

## The Misdiagnosis of Beta-Thalassemia Heterozygosity Led by Hyperthyroidism

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When patients have mild microcytic hypochromic anemia with slightly increased hemoglobin (Hb) A<sub>2</sub> fraction, the most likely diagnosis is beta-thalassemia heterozygosity. But herein we found a patient who had all these hematological parameters but did not have beta-thalassemia heterozygosity. He was a 14-year-old Thai who presented with fatigue and heat intolerance for 2 weeks. His physical examination revealed mild diffuse enlargement of thyroid gland. Blood tests showed Hb 120 g/L, mean corpuscular volume (MCV) 72.1 fL, mean corpuscular hemoglobin (MCH) 23.3 pg/cell, free triiodothyronine (FT<sub>3</sub>) > 20 pg/mL, free thyroxine (FT<sub>4</sub>) > 5.0 ng/dL, thyrotropin < 2.5 mIU/L, serum ferritin 51.3 µg/L, Hb A<sub>2</sub> 3.8%. Besides primary hyperthyroidism, he was diagnosed with beta-thalassemia heterozygosity. After being treated with antithyroid drug for 6 months, his blood tests showed subclinical hyperthyroidism, Hb 146 g/L, MCV 83.3 fL, MCH 26.3 pg/cell, Hb A<sub>2</sub> 3.0%. Not only the thyroid hormones levels but also the Hb concentration, MCV, and the Hb A<sub>2</sub> percentage became normal. Due to this inconsistency, the DNA analysis for beta-thalassemia genes was performed and found negative for numerous common and rare beta-thalassemia genes meanwhile beta-globin gene sequencing appeared normal. It should be concluded that hyperthyroidism could induce slightly elevated Hb A<sub>2</sub> percentage and mild hypochromic microcytic anemia in a normal individual, leading to the misdiagnosis of beta-thalassemia heterozygosity. In other words, Hb analysis should not be performed during hyperthyroidism and it should be delayed till achievement of the euthyroid stage.

**Keywords:** Hyperthyroidism, Fake Beta, Thalassemia, Heterozygosity

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## Introduction

Thalassemia is a group of hereditary disease due to genetic transmission of defect of globin chain synthesis. It is mainly classified into alpha and beta-thalassemia depending on the globin chain involved. Most of them are characterized by microcytic hypochromic anemia of that the degree can vary from asymptomatic to transfusion-dependent or still birth. For individuals with beta-thalassemia heterozygosity or traits, they usually have mild microcytic hypochromic anemia, hemoglobin (Hb) concentration ranging from  $106 \pm 19$  to  $133 \pm 17$  g/L,<sup>1</sup> mean corpuscular volume (MCV)  $63.8 \pm 4.2$  fL for beta(0),  $67.0 \pm 5.5$  fL for beta(+), and mean corpuscular hemoglobin (MCH)  $20.1 \pm 1.4$  pg/cell for beta(0),  $21.2 \pm 1.9$  pg/cell for beta(+) thalassemia traits.<sup>2</sup> Hb analysis mostly shows only mildly increased hemoglobin A<sub>2</sub> (alpha<sub>2</sub> delta<sub>2</sub>) percentage, 3.5 - 5.7 %, mean  $4.9 \pm 0.4$  %.<sup>3</sup> Although all these characteristics are genetically transmitted, they can be minimally modified by some acquired factors, for instance, in beta-thalassemia heterozygosity complicated by iron deficiency anemia: Hb concentration decreases from  $108 \pm 11$  to  $98 \pm 11$  g/L, MCV from  $67.9 \pm 4.8$  to  $64.0 \pm 6.4$  fL, MCH from  $21.5 \pm 3.0$  to  $20.0 \pm 3.0$  pg/cell, and Hb A<sub>2</sub> from  $5.8 \pm 0.9$  % to  $5.4 \pm 0.9$  %.<sup>4</sup>

On the contrary, we found a patient who had all these hematological parameters during hyperthyroidism that firstly led to misdiagnosis of beta-thalassemia heterozygosity but later they all became normal after hyperthyroidism was well controlled with medication.

## Case Report

A 14-year-old Thai man was definitely diagnosed with primary hyperthyroidism based on the history of 2-week fatigue, heat intolerance without weight loss and diffusely mild enlargement of thyroid gland, no exophthalmos, heart rate 100 beats/min regularly on the physical examination whereas his first thyroid function test showed free triiodothyronine (FT<sub>3</sub>) > 20.0 pg/mL (normal 1.71 - 3.71), free thyroxine (FT<sub>4</sub>) > 5.0 ng/dL (normal

0.7 - 1.48), thyrotropin < 2.5 mIU/L (normal 0.4 - 4.0), antithyropoxidase antibody 6.4 IU/mL (normal < 9), and antithyroglobulin antibody < 0.9 IU/mL (normal < 4).

Blood tests before starting antithyroid drug showed Hb 120 g/L, hematocrit (Hct) 37.2%, white blood cell (WBC)  $8.5 \times 10^9$ /L, platelet  $341 \times 10^9$ /L, neutrophil 56.6%, lymphocyte 31.5%, MCV 72.1 fL, MCH 23.3 pg/cell, mean corpuscular hemoglobin concentration (MCHC) 32.3 g/dL, red blood cell distribution width (RDW) 13.4%, and serum ferritin 51.3 μg/L. Hb analysis using the capillary zone electrophoresis method revealed A<sub>2</sub>A, Hb A<sub>2</sub> 3.8%, Hb A 96.2%. The interpretation was beta-thalassemia heterozygosity with or without alpha thalassemia trait. The polymerase chain reaction (PCR) for alpha thalassemia was performed and showed negative for alpha thalassemia-1 genes (Southeast Asian and Thai deletions), negative for Southeast Asian Ovalocyte (SAO) gene, negative for alpha-globin gene triplication and negative for alpha thalassemia-2 (3.7 kb and 4.2 kb deletions, Hb Constant Spring and Hb Pakse genes). Hence, the hematological diagnosis of beta-thalassemia heterozygosity was definitely concluded. And genetic counseling was offered to him.

He was treated with methimazole 30 mg, propranolol 20 mg as well as 2 iron tablets a day. He could tolerate drug well, no neutropenia in the first month: Hb 138 g/L, Hct 42.3%, WBC  $7.4 \times 10^9$ /L, platelet  $438 \times 10^9$ /L, MCV 75.9 fL, MCH 24.7 pg/cell, MCHC 32.5 g/dL, RDW 13.4%, so antithyroid drug and iron tablets were regularly continued until he achieved euthyroidism in 3 months and antithyroid drug was gradually tapered.

After completion of 6 months of treatment, his blood tests showed FT<sub>3</sub> 4.87 pg/mL, FT<sub>4</sub> 1.78 ng/dL, thyrotropin 0.143 mIU/L, Hb 146 g/L, Hct 45.8%, WBC  $7.5 \times 10^9$ /L, platelet  $336 \times 10^9$ /L, MCV 83.3 fL, MCH 26.3 pg/cell, MCHC 31.9 g/dL, RDW 12.9%, serum ferritin 193 μg/L. Whereas his hyperthyroidism had turned from full blown to subclinical, the mild anemia and microcytosis became normal, therefore Hb analysis using the old method was repeated A<sub>2</sub>A, Hb A<sub>2</sub> 3.0%, Hb F 0.3%.

Many hematological parameters that used to lead to the interpretation as beta-thalassemia heterozygosity during

hyperthyroidism became normal during euthyroidism. So, DNA analysis for beta-thalassemia was performed and found negative for all 10 common beta(0) and negative for 7 rare beta(+), and negative for 14 rare beta(0) thalassemia genes. And it was further confirmed with the whole beta-globin gene sequencing which was apparently normal. It was concluded that hyperthyroidism could temporarily induce many hematological parameters of a normal person to be closely similar to those of beta-thalassemia heterozygosity and all turned to normal after euthyroidism was achieved.

## Discussion

When definite diagnosis of primary hyperthyroidism had been concluded,<sup>5</sup> our case was misdiagnosed as beta-thalassemia heterozygosity because he had mild anemia, Hb 120 g/L, with mild microcytosis (MCV 72.1 fL), mild hypochromia (MCH 23.3 pg/cell), and mildly increased Hb A<sub>2</sub> percentage, 3.8%, that were perfectly consistent with parameters belonging to beta-thalassemia heterozygosity, MCV 67.0 ± 5.5 fL, MCH 21.2 ± 1.9 pg/cell, Hb A<sub>2</sub> 3.5 - 5.7 %, Hb concentration range from 106 ± 19 to 133 ± 17 g/L. His serum ferritin was normal (51.3 µg/L), more than 30 µg/L, hence iron deficiency anemia, another one of two common causes of microcytic anemia could be excluded.<sup>6</sup>

Basically, thyroid hormones can enhance erythropoiesis and delta globin chain synthesis.<sup>7</sup> Jaafer et al<sup>8</sup> showed the significant increase of Hb A<sub>2</sub> percentage in hyperthyroidism as compared with normal control, 2.77 ± 0.01 % vs 2.3 ± 0.01 %. But it did not reach 3.5%, the initial cut point for distinguishing beta-thalassemia heterozygosity from the control. In contrast, Hb A<sub>2</sub> in our case was 3.8% while the serum ferritin was adequate (51.3 µg/L) during hyperthyroidism. One of common causes of the decrease of percentage of Hb A<sub>2</sub> in beta-thalassemia heterozygosity is iron deficiency<sup>4</sup> anemia but it was not shown in Jaafer's study.

Characteristics of beta-thalassemia heterozygosity, Hct 35%, MCV 72 fL, MCH 27 pg/cell, MCHC 34 g/dL,

Hb A<sub>2</sub> 4.5% in Graves' disease used to be recognized in a Mediterranean man since 1994. After euthyroidism achievement, many parameters became normal, Hct 45% and Hb A<sub>2</sub> 2.8%<sup>9</sup> although the MCV and MCH were not mentioned. Moreover, the DNA analysis for beta-thalassemia was not demonstrated, either. In contrast, both MCV and MCH turned normal in euthyroidism in our case.

Besides hyperthyroidism, some authorities demonstrated the slightly higher percentage of Hb A<sub>2</sub> alone should not be generally the only one tool for making the diagnosis of beta-thalassemia heterozygosity because among 38 cases with beta-thalassemia traits which were proved with DNA sequencing; 7 cases had Hb A<sub>2</sub> < 3%, 3 cases had Hb A<sub>2</sub> 3.1 - 3.9 %, the remainder (28 cases) had Hb A<sub>2</sub> > 4%. On the contrary, among 189 normal cases, 179 cases had Hb A<sub>2</sub> < 3%, 8 cases had Hb A<sub>2</sub> 3.1 - 3.9 %, and 2 cases had Hb A<sub>2</sub> level above 4%.<sup>10</sup>

Hb concentration, MCV, and MCHC were found significantly low in only 38%, 8%, and 4% of hyperthyroidism, respectively, as compared with normal control.<sup>11</sup> Severity of the decrease of any parameter was not enough to suspect beta-thalassemia heterozygosity: Hb 124 ± 13 to 125 ± 14 g/L, MCV 81.3 ± 8.6 to 81.7 ± 8.3 fL, and MCH 26.9 ± 2.7 to 27.0 ± 3.6 pg/cell, as compared with 136 ± 12 g/L, 85.0 fL, 29.3 ± 2.9 pg/cell, respectively.<sup>12, 13</sup> In addition, because iron deficiency anemia can be found in 0.9% or more of Graves' disease patients,<sup>14, 15</sup> and it can contribute all these low parameters, so it should be concurrently explored in any microcytic anemic patient also.

Thyroid hormones could directly enhance erythropoiesis via the Krüppel-like factor 9 (KLF9) acting at thyroid hormone receptor-alpha that is essential for regulating erythropoiesis<sup>16</sup> or via increased erythropoietin through augmented accumulation of hypoxia-inducible factor-1.<sup>17</sup> Moreover, without iron deficiency, hyperthyroidism could induce microcytosis which would become normocytosis after euthyroidism achievement and the microcytosis could reappear again when the hyperthyroidism recurred,<sup>18</sup> the difference of MCV between hyperthyroidism and euthyroidism stages was 6 ± 3.5 fL.<sup>19</sup> The underlying mechanism of microcytosis in hyperthyroidism has not

been well understood but may be due to an ineffective erythropoiesis<sup>20</sup> or thyroid hormone itself because it was found in cats that the higher serum total thyroxine, the higher percentage of microcytosis was and there was no correlation between the time elapse from the first diagnosis of hyperthyroidism and the MCV.<sup>21-22</sup>

## Conclusions

Hyperthyroidism seemed to lead the misdiagnosis of beta-thalassemia heterozygosity in a normal person. Hence, Hb analysis should not be performed during hyperthyroidism.

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## ภาวะไทรอยด์เกิน ลงไปสู่การวินิจฉัยเบต้าธาลัสซีเมียแฝงเทียม

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ผู้ป่วยที่โลหิตจางเล็กน้อย มีขนาดเม็ดเลือดแดงเล็ก ดิสคีจาง และมีสัดส่วนฮีโมโกลบินเอ 2 (Hemoglobin [Hb] A<sub>2</sub>) เพิ่มขึ้นเล็กน้อย การวินิจฉัยที่เป็นไปได้มากที่สุดคือ ภาวะเบต้าธาลัสซีเมียแฝง ในรายงานนี้ แม้ผู้ป่วยจะตรวจพบสิ่งเหล่านี้ทั้งหมด แต่ไม่มีภาวะเบต้าธาลัสซีเมียแฝง ผู้ป่วยเป็นชายไทยอายุ 14 ปี มาพบแพทย์ด้วยอาการอ่อนเพลียและซีดร้อน เป็นเวลา 2 สัปดาห์ ตรวจร่างกายพบต่อมไทรอยด์โตเล็กน้อย ชีพจร 100 ครั้งต่อนาที ผลตรวจเลือดพบ Hb 120 g/L, mean corpuscular volume (MCV) 72.1 fL, mean corpuscular hemoglobin (MCH) 23.3 pg/cell, free triiodothyronine (FT<sub>3</sub>) > 20 pg/mL, free thyroxine (FT<sub>4</sub>) > 5.0 ng/dL, thyrotropin < 2.5 mIU/L, serum ferritin 51.3 µg/L, Hb A<sub>2</sub> 3.8% นอกเหนือจากภาวะฮอร์โมนไทรอยด์สูงปฐมภูมิแล้ว การวินิจฉัยอีกอย่างคือ ภาวะเบต้าธาลัสซีเมียแฝง แพทย์ให้การรักษาด้วยยาต้านไทรอยด์จนครบ 6 เดือน ตรวจเลือดซ้ำพบว่าฮอร์โมนไทรอยด์เกือบปกติ Hb 146 g/L, MCV 83.3 fL, MCH 26.3 pg/cell, Hb A<sub>2</sub> 3.0% ซึ่งไม่เพียงระดับฮอร์โมนไทรอยด์เท่านั้นที่ปกติ แต่ความเข้มข้นฮีโมโกลบินขนาดเม็ดเลือดแดง และสัดส่วนของฮีโมโกลบินเอ 2 ก็ปกติด้วย ผลการตรวจวิเคราะห์ยีนส์พบว่า ไม่พบยีนส์เบต้าธาลัสซีเมีย และลำดับยีนส์ของสายเบต้าโกลบินก็พบว่าปกติ จึงอาจสรุปว่าภาวะฮอร์โมนไทรอยด์ที่สูงเหนี่ยวนำให้คนปกติมีสัดส่วนของฮีโมโกลบินเอ 2 เพิ่มขึ้นเล็กน้อย มีโลหิตจางเล็กน้อยแบบเม็ดเลือดแดงมีขนาดเล็กและดิสคีจาง นำไปสู่การวินิจฉัยภาวะเบต้าธาลัสซีเมียแฝงเทียม อาจกล่าวได้อีกหนึ่งว่า การตรวจวิเคราะห์ชนิดของฮีโมโกลบินในผู้ป่วยที่มีภาวะฮอร์โมนไทรอยด์สูง ควรรองกว่าเข้าสู่ภาวะไทรอยด์ปกติ

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