

A blood transfusion leading to misdiagnosis of beta-thalassaemia carrier status

Peerapon Wong, Akamon Tapprom, Nangnoi Jermnim, Prissana Charoenporn, Somluck Kanthiyawong

Thalassaemia Research Unit, Faculty of Medicine, Naresuan University, Phitsanulok, Thailand

Thalassaemia is the leading genetic problem in the Thai population, with the prevalence of the carrier state being as high as 30–40%^{1,2}. Carriers live a normal life and can not be discriminated from the normal population unless blood investigations are performed. The diagnosis of a beta-thalassaemia carrier in thalassaemia screening and control programmes depends on the person's percentage of haemoglobin (Hb) A₂^{3,4}. The percentage of HbA₂ in normal adults ranges between 2.5 to 3.5%^{5,6}. To be diagnosed a beta-thalassaemia carrier, this value has to be higher than normal. Most institutes use a HbA₂ cut-off level of 3.5%³⁻⁵. The cause of the increase in HbA₂ in beta-thalassaemia carriers appears to involve both transcriptional and post-translational modifications of Hb synthesis⁶. Because a genetic diagnosis is not feasible in mass screening programmes, the percentage of HbA₂ is a critical surrogate for the diagnosis of beta-thalassaemia carriers.

Given the high prevalence of the carrier state in the Thai population, there is a chance of blood donation from thalassaemia carriers even with stringent blood bank criteria for donor selection. Here we report the interesting case of a patient misdiagnosed as a beta-thalassaemia carrier (elevated HbA₂) after having received a blood transfusion. The patient had chronic anaemia from renal insufficiency. She was treated with an erythropoiesis-stimulating agent and occasional packed red cell (PRC) transfusions. A previous Hb analysis appeared to be normal (HbA₂ 2.9%). Thereafter, the Hb analysis was repeated by accident and the woman was indicated to be a beta-thalassaemia carrier with a HbA₂ of 6.0%. However, subsequent beta-globin DNA sequencing did not reveal any beta gene mutations. The patient had been transfused one unit of PRC almost 2 months

prior to the finding of elevated HbA₂. Her blood donor was, therefore, traced back from the blood bank donor registry. A sample of blood product from this donor, which had been kept by routine in the blood bank, was rechecked with analysis of Hb. This donor appeared to be a HbE carrier.

We retrospectively reconsidered how transfused HbE led to a misdiagnosis of beta-thalassaemia carrier status. The issue involved in this patient was the misinterpretation of transfused HbE as high HbA₂ in the Hb analysis. Electrophoretic and chromatographic separation methods rely on differences in charges to resolve proteins from one another. HbA₂ and HbE have similar charge differences. Electrophoretic and conventional methods of column chromatography are, therefore, incapable of separating HbA₂ from HbE. High performance liquid chromatography (HPLC) with a cation exchange column produces good resolution of HbA₂ from a group of positively charged Hb with similar charge differences such as HbS and HbC⁶. However, HbA₂ and HbE are generally eluted from HPLC with the same retention time although in different amounts. The percentage of HbE in a HbE carrier is usually 28-30%⁷. The level of HbA₂ in a beta-thalassaemia carrier is above 3.5% but generally does not exceed 8%⁵. When the amount of HbA₂ from HPLC exceeds 8-10%, the Hb must be assumed to be HbE. The apparently high HbA₂ in our case was actually transfused HbE but with a dilutional effect.

One unit of PRC contains approximately 300 mL of red blood cell (RBC). Given that 30% of the Hb of a HbE carrier is HbE, one unit of PRC from a HbE carrier contains roughly 90 mL of HbE. Our patient, weighing approximately 60 kg, has an estimated blood volume of 4,200 mL (7% of body weight)⁸ and RBC volume of 840 mL (pretransfusion haematocrit 20%).

Therefore, after transfusion of one unit of PRC, she would have approximately 90 mL of HbE out of 1,140 mL RBC (840 mL + 300 mL) which is 7-8%. This percentage is reasonably close to the percentage of "HbA₂" found by Hb analysis after blood transfusion.

Once the mystery had been solved, we had the chance to collect the patient's blood for repeat Hb analysis almost 3 months after the transfusion of PRC from the HbE carrier. This third specimen still revealed an elevated HbA₂ (7.0%), confirming the results in the second specimen collected. Another 5 months later, her HbA₂ had returned to a normal level (3.0%) as expected.

A similar situation may happen in an everyday-life scenario in Thailand without detection. Without performing Hb analysis, a HbE carrier donor can not be discriminated clinically from a normal donor even with stringent blood bank selection criteria. Most HbE carriers have normal Hb and haematocrit levels. Furthermore, some HbE carriers can have a normal mean corpuscular volume. Given the high prevalence of HbE carriers in Thailand, misdiagnosis of beta thalassaemia carriers may not be uncommon in Thai blood transfusion recipients.

Key words: beta–thalassaemia, haemoglobin E, blood transfusion.

References

- 1) Tienthavorn V, Pattanapongsthorn J, Charoensak S, et al. Prevalence of thalassaemia carriers in Thailand. *Thai J Hematol Tranf Med* 2006; **16**: 307-12.
- 2) Wong P, Thanormrat P, Srithipayawan S, et al. Prevalence of thalassaemia trait from screening program in pregnant women of Phitsanulok. *Thai J Hematol Tranf Med* 2004; **14**: 181-6.
- 3) Olivieri NF. The β -thalassemias. *N Engl J Med* 1999; **341**: 99-109.
- 4) Cao A, Saba L, Galanello R, Rosatelli MC. Molecular diagnosis and carrier screening for β thalassaemia. *JAMA* 1997; **278**: 1273-7.
- 5) Brown BA. *Hematology: Principles and Procedures*. 6th ed. Philadelphia: Williams & Wilkins, 1993.
- 6) Steinberg MH, Adams JG. Hemoglobin A₂: origin, evolution, and aftermath. *Blood* 1991; **78**: 2165-77.
- 7) Sanchaisuriya K, Fucharoen G, Sae-ung N, et al. Molecular and hematologic features of hemoglobin E heterozygotes with different forms of α -thalassaemia in Thailand. *Ann Hematol* 2003; **82**: 612-6.
- 8) Cameron JR, Skofronick JG, Grant RM. *Physics of the Body*. 2nd ed. Madison: Medical Physics Publishing, 1999.

Received: 10 March 2009 - Revision accepted: 6 August 2009

Correspondence: Peerapon Wong MD

Thalassaemia Research Unit,

Faculty of Medicine, Naresuan University,

99 Moo 9, Tambon Thapoe, Amphur Mueang, Phitsanulok, Thailand 65000

e-mail: peeraponw@nu.ac.th
