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BRIEF REPORT

Challenges in Estimation of Glycated Hemoglobin in India

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Abstract

Glycated hemoglobin (HbA1c) is the most widely accepted index of long-term glycemic control. However, there are some clinical situations that make the accurate measurement of HbA1c difficult. Although some of these situations are general, others are more specific to some parts of the world like India. These conditions include hemoglobinopathies such as thalassemias and structural hemoglobin (Hb) variants such as HbS and HbD, as well as iron-deficiency anemia and the use of certain drugs. Because of the relatively frequent occurrence of some of these conditions in some parts of India, it is important that they are looked for when evaluating an inappropriately high or low HbA1c level. Alternative indices may have to be used for assessing glycemic control in these cases.

Introduction

Glycated hemoglobin (HbA1c) is widely accepted as the most reliable indicator of long-term glycemic control. There is sufficient evidence now to conclude that maintaining HbA1c levels as close as possible to the range found in those without diabetes can help patients with diabetes avoid chronic vascular complications.[1,2] There is also an increasing trend toward the use of HbA1c as a diagnostic tool for diabetes.[3]

HbA1c is formed by the nonenzymatic addition of glucose residues to valine moieties at the N-terminal of the β-chain of hemoglobin (Hb). HbA1c is part of the larger family of glycated hemoglobins, which also include HbA1a1, HbA1a2, and HbA1b. The concentration of HbA1c in an individual's blood is proportional to the mean ambient levels of blood glucose over the lifespan of the red blood cell (RBC) (i.e., 80–120 days). There are several methods for estimating HbA1c, some of which are designed to detect the differences in ionic charge between HbA1c and nonglycated Hb (e.g., ion exchange chromatography), whereas others differentiate between them based on structural differences (e.g., immunoassays). Also, some methods measure HbA1c specifically, whereas others (such as boronate affinity chromatography) measure total glycated hemoglobins (GHb).[4] In order to standardize the results of HbA1c among different laboratories and methods, the National Glycohemoglobin Standardization Program (NGSP) was set up in 1996. As of 2013, more than 140 methods and 120 clinical laboratories have been certified by the NGSP.[5]

Although HbA1c is usually a reliable indicator of long-term glycemic control, there are certain conditions that make its accurate measurement difficult or even impossible. In such cases, alternative methods of assessing glycemic control may need to be adopted. This report will discuss some of these conditions, with particular reference to the Asian Indian population.

Hemoglobinopathies and Hb Variants

Hemoglobinopathies are characterized by quantitative or qualitative abnormalities in hemoglobin. The World Health Organization estimates that about 5% of the world's population are carriers for inherited disorders of Hb.[6] The thalassemias are the prototype of quantitative Hb defect, in which defective synthesis of one or other of the two polypeptide chains of Hb leads to overproduction of the other chain, leading to precipitation of the chains in the RBC and the cell's accelerated destruction. β-Thalassemias account for more than 80% of the total thalassemia burden.[7] This condition is found in almost all parts of India. α-Thalassemia is much less common and is found in certain discrete geographical locations (e.g., west and central Gujarat and the Nilgiri Hills of Tamil Nadu in south India).[7] A recent multicentric study showed the overall prevalence of β-thalassemia trait to be 2.78% in six cities of India.[8]

Although thalassemias do not usually interfere with the assays used in HbA1c measurement, increased hemolysis and reduced RBC survival may lead to falsely depressed levels of HbA1c, necessitating the use of alternative methods of assessing glycemic control.[4]

Point mutations in the genes encoding the polypeptide chains of Hb lead to the formation of Hb variants, which are

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characterized by the substitution for one amino acid for another at different positions in the Hb molecule. Examples are HbS (glutamic acid replaced by valine at position 6 of the β-chain), HbC (glutamic acid replaced by lysine at position 6 of the β-chain), HbE (glutamic acid replaced by lysine at position 26 of the β-chain), and HbD Punjab (glutamic acid replaced by glutamine at position 121 of the β-chain). Although these mutations are clinically silent or cause only mild disease in the heterozygous state (usually called the "trait"), individuals homozygous for these mutations often have hemolytic anemia, which can be severe (called the "disease"). In addition, double heterozygotes also exist, having inherited one mutant allele from one parent and a different mutant allele from the other parent.

In India, the most common Hb variant is HbS, followed by HbE and HbD. HbS is widely distributed throughout India, whereas HbD is mainly found in the northwestern regions and HbE in the northeastern states of India.[7] The cumulative allele frequency of these three variants in India has been estimated to be 5.35%.[8]

Although individuals with "disease" due to Hb variants often have falsely low HbA1c levels because of reduced RBC survival, the effect of "traits" on HbA1c are more complex and depend on the assay used as well as the nature of the trait itself.[4] The actual magnitude of the problem of erroneous HbA1c results due to these conditions will depend on the prevalence of hemoglobinopathy in the concerned population, which in turn is a function of the geographical location. The presence of HbS and HbC, which alter the structure of Hb close to the N-terminus, can affect those methods of HbA1c estimation that depend on detecting structural differences (e.g., immunoassays). HbD and HbE, on the other hand, produce structural alterations far away from the N-terminus and therefore do not cause interference in immunoassays. Any of these four variants can affect the ionic charge of the Hb molecule, leading to interference with ion-exchange methods.

Because individuals with variant Hb "traits" are usually asymptomatic, it is important to look for them in the following situations:

- Wherever the HbA1c levels are found to be inappropriate for the level of glycemia
- When the HbA1c level is profoundly high (>15%)
- When there is a drastic change in the HbA1c level when tested using a different method[9]

Although Hb variants do interfere with the some of the commonly used high-performance liquid chromatography assays for HbA1c, careful observation of the chromatogram will allow the variant to be detected so that alternative methods of measuring HbA1c can be adopted. The presence of Hbs, HbD, and HbE can be inferred from the presence of the corresponding peaks on the chromatogram, distinct from HbA and HbF. Assays utilizing immunoturbidimetry and boronate affinity chromatography are usually not affected by the presence of Hb variants. However, these methods measure GHb and not HbA1c specifically. Fortunately, the formation of different types of GHb is proportional, thereby offering the opportunity to standardize results. Another alternative would be to use the capillary electrophoresis method; however, a different buffer needs to be used if variants are present.

Drug Use and HbA1c

Several drugs, some of them quite commonly used, have been known to interfere with accurate measurement of HbA1c.[10] Some of these are listed in Table 1.

Dapsone is a sulfonamide derivative, formerly widely used in the treatment of Hansen's disease (leprosy). Notwithstanding the sharp decline in the prevalence of this disease in India, there are still large numbers of patients who are on long-term therapy with this agent, either for this indication or for other conditions such as dermatitis herpetiformis. Dapsone is known to lower the HbA1c concentrations, by inducing hemolysis as well as by a hemolysis-independent effect on RBC survival. Recently, there have been a few reports of patients who presented with inappropriately low levels of HbA1c consequent to dapsone use in India.[11,12] The HbA1c usually returns to the expected range after cessation of therapy.

Ribavirin, antiretroviral agents, and trimethoprim–sulfamethoxazole alter HbA1c levels by inducing hemolysis, whereas hydroxyurea causes a shift from HbA to HbF, causing an apparent fall in HbA1c levels. Large doses of antioxidants such as vitamin C and vitamin E have also been reported to reduce HbA1c levels by interfering with Hb glycation, although the extent to which this is relevant in clinical practice is unknown.[13]

Anemia and HbA1c

Iron-deficiency anemia is endemic in India. It is particularly common in adolescents as well as in women of the reproductive age group. It has been estimated that more than 50% of women 15–49 years of age and more than 85% of pregnant women in India have Hb levels below the lower limit of normal for their age and sex.[14]

Hypoproliferative anemias such as iron-deficiency anemia prolong the lifespan of RBCs. In addition, malondialdehyde, which is increased in iron-deficiency anemia, can enhance the glycation of Hb. Both these factors can lead to falsely elevated HbA1c levels in iron-deficiency anemia. It has recently been shown that the use of HbA1c to diagnose diabetes in a rural Indian population led to an overestimation of the prevalence of both diabetes and prediabetes.[15] The authors attributed this to the high prevalence of iron-deficiency anemia, leading to spuriously elevated HbA1c levels in this population.

With iron-deficiency anemia and diabetes both being frequent in India, clinicians should be aware of this interaction

Table 1. Drugs Affecting Glycated Hemoglobin Levels

HbA1c finding	*Drug*
Falsely low	Dapsone Ribavirin Antiretrovirals Trimethoprim–sulfamethoxazole Hydroxyurea Vitamin C Vitamin E Aspirin (small doses)
Falsely high	Aspirin (large doses) Chronic opiate use

HbA1c, glycated hemoglobin.

when faced with an inappropriately elevated HbA1c value. Repeat measurement of HbA1c after correction of the anemia will clarify the picture.

Other Limitations in the Use of HbA1c

There are several other limitations to the use of HbA1c in assessing glycemic control. HbA1c levels can vary with age, time of year, and in the presence of conditions like uremia, hyperbilirubinemia, alcoholism, and pregnancy.[13] Because these conditions are not specific to India, they are not discussed further in this article.

Another practical problem in the Indian scenario is the lack of easily available, standardized assays for the measurement of HbA1c. The high cost of the assay may also prove to be prohibitive in many cases, particularly in the rural areas.

Hb1Ac for Diagnosis of Diabetes

In 2009, an International Expert Committee appointed by the American Diabetes Association, the European Association for the Study of Diabetes, and the International Diabetes Federation recommended that HbA1c could be used as a diagnostic tool for diabetes.[16] This stand has also been recently endorsed by the World Health Organization, with the caveat that conditions interfering with accurate measurement of HbA1c should be absent.[17] Although the use of HbA1c as a diagnostic tool is an attractive proposition, its use for this indication in India at present is not practical because of the high cost of the test, problems with standardization, and poor availability of the test in certain parts of the country.

Conclusions

Although HbA1c remains the gold standard for assessment of long-term glycemic control in diabetes, there are several frequently encountered clinical situations that can interfere with its accurate measurement. Unless clinicians are aware of these conditions, serious errors may be made in the diagnosis and treatment of diabetes. Use of alternative methods of measuring long-term glycemic control may be indicated in some of these situations. Some of the alternative indices of long-term glycemic control include serum fructosamine, glycated albumin, and 1,5-anhydroglucitol. However, the use of these methods is limited on account of issues with cost, availability and standardization. Development of less costly and more reliable indicators of chronic hyperglycemia is the need of the hour.

Author Disclosure Statement

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