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CASE REPORT

Hereditary persistence of hemoglobin F is protective against red cell sickling. A case report and brief review

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Abstract
Fetal hemoglobin (HbF) is a physiologic protein tetramer that is crucial for a developing fetus to survive in utero. Maternal hemoglobin has a relatively lower affinity for oxygen, and thus allows for an efficient transfer of oxygen from maternal to fetal blood. In addition to fulfilling a critical physiologic role, HbF is also known to alleviate symptoms of sickle-cell disease (SCD). The concentration of HbF depends on several factors. HbF is elevated in inherited conditions, such as hereditary persistence of HbF, hereditary spherocytosis, and thalassemia. The level of HbF is also increased in acquired states, such as pregnancy, aplastic anemia, thyrotoxicosis, hepatoma, myeloproliferative disorders, or hypoplastic myelodysplastic syndrome. It has been identified that some genetic loci have significant influence on HbF levels. The *Xmn*I polymorphism, the *HMIP* locus, and the *BCL11A* gene are responsible for 45% of variations in HbF levels. Although SCD has been well described in the subpopulations of Africa, it is less common in the subpopulations of India. We describe a case of SCD, in which a patient with high HbF level presented at a very late age (27 years old). We presume the patient's inherently elevated HbF levels were able to compensate for the hypoxic episodes associated with SCD. The onset of symptoms was delayed as a result of elevated HbF levels.

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Introduction

Sickle-cell disease (SCD) is one of the best characterized red-blood-cell disorders with 100,000 cases per year in the United States [1]. Clinically, SCD is manifested by vaso-occlusive events. An acute pain that may be due to temporary ischemia is usually the first symptom of SCD. In more severe cases, multi-organ failure occurs, which can be reversed by aggressive medical therapy and blood transfusions. Other complications that may be related to impaired circulation and blood flow include bone infarcts, ankle ulcers, strokes, and retinopathy. Auto-splenectomy may lead to infections with encapsulated bacteria, such as *Haemophilus influenzae* and *Streptococcus pneumoniae*. Additionally, acute chest syndrome (ACS) is the second most common cause of hospitalization of patients with SCD and is responsible for nearly 25% of all deaths associated with SCD [2]. One particular study followed 3751 SCD patients aged 0—66 years over a 5-years period. In that study, 25% of patients developed ACS. In addition, bacteremia was found in 3.5% of the patients [3,4]. Children, however, usually do not experience any symptoms throughout the first 6 months of life. After the levels of fetal hemoglobin (HbF) peak around 12 months, they start to gradually decline, and thus predispose children to symptomatic SCD. The mean age of SCD diagnosis is 27.33 months [5]. HbF decreases sickling in SCD patients. The presence of two gamma chains in HbF ($\alpha_2\gamma_2$) gives it a higher affinity for oxygen than adult hemoglobin (HbA) or hemoglobin S(HbS), which decreases the polymerization of deoxygenated HbS. By 12 months, the HbF concentration decreases, and in a normal adult, only about 1% of hemoglobin (Hb) is HbF ($\alpha_2\gamma_2$), 97% is HbA ($\alpha_2\beta_2$), and 2% is HbA_2 ($\alpha_2\delta_2$) [4]. Given the higher affinity of HbF for oxygen, there have been several attempts to stimulate the production of HbF in adults with SCD with the goal of higher oxygen saturation and decreasing hypoxic episodes. The most effective drugs that stimulate hematopoiesis are hydroxyurea, 5-azacytidine, and erythropoietin. Hydroxyurea stimulates the production of HbF by releasing nitric oxide. Nitric oxide stimulates a gene-transcribing pathway that ultimately stimulates the production of HbF de novo. An antineoplastic drug, 5-azacytidine, works by inhibiting DNA methylation, thus inhibiting the switch from HbF to HbA synthesis [6].

Erythropoietin, a natural substance, is a potent stimulator of HbF production. However, it is not as effective as Hydroxyurea [7].

Case report

A young Indian man in his late twenties presented to the emergency department with a chief complaint of pain in the right upper quadrant. The pain had been present for 3 days, and was described as aching and intermittent. The pain did not change with changes in position and did not radiate to any other abdominal quadrant. Further review of systems was significant for a lack of dyspnea, cough, fever, or changes in breathing. The family history was unremarkable.

Pertinent positive physical-exam findings included pain in the right upper quadrant and the epigastric area, as well as slight icterus. Pertinent negative physical-exam findings included a lack of surgical scars and negative Murphy's sign. The laboratory values were as follows: Hb, 8.5 g/dL; mean corpuscular volume, 92.4 fL; mean corpuscular hemoglobin, 30.7 pg; red-cell distribution width, 25.1%; platelet count, 584,000/mm^3; white-blood-cell count, 20,100/mm^3; aspartate aminotransferase, 43 U/L; total bilirubin, 3.2 mg/dL; and indirect bilirubin, 2.8 mg/dL. The ultrasound/computed-tomography imaging of the abdomen revealed a small spleen and no signs of cholecystitis. The red-blood-cell morphology was representative of target cells and sickle cells. The reticulocyte count and haptoglobin levels were 14% and <20 mg/dL, respectively. An Hb electrophoresis assay showed HbA_2 3.7%, HbF 8.4%, and HbS 87.9%. The patient developed an episode of ACS on the 3rd day and had a subsequent oxygen saturation of 88%. In addition, a chest X-ray revealed bilateral lower lobe infiltrates. The pain was treated with an exchange transfusion, and showed a marked improvement. Upon follow-up, the patient had a total of five admissions in 6 months for SCD. Of the five admissions, two required exchange transfusions. The patient was maintained on folic acid, yet denied treatment with hydroxyurea.

Discussion

Prevalence and biochemical properties of different human Hb

HbF is a major Hb during gestation, which is replaced almost completely by HbA by 12 months. HbF is a tetramer consisting of two α chains and two γ chains; γ-globin can be γ^G and γ^A, which are identical and differ in one amino acid at position 136: glycine (γ^G chains) or alanine (γ^A chains). In infants, γ^G is more prevalent, while γ^A is higher in adults. HbF has a higher affinity for oxygen than does HbA due to decreased binding of 2,3-diphosphoglycerate. The P_{50}, or partial pressure at which Hb is 50% saturated with oxygen, is 19 mmHg for HbF, compared to 27 mmHg for HbA. The high affinity for oxygen shifts the oxygen dissociation curve to the left, leading to Bohr effect 20% higher than that of HbA. HbF levels vary more than 20-fold in a healthy adult population [8,9]. Family studies showed that the HbF levels are inherited, but they do not obey Mendelian pattern of inheritance. It is believed that the HbF levels are multifactorial: 89% of variability is attributed to genetic factors, and 11% to age, sex, and environmental factors [10]. Certain β-globin haplotypes in SCD seem to have processes that regulate the production of HbF. For example, the Arab—Indian and Senegal haplotypes are associated with higher levels of HbF (over 20% in some cases). In one study of Senegalese patients, the mean HbF was 8.2%, and approximately half of the patients had a benign form of sickle-cell anemia [11].

Clinical significance of varying levels of Hb throughout life

In some inherited conditions, the HbF levels are elevated in adulthood. Most of the inherited conditions with increased HbF levels are associated with β-globin synthesis abnormalities. The inherited conditions include hereditary

persistence of HbF (HPFH), hereditary spherocytosis, and thalassemia. SCD is usually milder in patients with concurrent β-thalassemia because of the elevated HbF.

Delta—beta-thalassemia is a condition caused by deletions in δ- and β-globin genes ($\gamma^G\gamma^A[\delta\beta]^\circ$-thalassemia), or sometimes the γ^A gene ($\gamma^G[\gamma^A\delta\beta]^\circ$-thalassemia). These patients have elevated levels of HbF (usually 4—25%) and hypochromic microcytic erythrocytes [12].

HPFH is a benign asymptomatic condition, in which the HbF synthesis continues into adulthood. Erythrocytes usually have a normal morphology in HPFH. There are two types of HPFH: pan-cellular and heterocellular. Pan-cellular HPFH is caused by deletions of the β-globin gene or single point mutations in the γ-globin gene-promoter regions. It is inherited in Mendelian pattern. The HbF levels are usually 10—40%, but can reach up to 100% in the deletion type. The HbF level is uniform in all erythrocytes. Heterocellular HPFH has a milder increase in HbF levels (about 5%), and the concentration of HbF within red blood cells varies. This type does not obey Mendelian inheritance [13].

Some loci, chromosome point mutations, and post-transcriptional modifications have significant influence on HbF levels.

The *Xmn*I polymorphism on chromosome 11 (11p15), the *HMIP* locus on chromosome 6 (6q23), and single-nucleotide polymorphisms for the *BCL11A* gene on chromosome 2 (2q16) are responsible for 45% of variations in HbF levels. *Xmn*I polymorphism (rs7482144) is a single-nucleotide polymorphism (C → T) at position −158 upstream of the γ^G-globin gene. It is present in 32—35% of the population. It has a frequency of 14% among Caucasians with no underlying hemoglobinopathies. *Xmn*I polymorphism upregulates γ^G expression and leads to elevated HbF levels especially in situation of erythropoietic stress. It misbalances the γ^G:γ^A ratio resembling the ratio at birth (70:30), while usually γ^A is predominant in adults. Thus, *Xmn*I polymorphism leads to elevated levels of HbF, which is beneficial to patients with SCD and thalassemia, and leads to milder symptoms [14—17].

Genetic influences in Hg levels and formation

Quantitative trait locus (QTL) on chromosome 8, 8q region is suggested to influence HbF levels by encoding transcriptional factors, which bind to the *Xmn*I site [18]. *HMIP*, another QTL that regulates HbF levels, is found on chromosome 6 and is known to participate in the erythroid-maturation pathway [19]. (locus 22 vs locus 48) Another QTL regulating HbF levels is *HMIP* at 6q23. It is located on chromosome 6 between the *HBS1L* gene (codes elongation factors and regulates multiple cellular processes) and the *MYB* gene (encodes transcription factors, and participates in ontogenesis and erythropoiesis) [19]. This QTL codes factors that participate in the erythroid-maturation pathway. Wahlberg et al. demonstrated a correlation between 6q23 QTL and levels of HbF in Indian β-thalassemia patients. It is yet to be determined whether this correlation is secondary to a direct or indirect effect [9]. Craig et al. studied an Indian female population with β-thalassemia, and found that those with homozygous 6q23 QTL have a higher HbF concentration (24% compared to 10%). The same correlation was noticed among the healthy population in this study (3—1%). One more QTL associated with elevated HbF is *BCL11A*, located in the 2q16 region of chromosome 2. Fifteen percent of HbF variations are attributed to *BCL11A* QTL. *BCL11A* is more frequent in patients with β-thalassemia intermedia than in β-thalassemia major. Also, there is an association of the *BCL11A* QTL and HbF among Afro-American and Brazilian SCD patients. The *BCL11A* gene product is a zinc-finger transcription factor, which is thought to participate in turning off the γ-globin gene and activating the β-globin gene [20]. *BCL11A* QTL silences the expression of the γ-globin gene in K562 cells by binding to the gene-promoter central part. Another group led by Xu et al. reported that the *BCL11A* transcription factor silences the γ-globin genes by interaction with the β-globin gene-cluster chromatin and forming a chromosomal loop in the presence of the SOX6 transcription factor in the γ-globin gene-promoter regions. All these studies point toward the *BCL11A* transcription factor playing an essential role in silencing the γ-globin gene in adults [20,21].

Kruppel-like factor 1 (KLF1) is a transcription factor that plays an important role in erythropoiesis. The KLF1-coding gene is located on chromosome 19p13.3 (*ZBTB7A* locus) [22]. KLF1 activates the β-globin gene expression. It also interacts with *BCL11A*, leading to indirect silencing of the γ-globin gene. Mutations in the *KLF1* gene can change the DNA-binding domain of KLF1, which alters its regulatory action and leads to increases in HbF levels [23]. Various researchers reported 49—70% increase in HbF levels with a knockdown of ZBTB7A in CD34+ cells [22,24].

Role of drug therapy in SCD

Several medications, such as hydroxyurea, azacitidine, and Procrit, increase the levels of HbF. The Food and Drug Administration approved hydroxyurea for SCD treatment in 1998. Although the exact mechanism is not fully understood, it is postulated that hydroxyurea works by inhibiting the enzyme, ribonucleotide reductase, thus preventing the conversion of ribonucleotides to deoxyribonucleotides, a crucial step in the production of DNA. Hydroxyurea is used in the treatment of SCD, and decreases the number of hospitalizations, length of stay, and in-hospital analgesic use. Theoretically, it decreases the incidence of ACS and may lead to decreased mortality [25,26].

There are few new SCD treatment approaches under investigation at present. Gene therapy is a very promising field. Hacein-Bey-Abina et al. have used the transplantation of autologous hematopoietic stem cells with transduced lentiviral beta AT87Q-globin vector to treat patients with sickle cell and β-thalassemia. As a result, patients with severe SCD had 51.5% of antisickling Hb (HbA, HbF, and HbA2) 9 months after stem-cell transplantation. This study presents gene therapy as curative potential for SCD and thalassemia [27]. However, gene therapy poses certain risks. Incidence of leukemia in patients who underwent gene therapy for severe combined immune deficiency in London and

Paris trials raised many safety concerns for vector-transduction therapies [28].

Conclusion

Our medical team characterized the presentation of a 27-year-old patient with ACS and a severe vaso-occlusive crisis. The usual age of presentation of such patients is usually early in life, as the predominant form of Hb shifts from HbF to HbA. HbF has a naturally protective role against SCD. Although the exact genetic polymorphisms of our patient were not known, we suspect that elevated levels of HbF were responsible for delaying the symptomatic presentation of our patient. The levels of HbF could have been elevated secondary to HPFH. It is, however, unclear what factors had triggered his disease and are causing him to have multiple vaso-occlusive crises at the age of 27 years.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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