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A Rare Case: HaemoglobinS-Thalassemia in Adult

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Abstract
Hemoglobinopathy refers to a disease involving a qualitative or quantitative defect of the structure or synthesis of haemoglobin molecules. The HaemoglobinS- beta thalassemia occurs in a heterozygotes individual with beta-thalassemia and HaemoglobinS gene. A 29-year-old man came with severe anemia, thrombocytopenia, and history of repeated blood transfusions. Physical examination showed pale conjunctiva, pansystolic murmurs, and hepatosplenomegaly. The HaemoglobinS fraction was found in haemoglobin electrophoresis with increased HaemoglobinF and decreased HaemoglobinA_2 fraction. The peripheral blood smear shows abnormal erythrocytes morphologies such as pencil shapes, fragmentocytes, target cells, and sickle shapes. The patient was diagnosed with chronic anaemia caused by HaemoglobinS-beta thalassemia. It makes ineffective erythropoiesis, intravascular, and extravascular hemolysis. This haemoglobinopathy caused increased ferritin and transferrin saturation. The presence of renal failure indicate there is a complicated condition like microvascular obstruction of renal. In this case, there is a reduction of HaemoglobinA_2 fraction that is not common in HaemoglobinS-beta thalassemia. The patient with Haemoglobin S / beta$^+$ thalassemia shows intravascular hemolysis, ineffective hematopoiesis, and vaso-occlusive signs. Deoxyribo Nucleic Acid analysis is further needed to confirm the combination defect of haemoglobin synthesis disorders in conjunction with alpha thalassemia or Hereditary persistence of fetal haemoglobin.

Keywords: HaemoglobinS, hemoglobinopathy, Haemoglobin sickle-cell anemia, thalassemia

Kasus Jarang: HaemoglobinS-Thalassemia pada Dewasa

Abstrak
Hemoglobinopati merupakan penyakit yang disebabkan abnormalitas dari struktur atau sintesis molekul haemoglobin (Haemoglobin) yang menyebabkan defek kualitatif atau defek kuantitatif. HaemoglobinS-beta thalassemia adalah kondisi heterozigot dari gen beta-thalassemia dan gen HaemoglobinS. Laki-laki berusia 29 tahun dengan keterangan klinis anemia gravis dan trombositopenia disertai riwayat transfusi berulang. Konjungtiva pasien tampak anemis, dijumpai murmur pansistolik dan hepatosplenomegali. Pada analisis Haemoglobin dijumpai fraksi HaemoglobinS disertai fraksi HaemoglobinF tinggi, fraksi HaemoglobinA_2 rendah. Dari gambaran darah tepi didapatkan sel pensil, fragmentosit, sel target, dan sel sabit. Pasien didiagnosis anemia kronik yang disebabkan oleh HaemoglobinS/beta$^+$ thalassemia. Hal ini menyebabkan eritropoiesis inefektif, hemolisis intravaskular dan ekstravaskular. Hemoglobinopati ini menyebabkan meningkatnya ferritin dan menjenuhkan transferrin. Adanya gagal ginjal mengindikasikan bahwa terjadi komplikasi berupa sumbatan mikrovaskular ginjal. Pada kasus ini didapatkan fraksi HaemoglobinA_2 rendah berbeda dari kriteria yang seharusnya terdapat peningkatan fraksi HaemoglobinA_2. Pasien didiagnosis HaemoglobinS/beta$^+$ thalassemia mengalami hemolisis intravaskular, hematopoiesis inefektif, dan tanda vaso-oklusif. Perlu pemeriksaan analisis Deoxyribo Nucleic Acid untuk menentukan kombinasi gangguan sintesis hemoglobin dengan alpha thalassemia atau Hereditary persistence of fetal haemoglobin.

Kata Kunci: *HaemoglobinS, hemoglobinopati, Haemoglobin sel sabit, thalassemia*

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Introduction

The HaemoglobinS-beta thalassemia involves the beta-thalassemia gene and the HaemoglobinS gene. Both qualitative and quantitative defects occur in this globin chain. The clinical manifestations of HaemoglobinS-beta thalassemia depend on the type of beta-thalassemia. The severity of the symptom of HaemoglobinS- beta thalassemia is classified into three parts. For example, HaemoglobinS/beta0 Type 1 (severe), Haemoglobin S/beta+-Type 1 (moderate), and HaemoglobinS/beta+-Type 2 (asymptomatic).[1,2] HaemoglobinS is the most common Haemoglobin variant in Africa (>20%), followed by the Mediterranean, Middle East, India, Nepal & America. In African Americans, the incidence of sickle cell disease is 0.3-1.3% and the sickle cell trait 8-10%.[3] The incidence of beta-thalassemia is high in Indonesia. However, the case of HaemoglobinS-beta thalassemia is rarely reported.

The clinical manifestations of patients with sickle cell disease are anaemia, vaso-occlusive crisis, bacterial infection, acute spleen sequestration, acute chest syndrome, and iron overload.[2] First, polymerised erythrocytes can return to their original shape if the oxygenation is adequate (reversible). At the end of the stage, those erythrocytes could not return to their original form (irreversible). Several factors influencing the polymerization process are oxygen, the blood concentration of Haemoglobin S in the blood, and temperature. Some haemoglobin can inhibit polymerization, such as Haemoglobin F and Haemoglobin A.[1,2,4]

Case Report

A 29-year-old man came with clinical manifestations pale and weak. There is no bleeding history. The patient had repeated blood transfusions in 2018 and 2014 with a Haemoglobin level <10 g/dL but never had further tests. No family member suffered from recurrent anaemia. In 2013 the patient complained of fever and nosebleeds while living in a malaria-endemic area, but was not diagnosed with malaria. Physical examination showed a blood pressure of 140/90 mmHg, the conjunctival anaemic, pan systolic murmur of the apex region of the heart, the liver was palpable in 2 fingers under the arch of the ribs, and there was Schuffner IV splenic enlargement.

The laboratory tests (Table 1) showed a decrease in haemoglobin levels, eosinophilia, and thrombocytopenia. The peripheral blood (Figure 1B) showed microcytic hypochromic erythrocytes with anisopoikilocytosis consist of target cells, pencil cells, sickle cells, stomatocytes, fragmentocytes, and polychromatic erythrocytes. This condition was suitable for the wide RDW accompanied by normal MCV. Electrophoresis haemoglobin (Figure 1A) showed HaemoglobinS fraction with a high HaemoglobinF fraction and a low HaemoglobinA2 fraction. Besides, there was a discrepancy between normal iron reserves and low Ret-He levels.

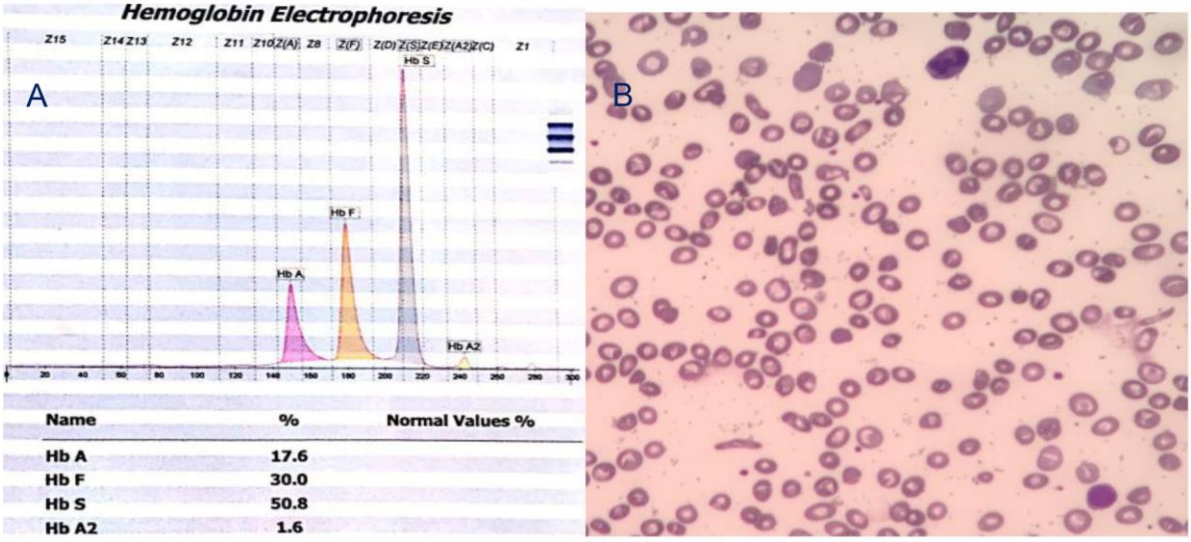

Figure 1. (A) Haemoglobin Electrophoresis result,
(B) Morphology of peripheral blood, magnification 10x40, Wright stain

The blood chemistry examination showed an increase in total bilirubin and Laktat dehidrogenase. The increased bilirubin is dominated by indirect bilirubin. There is hemoglobinuria which indicates that hemolysis has occurred. Coombs' test was negative. CD55 and CD 59 as a marker for Paroxysmal Nocturnal Hemoglobinuria (PNH), showed no PNH clones. The glomerular filtration rate also decreases.

Table 1. Laboratory Results of The Patient

Type Of Examination	**Result**	**Unit**	**Reference Value**
Darah Perifer Lengkap			
Haemoglobin	**4.8**	g/dL	12.0 – 14.0
Haematocryte	**15.5**	%	37.0 – 43.0
Erythrocyte	**1.71**	10^6/µL	4.00 – 5.00
MCV	90.6	fL	82.0 – 92.0
MCH	28.1	pg	27.0 – 31.0
MCHC	**31**	g/dL	32.0 – 36.0
Thrombocyte	**71**	10^3/µL	150 – 400
Leucocyte	8.97	10^3/µL	5.00 – 10.00
Diff count			
Basophil	0.2	%	0 – 1
Eosinophil	**8.4**	%	1 – 3
Neutrophil	68.9	%	52.0 – 76.0
Lymphocyte	**15.6**	%	20 – 40
Monocyte	6.9	%	2 – 8
RDW-CV	**19.8**		11.5 -14.5
RDW-SD	61.5		
RET-He	**19.7**	pg	31.12-36.24
Reticulocyte	**15.2**	%	0.5-2.00
Blood Chemistry			
Total Bilirubin	**2.6**	mg/dL	0.2 - 1.2
Direct Bilirubin	0.49	mg/dL	0.0 - 0.5
Indirect Bilirubin	**2.11**	mg/dL	0.2 - 0.8
LDH	**611**	U/L	125-220
Serum iron	87	µg/L	65-175
UIBC	**54**	µg/L	112-346
TIBC	**141**	µg/L	228-428
Transferin saturation	**62**	%	15-45
Ferritin	218.22	ng/L	20-500
Creatinine	**2.3**	mg/dL	0.60 – 1.20
eGFR	**37**	mL/menit/1.73m^2	86.00 – 128.00
Ureum	**121**	mg/dL	<50

Discussion

HaemoglobinS-beta thalassemia is a rare condition in Indonesia. Thalassemia-beta is a quantitative defect in the beta-globin chain due to a mutation in the beta-globin gene located on chromosome 11. The type of beta-globin chain mutation varies from a defect in the beta globin gene that causes insufficient production of beta-globin chains (beta$^+$) to no production at all (beta^0).[3,5] HaemoglobinS is a qualitative defect in the beta-globin chain that occurs due to the substitution of the 6th amino acid of chromosome 11 in the helix chain A3 globin beta (beta6 [A3] Glu to Val) from glutamic acid (polar) to valine (non-polar). This condition has an impact on the morphology of erythrocytes. Also, the interaction of erythrocytes causes HaemoglobinS to bind other HaemoglobinS. It will form a spiral chain resembling a rope when it undergoes deoxygenation, known as polymerization. This process made erythrocytes no longer biconcave but resemble a sickle.[1-3,5]

There was a history of repeated transfusions with anaemic conjunctiva and pan systolic heart murmur in the apex region suggest chronic

anaemia. Erythropoiesis is ineffective, sequestration of the spleen and liver cause hepatosplenomegaly.[1,6] The complete blood count shows low haemoglobin level, increased reticulocytes, eosinophilia, and thrombocytopenia. The peripheral blood image shows hypochromic microcytic erythrocytes and abnormal morphology of erythrocytes. This condition may be due to iron deficiency or hemoglobinopathy. Value of mean corpuscular volume was in the normal range followed by wide red cell distribution width, possibly due to various size of erythrocytes so that the mean erythrocyte volume was still in the normal range.[7] The clinical features and laboratory results lead to a diagnosis of thalassemia or sickle cell anaemia.

In Haemoglobin analysis, the fractions of 17.6% HaemoglobinA, 1.6% HaemoglobinA2, 30% HaemoglobinF, and 50.8% HaemoglobinS were obtained. A high HaemoglobinF fraction accompanied by a low HaemoglobinA fraction proves that there is a combination of Haemoglobin synthesis disorders in the form of HaemoglobinS/beta$^+$ thalassemia. According to Figueiredo quoted from Serjeant et al show that the classification of HaemoglobinS/beta$^+$ thalassemia based on HaemoglobinA levels divided into type 1 (Haemoglobin A: 1-7%), II (Haemoglobin A: 7-14%), or III (Haemoglobin A: 14-25%).[8] The study of Serjeant et al (2011) showed that the HaemoglobinS/beta$^+$ thalassemia classification of this patient was type III. However, this classification is not widely accepted. According to the criteria from Rodak's Hematology Clinical Principles and Applications (Table 2), this patient fulfilled some criteria of HaemoglobinS/beta$^+$ thalassemia.[3]

Table 2. Thalassemia with Globin chain disorder[5]

Genotype	HaemoglobinA	HaemoglobinA$_2$	HaemoglobinF	Other Haemoglobin	RBC Morphology	Clinical Manifestations	Treatment
HaemoglobinS/ beta$^+$ thalassemia	↓↓	↑	N to ↑	HaemoglobinS> HaemoglobinA	Microcytes, sickle cells, target cells	Ranges from mild to severe anaemia with recurrent vasoocclusive crises	Ranges from no treatment to transfusion support and pain control
HaemoglobinS/ beta^0 thalassemia	0	↑	N to ↑	HaemoglobinS			

↑, increased; ↓, decreased; 0, absent; Haemoglobin, hemoglobin; N, normal; RBC; red blood cell

In this case, there is a discrepancy with the HaemoglobinS/beta$^+$ thalassemia criteria in Table 2 because of the low Haemoglobin A2 fraction. The iron deficiency anemia and alpha/delta thalassemia cannot be excluded yet. In homozygous beta^{s}beta^s with alpha-thalassemia, the clinical severity of sickling will reduce. It happens because the risk of HaemoglobinS polymerization decreased. The occurrence of erythrocyte hemolysis and vaso-occlusion symptoms were less. Fetal haemoglobin is also known to ameliorate the clinical complications of sickle cell disease (SCD).[1,9] A low mean corpuscular hemoglobin concentration value and low level of HaemoglobinA2 fraction support the diagnosis of alpha thalassemia. Analysis of DNA is needed to confirm the diagnosis.[10,11] There were so many abbreviation in the beginning of sentence in this article that are not allowed. Would you please correct them one by one?

The haemoglobin electrophoresis used electrophoresis microcapillary with alkaline pH. The migration pattern of Haemoglobin S found in zone (S) were the same as the migration patterns of Haemoglobin Hasharon, Haemoglobin Handsworth, and denaturated Haemoglobin O-Arab. The four types of haemoglobin variants are distinguished by epidemiology, clinical symptoms, and other laboratory markers. In Haemoglobin Hasharon, there was no sign of hemolysis without any abnormalities at peripheral blood picture. In Haemoglobin Handsworth, there was microcytic hypochromic anaemia without clear evidence of iron deficiency. Both haemoglobin variants have ferritin levels of the normal range.[12,13] The sickling test is needed to rule out a diagnosis of denaturated HaemoglobinO-Arabic because the sickle cell image is still unclear.[1,3,11]

On iron profile examination, ferritin and serum iron levels were in the normal range, accompanied by low total iron binding capacity and increased transferrin saturation were in concordance thalassemia. The other reason was hemoglobinopathy leads to increased ferritin and transferrin saturation.[3,6] Reduced synthesis of transferrin in the liver due to chronic disease is the most contributing factor to increased transferrin saturation.[14] Low Reticulocyte haemoglobin levels

leads to diagnosis of iron deficiency in this patient still cannot be ruled out. An increased in the absolute value of reticulocytes can prove that the bone marrow response is good.[1,3,5]

In this patient, there was an increase in Laktat dehydrogenase levels, indirect bilirubin and total bilirubin with the presence of fragmentocyte in the peripheral blood smear. This condition caused by the evidence of hemolysis both intravascular and extravascular.[1,5] Hemoglobinuria support intravascular hemolysis. Splenomegaly caused by erythrocyte sequestration or vaso-occlusion in the microvascular spleen.[1-4,10] Examination of clonal parameters for paroxysmal nocturnal hemoglobinuria and coombs test were negative. Both parameter support that intravascular hemolysis is not due to an autoimmune process.

Absolute eosinophilia occurs in this patient can caused by VLA-4, LFA-1, and Mac-1 integrins which mediated the adhesion of eosinophils with fibronectin in sickle cell anemia.[15,16] Thrombocytopenia in this patient might be caused by vascular endothelium dysfunction process. This process will cause excessive platelet activation (consumptive coagulopathy) or an aplastic crisis in sickle cell anemia.[6,16,17]

Increased levels of creatinine and blood urea are a sign of renal failure, possibly due to renal microvascular stagnation/blockage. Vaso-occlusive is one of an etiology. Inflammation is also key to the initiation of vasoocclusion.[18,19] It detained sickle cells to pass the microvascular.[1,2,5,6] An increase in blood creatinine> 1.5 times upper limit normal, decreasedestimated glomerulus filtration rate, and hypertension support the renal impairment. The suspicion of acute on chronic renal failure still needs to be followed up because at the moment there is no data on kidney function 48 hours and three months previously (diagnostic criteria for acute on chronic renal failure according to KDOQI 2012).[20]

Conclusion

A 29 year old-male with a clinical description of anaemia gravis and thrombocytopenia. On the haemoglobin analysis, the fraction of HaemoglobinA was 17.6%, HaemoglobinA2 1.6%, HaemoglobinF 30%, and HaemoglobinS or variant Haemoglobin was 50,8%. The presence of hypochromic microcytic erythrocytes with intravascular hemolysis, ineffective hematopoiesis, and vaso-occlusive signs support the diagnosis of HaemoglobinS / beta^{+} thalassemia. Analysis of DNA is further needed to confirm the combination defect of haemoglobin synthesis disorders in conjunction with alpha thalassemia or HPFH that cannot be excluded in the examination. The "sickling test" also needed to rule out other hemoglobinopathies such as denatured HaemoglobinO-Arab.

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