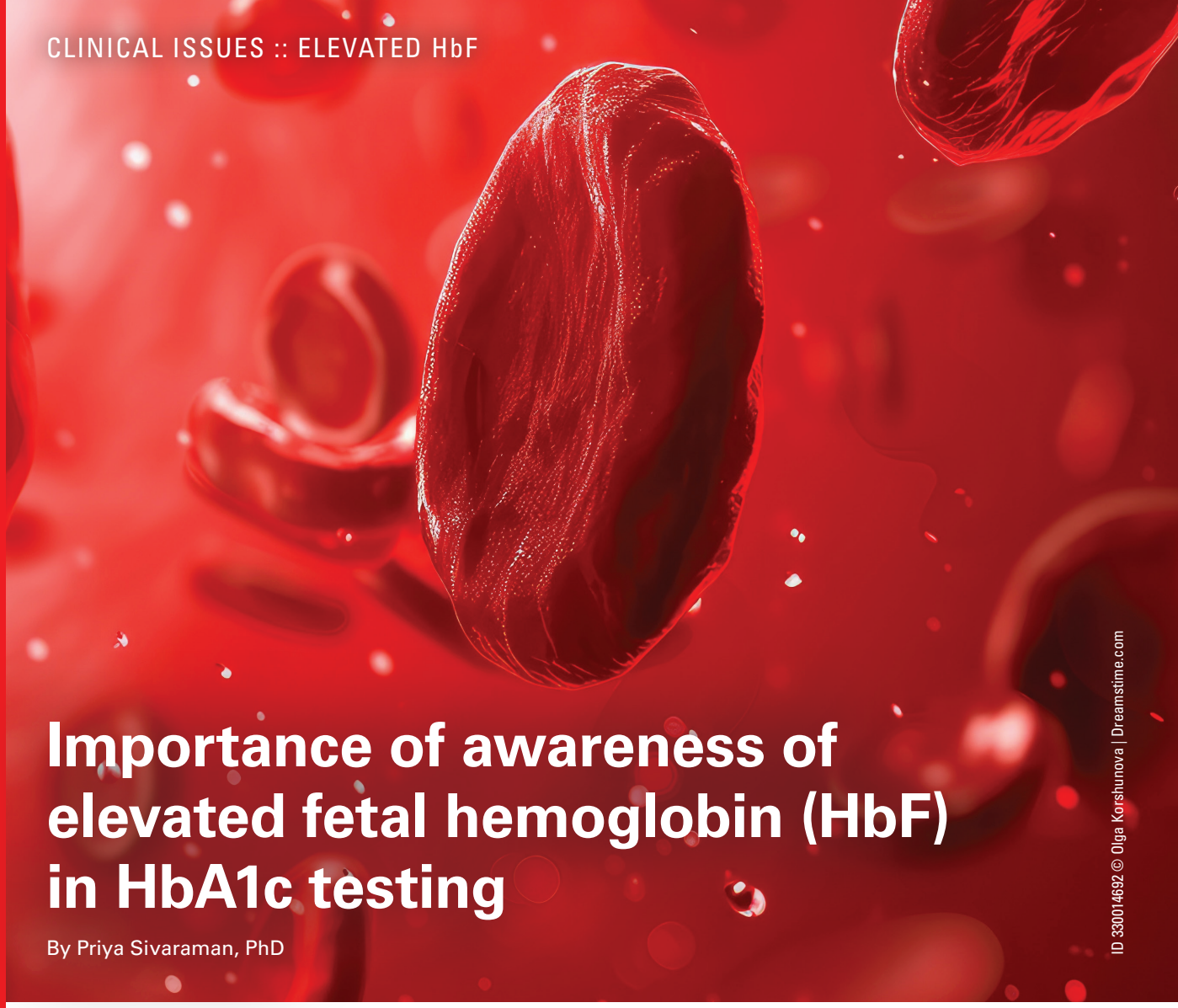

Importance of awareness of elevated fetal hemoglobin (HbF) in HbA1c testing

By Priya Sivaraman, PhD

Elevated fetal hemoglobin (HbF) in adults, though well-documented, remains a point of interest in clinical diagnostics, particularly in HbA1c testing. HbF is a type of hemoglobin made of two alpha and two gamma subunits, in contrast to adult hemoglobin (HbA), which consists of two alpha and two beta subunits. Typically present in fetuses and newborns, HbF production usually decreases by six months of age. However, several conditions lead to elevated HbF levels in adults, potentially affecting the accuracy of A1c test results, a key tool for monitoring diabetes.[1] The illustration in **Figure 1** (on page 12) describes the process.[2]

Some conditions and situations associated with elevated HbF in adults

- **Sickle cell disease (SCD):** Sickle cell disease, a genetic disorder affecting hemoglobin, causes red blood cells to become rigid and sickle-shaped, obstructing blood flow. This condition is prevalent in African and African American populations, with 1 in 365 African American babies born with sickle cell disease. HbF, which typically accounts for less than 1% of total hemoglobin in adults, is elevated in individuals with SCD, ranging from 5% to 8%. Higher HbF levels in these patients may provide some protection against the severe effects of sickle cell anemia by reducing red cell sickling.[3]
- **β-thalassemia:** β-thalassemia is an inherited blood disorder caused by mutations in genes responsible for hemoglobin production. There are two main types: β-thalassemia major, characterized by severe anemia requiring regular blood transfusions, and β- thalassemia minor, which leads to mild anemia. In some forms of β-thalassemia, such as HbE/ β-thalassemia, HbF levels are elevated, potentially providing a survival advantage by increasing the number of red blood cells with HbF (F cells). This elevation is driven by increased erythropoietin production, a hormone that promotes red blood cell production.[4,5]
- **Hereditary persistence of fetal hemoglobin (HPFH):** HPFH is a benign condition characterized by the continued production of significant levels of HbF into adulthood. This occurs due to mutations in genes that regulate hemoglobin production, allowing HbF levels to remain elevated even after birth.[6]
- **Malignancies:** Elevated HbF levels can also be seen in individuals with certain malignancies and among specific ethnic groups. For example, some populations of Mediterranean, Southeast Asian, or African descent may naturally have higher HbF levels.[7]
- **Therapeutic factors:** HbF is being explored as a potential treatment option in certain conditions. Recent research

has identified a protein, hypoxia-inducible factor 1α (HIF1α), that activates the production of fetal hemoglobin (HbF) in adult blood cells. Additionally, CRISPR-Cas9 gene editing can be used to increase HbF levels in patients with β-thalassemia and correct the primary gene mutations causing the condition. Methods to enhance the transcription of the gamma-globin (HBG) gene are being explored, allowing patients with β-hemoglobinopathies to produce more HbF. HbF is also being investigated as a potential treatment for severe inherited blood disorders like sickle cell disease and β-thalassemia.[8,9,10]

Does elevated HbF affect HbA1c results?

Whether it is a condition that may or may not cause health problems or a therapy approach considered in treating a condition, it is important to be aware that the presence of elevated HbF in individuals can impact the accuracy of HbA1c test results. Since HbA1c testing measures glycated hemoglobin to estimate average blood glucose levels, elevated HbF may lead to either falsely high or falsely low HbA1c values, depending on the testing method used.[11]

This raises challenges for laboratories, as not all HbA1c testing methods can detect or account for elevated HbF and hence show HbF interference. Methods for HbA1c testing include cation-exchange HPLC, boronate affinity, capillary electrophoresis, immunoassays, and enzymatic methods. Boronate affinity, immunoassay, and enzymatic methods have been reported to exhibit lower tolerance to elevated HbF levels compared to ion-exchange HPLC and capillary electrophoresis. Boronate affinity and immunoassay methods leverage structural differences in hemoglobin molecules.[12]

Boronate affinity methods measure total glycated Hb and results are reported as a corrected HbA1c equivalent. HbF has gamma chains, for which the terminus is a glycine residue, in place of β-chains where the valine terminal residues can be glycated to form HbA1c. Thus, for boronate affinity methods, since HbF is glycated to a lesser extent, the glycated fraction will be lower than for people without elevated HbF. The interference of HbF with HbA1c results is likely due to a lower glycation rate for HbF compared with HbA. Because boronate affinity measures the ratio of glycated to non-glycated hemoglobin regardless of species, the presence of elevated HbF causes a false lowering of the HbA1c result. With boronate affinity methods, HbF at levels of 10–15% interfere with HbA1c results. Even though the user is able to detect the glycated and the non-glycated peak in the form of a chromatogram, the method lacks the ability to detect or presumptively identify HbF, making it impossible to know if the HbA1c results needs a second opinion and/or verification.[13,14,15]

Immunoassays for HbA1c measurement also leverage structural differences between hemoglobin molecules. The system measures both A1c and total hemoglobin (THb), using antibodies that specifically bind to the HbA1c epitope, which includes the glycated N-terminal valine and the next three amino acids of the β-chain. The system detects the antigen–antibody complex; and the HbA1c percentage is calculated as the ratio of measured glycated HbA to THb. In patients with elevated HbF, lack of glycation at the terminus of the gamma chain of HbF results in less antibody binding, while the total hemoglobin measurement includes HbF as well as HbA, leading to a lower HbA1c result.[14] This limitation is acknowledged in the instructions for use of immunoassay systems. With immunoassays, HbF as low as 10–15% can interfere with HbA1c results.[16]

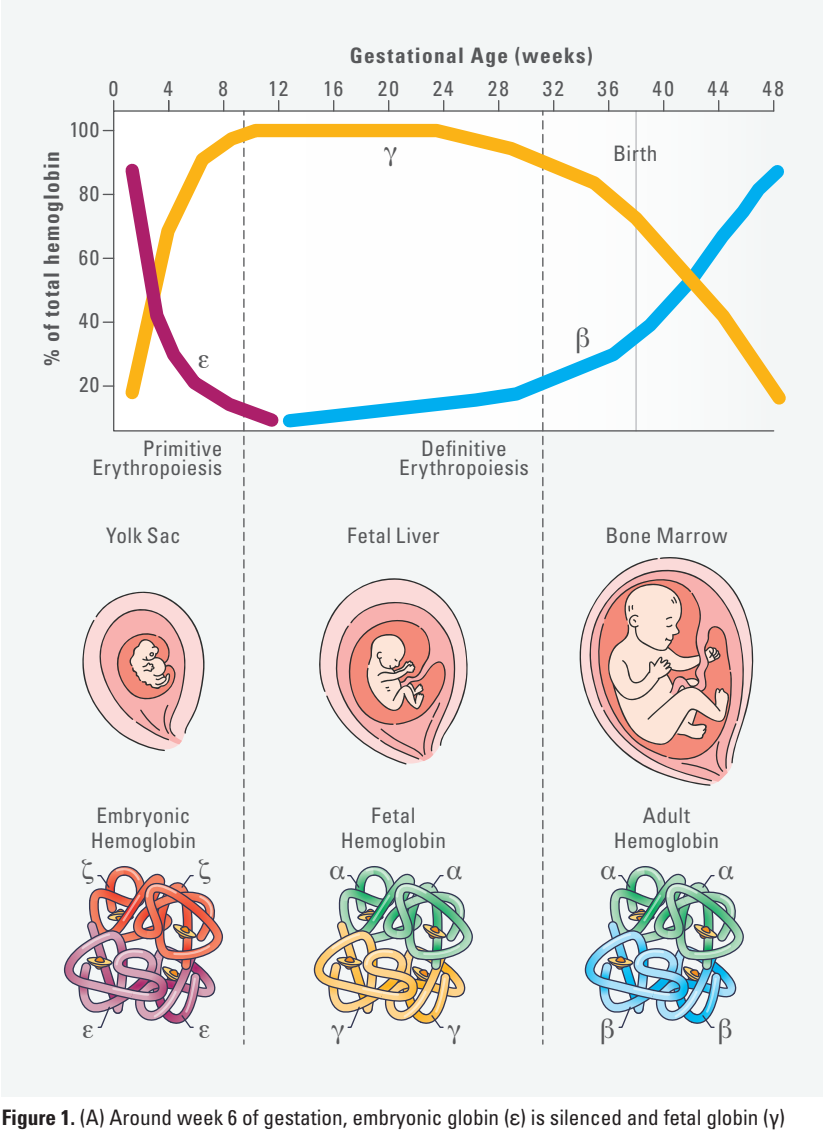

Figure 1. (A) Around week 6 of gestation, embryonic globin (ε) is silenced and fetal globin (γ) starts to be expressed. Perinatally the switch to adult globin (β) occurs. (B) Erythropoiesis occurs in the blood islands of the yolk sac in the first 8 weeks of gestation, then in the fetal liver between 8 and 32 weeks, and finally in the bone marrow from 32 weeks on. (C) Structure of the main human hemoglobins expressed during development. Embryonic globin, fetal hemoglobin and adult hemoglobin.

Some immunoassay manufacturers notify users that their method interferes with HbA1c results at levels as low as 7% HbF, leading to inaccurately lower reported HbA1c values.[17]

Enzymatic methods measure HbA1c and total hemoglobin (THb) from whole blood specimen. Some methods specifically measure N-terminal fructosyl dipeptides of the β-chain of HbA1c cleaved by a fructosyl peptide oxidase. The HbA1c concentration is calculated from the ratio of HbA1c and THb: HbA1c. These methods like immunoassays provide the laboratorian with just a HbA1c number with no detection of HbF or other variant hemoglobins. Just as in immunoassays, enzymatic assays also show interference with HbA1c results at HbF concentration of 10–15%.[16]

Cation exchange HPLC for HbA1c testing separates hemoglobin molecules based on charge. The method employs a column with negatively charged stationary phases that interact with the positively charged amino groups of hemoglobins. As the sample passes through the column, hemoglobin fractions are separated based on their charge differences. HbA1c elutes at a specific retention time and is detected via absorbance, typically at 415 nm. An HPLC chromatogram is a graphical representation of the separation of compounds as they pass through the HPLC system. Each peak on the chromatogram corresponds to a different fraction in the sample.[12] In the context of HbA1c testing, the chromatogram shows distinct peaks for various hemoglobin fractions including HbF.

A laboratory within the Texas hospital system uses a cation exchange HPLC analyzer for HbA1c testing in their laboratory. They see a fair amount of adult patient whole blood samples that are presumptively identified as HbF on their HPLC analyzer. The analyzer claims no interference with HbA1c results from HbF at levels up to 25% (**Figure 2**). At levels beyond 25%, the analyzer will provide a presumptive identification of the HbF, however, it will not provide an HbA1c result (**Figure 3**). The advantage of an ion exchange HPLC analyzer is the ability to detect a peak in the HbF window highlighted on the chromatogram.

Summary

As the incidence of elevated HbF in testing samples rises, laboratories need to remain vigilant and assess the potential impact on HbA1c measurements. Accurate diagnostics ensure appropriate diabetes management and reduce the risk of misdiagnosis or inadequate treatment.

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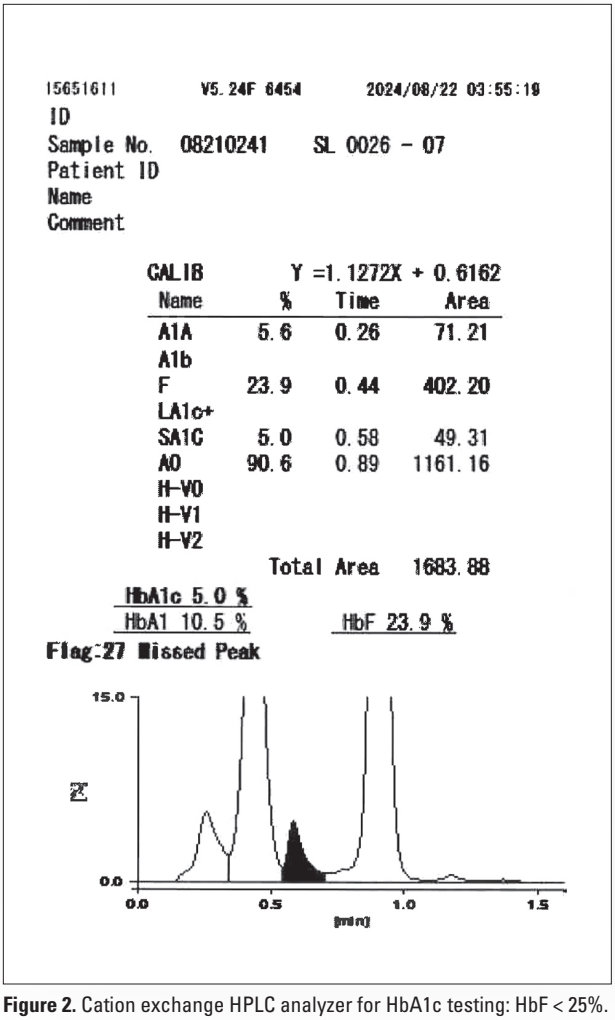

Figure 2. Cation exchange HPLC analyzer for HbA1c testing: HbF < 25%.

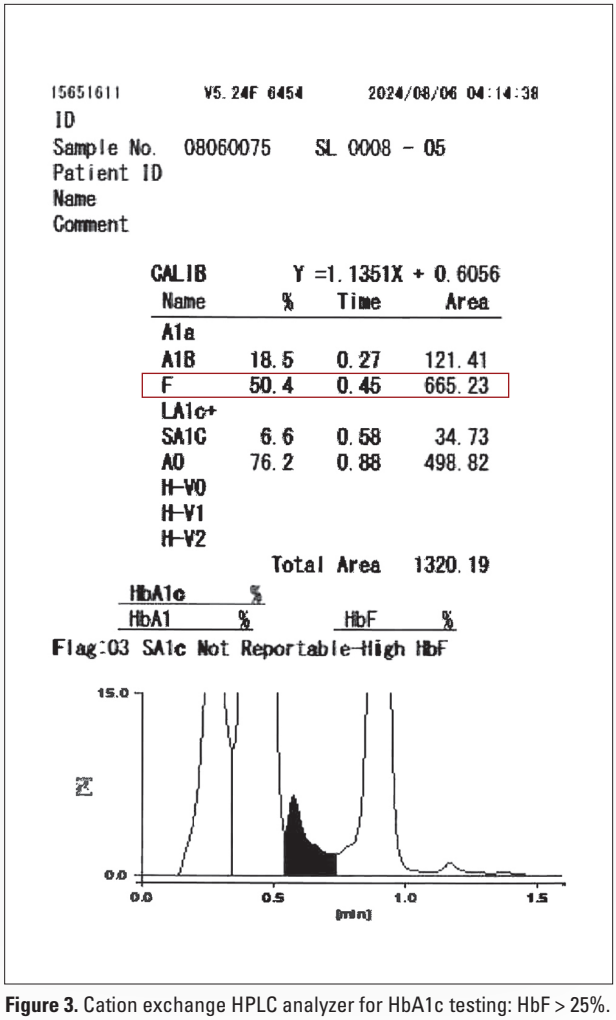

Figure 3. Cation exchange HPLC analyzer for HbA1c testing: HbF > 25%.

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