ผลกระทบจากการรับเซลล์เม็ดเลือดแดงต่อการตรวจวิเคราะห์ฮีโมโกลบิน ของผู้ป่วยโรคเบต้าศูนย์ธาลัสซีเมียฮีโมโกลบินอี และโฮโมไซกัสฮีโมโกลบินอี

The case report: Effect of red blood cell transfusion on hemoglobin (Hb) analysis of β^0-Thalassemia/HbE disease and homozygous HbE

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บทคัดย่อ

การให้เลือดเป็นการรักษาหลักในผู้ป่วยธาลัสซีเมียและผู้ที่มีฮีโมโกลบินผิดปกติชนิดรุนแรง อย่างไรก็ตาม การให้เลือดมีผลกระทบต่อการตรวจวิเคราะห์ฮีโมโกลบิน บทความนี้รายงานการปรากฏของฮีโมโกลบินเอ บนอิเล็กโทรโฟรีแกรมของการตรวจด้วยวิธีแคปิลลารีอิเล็กโทรโฟรีซิส ในผู้ป่วยโรคเบต้าศูนย์ธาลัสซีเมียฮีโมโกลบินอี และผู้ป่วย โฮโมไซกัสฮีโมโกลบินอีที่ได้รับเซลล์เม็ดเลือดแดง ซึ่งทำให้การแปลผลการตรวจวินิจฉัยผิดพลาดเป็นเบต้าบวกธาลัสซีเมียฮีโมโกลบินอี และเฮเทอโรไซกัสฮีโมโกลบินอี ตามลำดับ ดังนั้น เพื่อเป็นการป้องกันการแปลผลที่ผิดพลาด จึงควรตรวจวินิจฉัยชนิดของฮีโมโกลบินก่อนการรับเลือดหรือภายหลังได้รับการรับเลือดแล้วอย่างน้อย 3 เดือน และหากมีความจำเป็นที่จะต้องตรวจวิเคราะห์ภายในระยะเวลา 3 เดือน ควรมีประวัติการได้รับเลือดประกอบการวินิจฉัย นอกจากนี้ ควรตรวจวิเคราะห์ทางอณูวิทยาร่วมด้วยเพื่อวินิจฉัยชนิดของธาลัสซีเมีย

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คำสำคัญ: การรับเลือด เบต้าศูนย์ธาลัสซีเมีย ฮีโมโกลบินอี การแปลผลผิดพลาด

Abstract

Red blood cell (RBC) transfusion is a medical therapy in patients with severe thalassemia and hemoglobinopathy. However, it also affects the hemoglobin (Hb) analysis. We report here the presentation of HbA peak on capillary electrophoresis (CE) electrophoregrams of β^0-thalassemia/HbE and homozygous HbE patients who received RBC transfusions. The misinterpretations of β^+-thalassemia/HbE and heterozygous HbE, respectively, were occurred. Therefore, to avoid misdiagnosis, hemoglobin analysis should be determined prior to or after 3 months of blood transfusion. When hemoglobin typing is needed within the 3 month period mentioned, history of transfusion is required to accompany the diagnosis. Moreover, molecular analysis for identification of thalassemia genotype should be performed.

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Keywords: Blood transfusion, β^0-thalassemia, HbE, misinterpretation

Case Report

Case 1

A 23-year-old Thai female was found fatigue and dizziness by the physician at a private hospital in Chiang Mai, Thailand. On physical examination, she had hepatosplenomegaly and anemia. Her RBC counts, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and red blood cell distribution width (RDW), measured by automated blood cell counter (Sysmex KX-21, Sysmex Corporation, Kobe, Japan) at the hospital, were $3.9x10^{12}$ cells/L, 77 g/L, 25%, 62 fL, 19.4 pg, 29.1%, respectively. Two units of RBCs were given at the day of initial admission. Four days after transfusion, her blood sample was collected and sent for thalassemia investigation at the Associated Medical Sciences (AMS) Clinical Service Center, Chiang Mai University, Chiang Mai, Thailand. In the thalassemia laboratory, hemoglobin typing, which included HbA_2 (for β-thalassemia detection) and hemoglobinopathies, was analyzed by the capillary electrophoresis (CE, Capillarys™ 2 Flex Piercing, Sebia, Norcross, Georgia, USA). At the same time, molecular analysis for α-thalassemia-1 is carried out. Genomic DNA was extracted from whole blood sample using the NucleoSpin® kit (Macherey-Nagel, KG., Duren, Germany) according to manufacturer's instructions. Real-time PCR with SYBR Green1 and high resolution melting (HRM) analysis for detection of the α-thalassemia-1 South-East Asian (SEA) and Thai type deletion was performed.[5,6] The results showed that her HbA, HbF, HbE and HbA_2 were 38.4, 18.0, 39.5 and 4.1%, respectively (Figure 1). DNA analysis was negative for α-thalassemia-1 SEA and Thai type deletions. She was, therefore, diagnosed as β^+-thalassemia/HbE. Since she received the red blood cell transfusions, the β^0-thalassemia/HbE was doubted. Thus, the β^0-thalassemia codons 71/72(+A) and 41/42(-TCTT) mutations, IVSI-nt1 (G>T) and codon 17(A>T) mutations which are commonly found in Thai population were analyzed by multiplex amplification refractory mutation system (MARMS)-PCR as previously described protocol.[7] The 439 bp amplified fragment of β^0-thalassemia codons 41/42 mutation was observed (Figure 2). Therefore, she was finally diagnosed as β^0-thalassemia/HbE disease. The peak of HbA on CE electrophoregram was due to RBC transfusions.

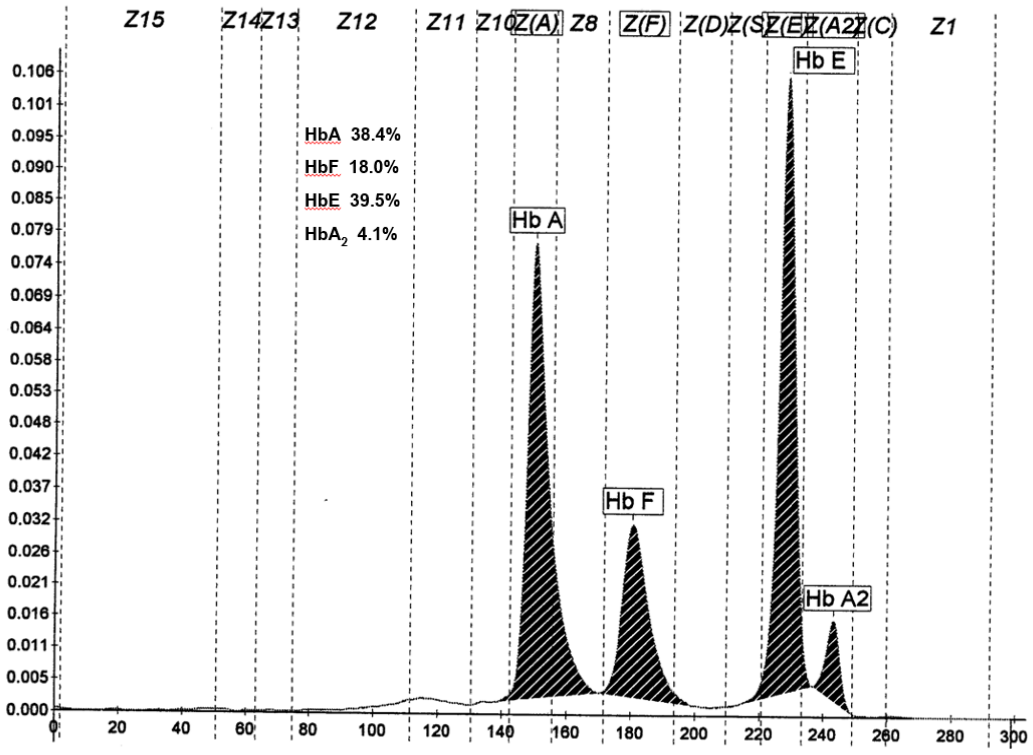

Figure 1. *CE electrophoregram of patient 1.*

Case 2

EDTA blood sample of 39-year-old Thai male was sent from the private hospital in Lamphun, Thailand, to the AMS Clinical Service Center, Chiang Mai University, Chiang Mai, Thailand for thalassemia diagnosis. His RBC counts, Hb, Hct, MCV, MCH and RDW, measured by automated blood cell counter (Sysmex KX-21, Sysmex Corporation, Kobe, Japan) at the hospital, were $4.2x10^{12}$ cells/L, 105 g/L, 32%, 77 fL, 25.0 pg and 15.9%, respectively. In the laboratory, hemoglobin analysis was performed by CE and found that HbA, HbE and HbA_2 levels were 49.2, 47.1 and 3.7%, respectively (Figure 3). DNA analysis for α-thalassemia-1 SEA and Thai type deletion, which is routinely performed at the same time the hemoglobin analysis was carried out, showed negative result. His HbE/A_2 (50.8%) was higher than HbA (49.2%) and it was not in the ranges which normally found in HbE trait (25-30%).[8] His blood transfusion history was verified and found that 5 days prior to drawing a blood for thalassemia diagnosis, he was admitted to the emergency room because of car accident that caused a severe blood loss. He received 2 units of RBCs. Therefore, he was finally diagnosed as homozygous HbE. The presentation of a peak of HbA on CE electrophoregram was resulted from RBC transfusions. The amplification refractory mutation system (ARMS)-PCR for characterization of HbE genotype was also performed according to the previously described protocol.[7] PCR product from HbE mutant allele with a size of 267 bp but not from wild type allele was observed (data not shown). Moreover, the MARMS-PCR for detection of β^0-thalassemia codons 71/72(+A) and 41/42(-TCTT) mutations, IVSI-nt1 (G>T) and codon 17(A>T) mutations and the real-time PCR with SYBR Green1 and HRM analysis for detection of β^0-thalassemia 3.4 kb deletion were also performed, according to protocols described previously.[7,9] The amplified fragments from these β^0-thalassemia mutations were not found (data not shown). Thus, these results insisted that the patient had homozygous HbE.

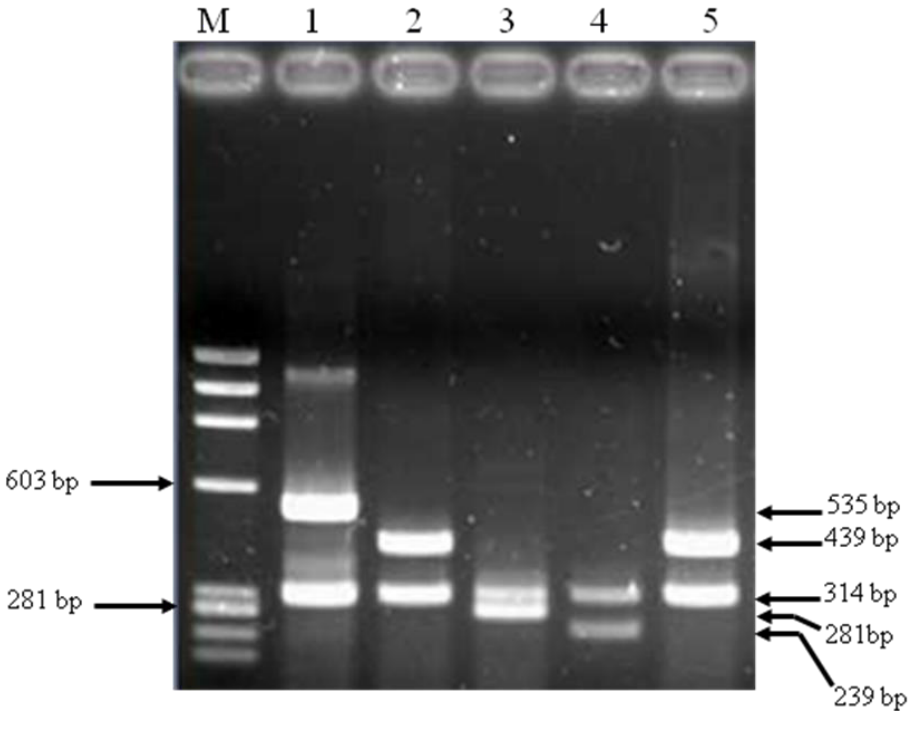

Figure 2. *Multiplex amplification refractory mutation system (MARMS)-PCR for identifying of β^0-thalassemia mutations. The amplified fragments were separated by 2.0% agarose gel electrophoresis and visualized under UV-light after ethidium bromide staining. M represents φX174 size marker DNAs. The 314 bp internal control fragment, 535, 439, 281 and 239 bp amplified fragments from β^0-thalassemia codons 71/72 and 41/42 mutations, IVSI-nt1 and codon 17 mutations, respectively are indicated. Lanes 1-4 show analysis results of control DNA of β^0-thalassemia codons 71/72 and 41/42 mutations, IVSI-nt1 and codon 17 mutations, respectively. Lane 5 shows analysis result of patient 1.*

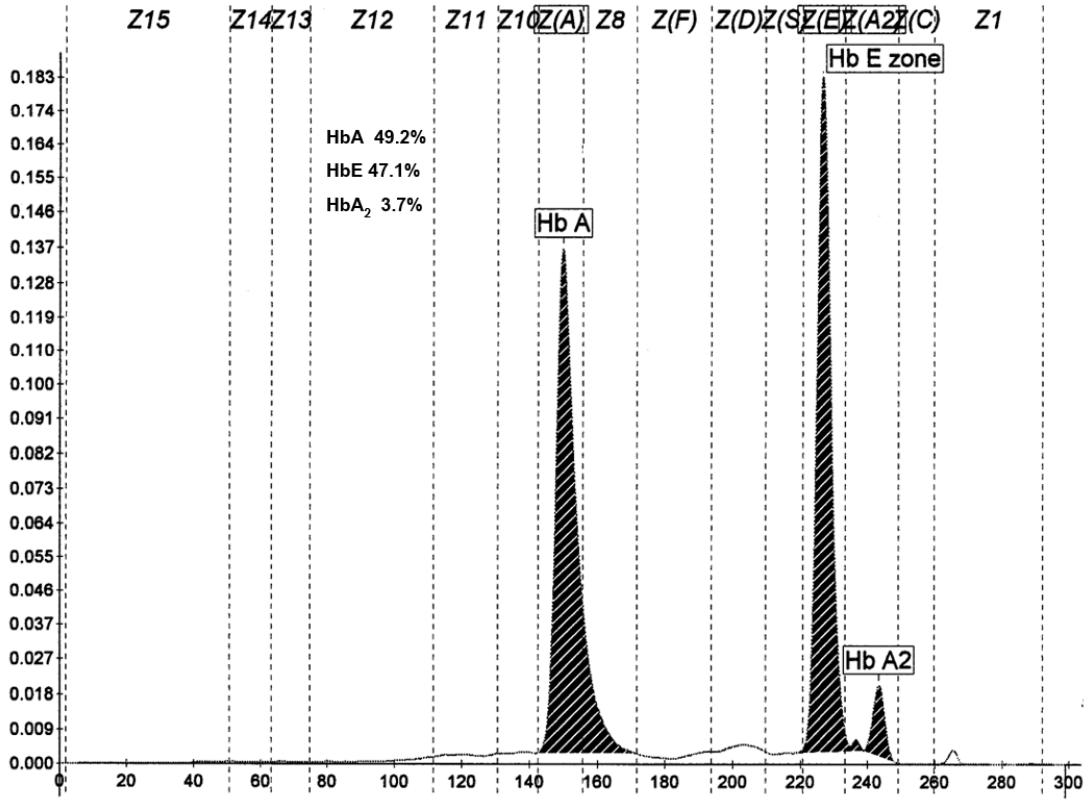

Figure 3. *CE electrophoregram of patient 2.*

Discussion

RBC transfusion is the key therapy in patients with severe thalassemia and hemoglobinopathy. However, it can also affect the hemoglobin analysis. This report demonstrates an effect of HbA from non-thalassemia blood donors on the hemoglobin analysis of patients with β^0-thalassemia/HbE disease and homozygous HbE. In β^0-thalassemia/HbE disease and homozygous HbE, β^A-globin chains are not synthesized and the disease is characterized by the HbE and HbF production with undetectable HbA. HbE level varies from 30% to 70% of total hemoglobins in β^0-thalassemia/HbE disease and >80% in homozygous HbE with the remaining HbF. Whereas, in β^+-thalassemia/HbE, the variable amounts of HbA could be detected besides HbE and HbF.[8] Due to the presentation of HbA, β^+-thalassemia/HbE and HbE trait might be considered in patient 1 and 2, respectively. The previous study reported the CE has a high efficiency to prevent misinterpretation of hemoglobin analysis in patients who received HbE trait blood transfusion.[10] However, in patients receiving normal RBC transfusions, this method cannot differentiate β^+-thalassemia/HbE from β^0-thalassemia/HbE disease or HbE trait from homozygous HbE. To avoid misdiagnosis and unnecessary genetic counseling, hemoglobin analysis should be performed prior to or 3 months after blood transfusion. However, when hemoglobin typing is needed within 3 months of mentioned period, correct interpretation of hemoglobin analysis results requires the information on the patient's age and history of transfusion. Thus, this information should be stated in the requisition form. Moreover, molecular analyses for characterization of thalassemia genotype should be also performed in these cases.

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