

CASE REPORT

HEMOGLOBIN PYRGOS WITH HEMOGLOBIN H DISEASE: NEW TRIPLE HETEROZYGOSITY

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Abstract. A 19-year old Thai male presented to the hospital with fever, acute hemolysis, pallor and jaundice without hepatosplenomegaly. On admission his hematocrit was 17.4% and a blood smear showed moderate hypochromia with mild anisopoikilocytosis. Hemoglobin (Hb) electrophoresis revealed Hb A₂, ABart's Hb H with an abnormal band, which on PCR proved to be Hb Pyrgos ($\beta^{83, \text{glycine} \rightarrow \text{aspartic acid}}$). The patient inherited β^{Pyrgos} globin from his mother and α -thalassemia-1 from his father. He was diagnosed as having Hb H (α -thalassemia-1/ α -thalassemia-2) heterozygous Hb Pyrgos. He was treated with a transfusion of packed red blood cells. During follow-up his hematocrits ranged from 31 to 34%. The Hb Pyrgos did not add any deleterious effect to his Hb H disease.

Keywords: Hb Pyrgos, Hb H disease, triple heterozygosity

INTRODUCTION

Hemoglobin (Hb) Pyrgos is a Hb variant caused by a missense mutation at codon 83 of the β globin gene (GGC to GAC) resulting in the substitution of glycine by aspartic acid ($\beta^{83 \text{ Gly} \rightarrow \text{Asp}}$). On Hb electrophoresis, Hb Pyrgos moves faster than Hb A but more slowly than Bart's Hb (Jetsrisuparb *et al*, 2002). Its oxygen (O₂) affinity is normal (Yamada *et al*, 1977).

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Hb Pyrgos was first identified in a 3-year old Greek boy who also had Hb S without anemia (Tatsis *et al*, 1976). Since then it has been occasionally reported in Japan (Yamada *et al*, 1977), Turkey (Akar *et al*, 2003) and Inner Mongolia (Qin *et al*, 1994). In Thailand, the first reported case of Hb Pyrgos was in a heterozygote individual (Pravatmuang *et al*, 1996) followed by a double heterozygote individual with Hb Pyrgos and Hb Tak (Fucharoen *et al*, 1997) and in various combinations of triple heterozygotes: Hb Pyrgos + α -thalassemia-1 + Hb Constant-Spring (CS), or Hb Pyrgos + Hb E + Hb CS, or Hb Pyrgos + Hb E + α -thalassemia (Sawangareetrakul *et al*, 2002).

We report a new case of triple heterozygosity with Hb Pyrgos + α -thalassemia-1 + α -thalassemia-2 in a young Thai man.

CASE REPORT

The case is a 19-year old Thai male who presented to the hospital with sudden onset fever, dark urine for several days, fatigue and dizziness. During the febrile illness, he had no common cold type symptoms or bleeding and had not taken any medication. His past history revealed no hemolytic episodes or transfusions. The family history was negative for hemolytic disease and there was no consanguinity. On examination, his height was 1.78 m, and his weight was 60 kg. He had moderate pallor, mild jaundice and no hepatosplenomegaly. On admission, his Hb was 5.6g%, Hct 17.4%, RBC $2.4 \times 10^6/\text{mm}^3$, WBC $8,100/\text{mm}^3$, platelets $233,000/\text{mm}^3$, MCV 71.5 fl, MCH 23.0, MCHC 32.1, RDW 18.6, NRBC 6/100 WBC, neutrophils 60%, lymphocytes 26%, monocytes 4%, eosinophils 4%, atypical lymphocytes 6%, hypochromia 2+, anisopoikilocytes 1+, microcytes 1+, macrocytes 1+, ovalocytes 1+, a few tear drop cells; his BUN was 13 mg%, creatinine 1.12 mg%, albumin 4.2 g%, globulin 3.6 g%, indirect bilirubin 2.9 mg%, direct bilirubin 0.3 mg%, AST 25 IU/l, ALT 12 IU/l, alkaline phosphatase 75 IU/l, cholesterol 100 mg%, triglycerides 129 mg%, LDL 35 mg% and HDL 39 mg%; his hemoculture was negative for bacterial growth. His urinalysis was unremarkable. His dengue titer and anti-HIV antibody were negative. His direct and indirect Coombs' tests were negative; G-6-PD screening was negative. Hb electrophoresis revealed A₂ 1.3%, A 39.3%, Hb H, Hb Bart's, an abnormal Hb band 57.7%, later identified by PCR as Hb Pyrgos, and Hb

F 1.3%. PCR for an α -thalassemia-1 gene was positive for Southeast Asian (SEA) type and α -thalassemia-2 was also positive. The final diagnosis was Hb H disease with co-inheritance of Hb Pyrgos trait, which resulted in sudden hemolytic crisis during the febrile illness.

Hb Pyrgos was identified using a multiplex allele specific PCR to differentiate between Hb Pyrgos and Hb J Bangkok ($\beta^{56,\text{Gly} \rightarrow \text{Asp}}$) as described previously (Fucharoen *et al*, 2005). In brief, the Hb Pyrgos specific primer (5' CAC CTG GAC AAC CTC AAG GA 3') was used with a common primer (5' TCC CAT AGA CTC ACC CTG AA 3') located downstream to produce an 89 bp Hb Pyrgos specific fragment. During the PCR, two additional PCR primers (5' GGC CTA AAA CCA CAG AGA GT 3' and 5' CCA GAA GCG AGT GTG TGG AA 3'), which produce a 578 bp fragment of the γ -globin gene promoter were also included to produce an internal control fragment. The PCR mixture contained 100 ng genomic DNA, 15 pmol of each primer, 200 μM dNTPs, and 1 U *Taq* DNA polymerase in 10 mM Tris-HCl buffer at a pH of 8.3, with 50 mM KCl and 3 mM MgCl_2 . Thermal cycling (conducted in a T-Personal Thermocycler, Biometra, Gottingen, Germany) consisted of 30 cycles (after an initial heating to 94°C for 3 minutes) of 94°C for 1 minute and 65°C for 1.5 minutes. The amplicon was analyzed with 1.5% agarose gel electrophoresis and visualized under UV light after ethidium bromide staining.

The cause of the infection in this patient could not be determined, but since the fever resolved spontaneously within 2 days and the hemoculture revealed no growth, no antibiotics were given. The patient was treated by transfusion with 2 units of packed red blood cells and discharged home on folic acid supplementation.

At 1-month follow-up, his CBC showed a Hb of 10.3 g%, Hct 34.6%, RBC $4.8 \times 10^6/\text{mm}^3$, WBC $6,700/\text{mm}^3$, N 56%, L 32%, E 14%, platelets $375,000/\text{mm}^3$, MCV 71.3, MCH 21.6, MCHC 29.4, RDW 19.5, hypochromia 1+, and anisopoikilocytosis 1+. His hematocrit remained stable between 31 and 34% during successive follow-up visits.

A CBC in the patient's father showed a Hb of 12.3 g%, Hct 37.7%, WBC $6,000/\text{mm}^3$, platelets $235,000/\text{mm}^3$, MCV 72.1, MCH 23.6, MCHC 32.7 and RDW 15.6; Hb electrophoresis showed a Hb A₂ 3.0%, Hb A 96.4% and Hb F 0.6%. A CBC in the patient's mother showed a Hb of 12.0 g%, Hct 37.7%, WBC $8,000/\text{mm}^3$, platelets $246,000/\text{mm}^3$, MCV 79.9, MCH 25.6, MCHC 32.0 and RDW 13.6; Hb electrophoresis showed a Hb A of 0.2%, Hb F 2.6%, Hb E 20.5% and Hb Pyrgos 75.1%. A PCR for α -thalassemia-1, SEA type, was positive in the father but detection of α -thalassemia-2 was not carried out.

DISCUSSION

The patient had Hb Pyrgos and Hb H disease, a triple heterozygote of Hb Pyrgos, α -thalassemia-1 and α -thalassemia-2. The RBC indices and morphology were that of classical Hb H disease (Sutcharitchan *et al*, 2005). The triple heterozygote still retained an important characteristic of classical H disease, namely, development of an acute hemolytic crisis during exposure to fever (Fucharoen and Viprakasit, 2009).

Heterozygous Hb Pyrgos does not manifest any clinical symptoms, having Hb concentrations within the normal range (Waye *et al*, 2001). When combined with other β -globin variants, such as Hb S or Hb E, Hb Pyrgos still does not aggravate any

clinical or hematological problems (Tatsis *et al*, 1976; Jetsrisuparb *et al*, 2002). The proband's mother had Hb E trait, Hb Pyrgos trait and presumably homozygous or heterozygous α -thalassemia-2, but her Hb level was still normal, with only a slightly low MCV and a low MCH.

When Hb Pyrgos is in triple heterozygosity with Hb CS and Hb E, it does not contribute any deleterious effects on the patient (Fucharoen *et al*, 2005), but when Hb Pyrgos is combined with Hb H and Hb CS, the patient has anemia and needs transfusions during childhood (Jetsrisuparb *et al*, 2002). The anemia in a patient with Hb H and Hb CS is always more severe than in classic Hb H disease (Fucharoen and Viprakasit, 2009). However, when Hb H disease is combined with other β -globin hemoglobinopathies, such as Hb E (Hb AEBart's disease), our patient has a better hematological outcome; half of Hb AEBart's cases have only mild hepatosplenomegaly, with a mean Hb level of 7.6 ± 1.3 g% (Fuchareon *et al*, 1988). Triple heterozygosity with Hb H, Hb CS and heterozygous Hb J Bangkok is more severe (Hb concentration of 8.1 g% at stable state) than in our patient (Fucharoen *et al*, 2001).

The α globin chain is believed to prefer combining with β^{Pyrgos} more than $\beta^{\text{J Bangkok}}$ because the percent of Hb Pyrgos in Hb Pyrgos trait is greater than Hb J Bangkok in Hb J Bangkok trait (Fucharoen *et al*, 2005). This may be the reason why triple heterozygosity including Hb H and Hb J Bangkok is more severe than triple heterozygosity including Hb H and Hb Pyrgos.

This is the first report of a triple heterozygote with Hb Pyrgos, α -thalassemia-1 and α -thalassemia-2. The clinical and hematologic manifestations in this

patient were similar to those of classical Hb H disease.

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