Anemia in hemoglobin E traits resistant to treatment: report of two cases

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Abstract:

Introduction: The hemoglobin E traits are always asymptomatic; they mostly have normal hemoglobin concentration and normal MCV. Their hemoglobin analysis usually reveals only Hb A and E. But herein we reported two cases of supposed Hb E traits that had moderate microcytic anemia which could not be simply corrected. Case Presentation: Case 1. A 40-year-old Thai woman had moderate microcytic anemia without hepatosplenomegaly. Her blood tests showed: Hb 9.1 g%, MCV 52.3 fl, MCH 16.3 pg, ferritin 444.3 ng/ml, Hb analysis using the high performance liquid chromatography method: Hb AE, Hb E 15.2 %, Hb F 1.2 %. Case 2. A 36-year-old Thai woman had no anemic symptom and no hepatosplenomegaly. The blood tests included: Hb 9.3 g%, MCV 46.2fl, MCH 15.3 pg, ferritin 198 ng/ml, Hb analysis using the capillary zone electrophoresis method: Hb AE, Hb A 82.3 %, Hb E 12.0 %. They both were initially diagnosed as having Hb E heterozygosity with moderate anemia from unknown causes. They were supportively treated with the iron tablets and folic acid without the improvement within three months. Because both patients had some clues, viz. the Hb, MCV, MCH and the Hb E level which were all too low to be solely attributed by Hb E heterozygosity, the PCR for alpha thalassemia genes was performed and revealed the existence of Southeast Asian (SEA) and 3.7 kb deletions similarly in both patients. Their diagnoses were finally corrected to be Hb AEBart disease, the co-inheritance of Hb E heterozygosity and Hb H disease. Conclusion: The absence of Hb Bart in Hb AEBart disease could lead to the wrong diagnosis. To correct the diagnosis in this situation, it needs the clinical competence of the physician and the sophisticated test such as genotypes study.

Key Words: Hemoglobin E trait, Hemoglobin AEBart disease, Absence of hemoglobin Bart
Introduction

Hemoglobin (Hb) E is genetically resulted from the combination of two normal alpha globin chains and two abnormal beta globin chains of which glutamic acid at the 26th position of polypeptide is substituted by lysine. For Hb E traits, they are usually asymptomatic, mean Hb 12.8±1.5 g%, mean corpuscular volume (MCV) 84±5 fl, mean corpuscular hemoglobin (MCH) 30±2.4 pg, and the percentage of Hb E is found around 29.4±2.3(1). If the Hb E level is less than 25% in Hb E traits, it denotes other contributing factors, congenital or acquired. In case of double heterozygosity of Hb E and alpha thalassemia-1 genes, the fraction of Hb E is found around 20.7±1.2%, in triple heterozygosity of Hb E, alpha thalassemia-1 and thalassemia-2 genes or Hb AEBart disease, the fraction of Hb E will be lowered to 13±2.1(2). The acquired contributing factors may include the iron deficiency anemia which
can lower the percentage of Hb E in Hb E traits to 20.5 % whereas the Hb concentration can be lowered to 9.2 g\%\(^d\). The diagnosis of the Hb E heterozygosity, thalassemia or other hemoglobinopathies mostly depends on the hemoglobin analysis\(^d\) in combination with clinical background. In some complicated cases, it may need other sophisticated laboratory tests such as DNA analysis. Solely depending on the interpretation of Hb analysis may lead to the missed diagnosis as found in our two patients.

**Case Report**

**Case 1:** A 40-year-old Thai woman was accidentally found to have moderate anemia during check-up. Her physical examination revealed only pallor, no hepatosplenomegaly. Her blood tests showed Hb 9.0 g\%, Hct 28.6 %, MCV 52.3 fl, MCH 16.5 pg, RDW 20.1 %, WBC 6,000/mm\(^3\), platelet 209,000/mm\(^3\), reticulocyte 0.8 %, ferritin 444.3 ng/ml, normal serum creatinine, Hb analysis using the high performance liquid chromatography (HPLC), Bio-Rad\(^\circledast\): Hb AE, Hb E 15.6 %, Hb F 1.4 %. The initial diagnosis was Hb E trait with microcytic anemia of unknown cause and she was treated with iron tablets and folic acid. Three months later, her blood test was repeated: Hb 9.1 g\%, Hct 29.2 %, MCV 52.3 fl, MCH 16.3 pg, reticulocyte 0.9 %, Hb analysis with the old method: Hb AE, Hb E 15.2 %, Hb F 1.2 %, the PCR for alpha thalassemia was positive for Southeast Asian (SEA) and 3.7 kb deletions. Her final diagnosis was Hb AEBart disease and her treatment was folic acid and genetic counseling.

**Case 2:** A 36-year-old Thai woman noticed herself have transient frank anemia every time after recovery from fever despite no obvious blood loss. The physical examination revealed only pallor, no hepatosplenomegaly. The blood tests showed: Hb 9.3 g\%, Hct 28.0 %, WBC 4,080/mm\(^3\), platelet 214,000/mm\(^3\), MCV 45.3 fl, MCH 15.3 pg, RDW 20.1 %, ferritin 198 ng/ml, normal creatinine level. Her Hb analysis using the capillary electrophoresis method, Bria\(^\circledast\): Hb AE, Hb A 82.1 %, Hb E 11.9 %, Hb A\(_2\) 3.6 %, Hb F 2.4 %. Her initial diagnosis was Hb E trait with microcytic anemia of unknown cause. And her treatment was iron tablet and folic acid. Three month later, her blood was tested again: Hb 9.3 g\%, Hct 28 %, MCV 46.2 fl, MCH 15.3 pg, Hb analysis using the old method: Hb AE, Hb A 82.3 %, Hb E 12.0 %, Hb A\(_2\) 3.5 %, Hb F 2.2 %, the PCR for alpha thalassemia was positive for Southeast Asian (SEA) and 3.7 kb deletions. Her final diagnosis was Hb AEBart disease and her treatment was folic acid and genetic counseling.

**Discussion**

From CBC tests, both patients had definite microcytic anemia, viz., Hb less than 12 g\%, MCV less than 80 fl of which two major causes were the iron deficiency anemia and thalassemia and/or hemoglobinopathy\(^d\). Our cases had both normal serum ferritin, the representative of the body iron storage, viz., more than 30 ng/ml, therefore the diagnoses of iron deficiency anemia could be definitely excluded whereas thalassemia and/or hemoglobinopathy was strongly possible although they did not have jaundice or hepatosplenomegaly.

The Hb analysis in both patients despite using different methods for two times in each case revealed only Hb E heterozygosity. Because their many hematologic parameters were too low to be
contributed by Hb E heterozygosity alone, for instance, Hb concentration 9.1-9.3 g%, MCV 46.2-52.3 fl, MCH 15.3-16.3 pg and the percentage of Hb E 12.0-15.2 %, other sophisticated tests were in need to explore other contributing factors. Finally alpha thalassemia-1, SEA deletion type and alpha thalassemia-2, 3.7 kb deletion, were demonstrated in both patients. Therefore the definite diagnoses in both cases were Hb AEBart disease\(^7\). Both SEA and 3.7 kb deletions were the most common alpha thalassemia-1 and alpha thalassemia-2 genes, respectively in Thailand\(^8\).

Hb AEBart disease is resulted from a genetic co-transmission among three heterozygositites: Hb E, alpha thalassemia-1 and alpha thalassemia-2. Hb analysis in this disease would show the combination of Hb A, Hb E and Hb Bart\(^9\), the percentages of Hb E was 13±2.1 %, Hb Bart was 2.2±1.8 % whereas other parameters were: Hb concentration 9.1±1.1 g%, MCV 60±3 fl, MCH 17±2 pg\(^1\). The existence of Hb Bart was one of important clues to distinguish between Hb E trait and Hb AEBart disease. Our patients were repeatedly found to have only Hb A and Hb E, no Hb Bart therefore their initial diagnoses were only Hb E trait. Until alpha thalassemia-1 and alpha thalassemia-2 genes were documented, the diagnosis was definitely corrected to be Hb AEBart disease. The amount of Hb Bart might be too small until it could be simply overlooked even by the different methods of analysis.

Sanchaisuriya et al studied Hb E traits in combination with various forms of alpha thalassemia. From 202 cases, all 18 patients who truly have Hb AEBart disease by the PCR study were firstly diagnosed as Hb E traits when they were tested with 2 different methods of Hb analysis\(^10\). Actually Hb Bart in Hb AEBart disease should have been found around 7.7 ±5.8 %\(^11\). It seemed to suggest the Hb analysis might not be the appropriate method enough for being the diagnostic investigation in some cases of Hb AEBart disease.

**Conclusion**

Two Thai women with moderate microcytic anemia were initially found to be Hb E trait by different methods of Hb analysis. But many hematologic parameters were too low to be solely contributed by Hb E heterozygosity, they were genotypically proved to truly have Hb AEBart disease. To diagnose Hb AEBart disease in these patients needs the clinical competence of the physicians to recognize many low hematological parameters that belong to Hb AEBart disease instead of Hb E trait alone.

**References**