cells results to anemia. One study showed HS reticulocytes level was consistently lower and hemoglobin concentration consistently higher than those of either normal or auto immune hemolytic anemia (AIHA) reticulocytes. The filtration function of the spleen is well known. The spherocytes are trapped in "red" pulp of the cords of Billroth because they are less deformable than normal red cells and thus not able to transverse through the endothelium of splenic sinuses. The trapped cells are subsequently destroyed by the macrophages. The life span of red cells in HS is significantly increase following splenectomy.[18]

Anemia, jaundice, and splenomegaly are the clinical features of HS. However, signs and symptoms are highly variable. Anemia or hyperbilirubinemia may be of such magnitude as to require exchange transfusion in the neonatal period. The disorder may escape clinical recognition altogether. Anemia is usually mild to moderate; however, it may be very severe and sometimes absent.[3] Clinical severity and response to splenectomy is roughly parallel to the degree of spectrin (or ankyrin) deficiency. Two thirds to three fourths of HS patients have incomplete compensated hemolysis and mild to moderate anemia.[10] Hemoglobin values are usually normal at birth, yet decrease sharply during the subsequent 20 days, which leads, in many cases, to a transient and severe anemia. The anemia is severe enough to warrant blood transfusions in a large number of infants. The presence of anemia at birth, or an abrupt decrease in Hb values during the first weeks of life, is a likely predictor of transfusion needs in the future.[19] Delhommeau *et al*[19] concluded that the evolution of Hb and reticuloctyes values should be monitored carefully every other day during the first 10 days of life. They suggest that monitoring infants with HS during the first 6 months of life is important for appropriate clinical management.[19] Partial splenectomy appears to be a reasonable treatment option for management of patients with HS, especially young children.[20]

We could not detect abnormality in 4 of 13 patients since it was masked by previous transfusions. Both blood smear and hemoglobin electrophoresis were normal. These findings give explanation that regular and repeated blood transfusions will suppress endogenous erythropoesis resulting in an inappropriate hemoglobin electrophoresis pattern.[21] As of yet, we still could not find the etiology of hemolytic anemia since all examinations showed normal findings. DNA analysis should be performed in 2 of 13 patients.[15]

We concluded that in facing patients with signs and symptoms of hemolytic anemia and normal Hb electrophoresis as well as RBC enzyme level, the possibility of red cells membrane defect as a cause of this disorder should be taken into account. The clinical features and laboratory findings of patients with red blood cells membrane defect are highly variable. Occasionally, special hematologic investigations are necessary.

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