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Newsletter

HbA1c MEASUREMENT AND ASSAY INTERFERENCES

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Introduction

Haemoglobin is the iron containing oxygen transport protein in red blood cells. Adult haemoglobin (HbA) consists of two α- and two β-chains. These constitute about 97% of most normal haemoglobin. A1c is the most abundant minor haemoglobin component. Haemoglobin A1c (HbA1c) is formed as a result of the non-enzymatic attachment of a hexose molecule to the N-terminal amino acid of the haemoglobin molecule. This occurs continually over the lifespan of the erythrocyte (average 120 days) and is dependent on blood glucose concentrations.

HbA1c provides a better index of long term glycaemic control than blood and urinary glucose estimations. Other advantages are that no fasting is required prior to specimen collection, the intra-individual variability is low, and there is good sample stability. It is used clinically as an important predictor of diabetes-related outcomes, as well as a criterion for the diagnosis of diabetes.

Measurement

There are two approaches to measuring HbA1c. The first is to separate A1c from other haemoglobin fractions, using methods such as chromatography and electrophoresis. The second approach targets A1c as an antigen, using methods such as immunochemistry.

There are certain clinical situations in which the use of HbA1c levels alone for either diagnosis of diabetes or as a measure of glycaemic control, is not recommended and may lead to errors. Whenever a clinician notices that the glycaemic control of their patient (as estimated from continuous glucose monitoring system data, point of care glucose readings, fructosamine or glycated albumin) is discordant with their HbA1c level, this should prompt an investigation as to whether the patient has a condition in which the HbA1c data is misleading.

Conditions associated with falsely elevated or lowered HbA1c

Conditions associated with either a falsely elevated or false lowered HbA1c measurement are summarised in Table 1.

Table 1. Conditions associated with falsely elevated or lowered HbA1c*

Condition	Effect on A1c	Comments
Anaemias associated with decreased red cell turnover	False increase	E.g. iron deficiency, vitamin B12, folate deficiency anaemias
Asplenia	False increase	Increased erythrocyte lifespan
Uraemia	False increase	Formation and detection of carbamyl-haemoglobin
Severe hypertriglyceridaemia	False increase	When levels > 19.78 mmol/L
Severe hyperbilirubinaemia	False increase	When levels > 342 µmol/L
Chronic alcohol consumption	False increase	Formation of acetaldehyde-HbA1 compound
Chronic salicylate ingestion	False increase	Mechanism uncertain, may interfere with assay
Chronic opioid ingestion	False increase	Mechanism uncertain
Lead poisoning	False increase	Mechanism uncertain
Anaemia from acute or chronic blood loss	False decrease	Includes haemolytic anaemia
Splenomegaly	False decrease	Decreased erythrocyte lifespan
Pregnancy#	False decrease	Decreased erythrocyte lifespan
Vitamin E ingestion	False decrease	Reduced glycation
Ribavirin and interferon-alpha	False decrease	Possibly due to haemolytic anaemia
Red blood cell transfusion‡	False increase OR False decrease	High glucose concentration in storage medium (False increase) Dilutional effect (False decrease)
Haemoglobin variants	False increase OR False decrease	Depends on method and analyser used. A1c generally reliable for heterozygous variants, but not homozygous variants.
Vitamin C ingestion	False increase OR False decrease	May increase A1c when measured by electrophoresis. May decrease A1c when measured by chromatography due to competitive inhibition of glycosylation.

* Adapted from Radin MS. J Gen Intern Med 2014; 29(2): 388 – 394.
# Expect falsely low A1c values through the 2nd trimester, but may rise during the 3rd trimester.
‡ Typically reported to falsely elevate A1c, but may also result in false decrease.

Falsely elevated HbA1c

Any condition that prolongs the lifespan of the erythrocyte, or is associated with decreased red cell turnover, exposes the cell to glucose for a longer period of time, resulting in higher HbA1c levels. Iron deficiency anaemia is commonly associated with falsely elevated HbA1c. Studies in patients with and without diabetes have demonstrated that the treatment of iron deficiency lowers HbA1c, although the exact mechanism remains unclear. Other conditions associated with decreased red cell turnover and falsely elevated HbA1c are vitamin B12 and folic acid anaemias, and asplenia.

There are conflicting reports about the effects of recent blood transfusion on HbA1c. Traditionally, the perception was that the exposure of red cells to the high glucose concentrations of the storage medium resulted in a falsely elevated HbA1c in the transfused patient. However, more recent data suggest that the dilutional effect from the significant volume of red cells transfused from a person without diabetes can result in a falsely decreased HbA1c. Until there is clarification from these conflicting reports, HbA1c results from a recently transfused patient should be considered uninterpretable.

Severe hypertriglyceridaemia, hyperbilirubinaemia, uraemia and the medications listed in Table 1 may falsely increase HbA1c levels.

Falsely lowered HbA1c

Any condition that shortens the lifespan of the erythrocyte or is associated with increased red cell turnover shortens the exposure of the cell to glucose, resulting in lower HbA1c levels. These include acute and chronic blood loss, haemolytic anaemia and splenomegaly.

The clinician should be aware that the interpretation of HbA1c levels in end stage renal disease may be complicated. Generally, patients have falsely low HbA1c levels due to associated chronic anaemia with decreased red cell survival. However, the complex interplay of other contributing factors such as erythropoietin therapy, and the presence of anaemia, can have differing effects that further influence HbA1c. The clinician should consider using an alternative index to monitor glycaemic control in these patients.

HbA1c may not be a true reflection of glycaemia during pregnancy because of the decreased lifespan of the red blood cell from 120 to 90 days, as well as increased erythropoietin production. Since values are generally falsely low during pregnancy, do not use this parameter for the diagnosis of gestational diabetes. The oral glucose tolerance test is advocated for screening and diagnosis, and self-monitoring of blood glucose levels is advised for management during pregnancy.

Monitoring HIV-infected patients with diabetes

The increase in inflammatory mediators and cytokines associated with HIV infection, the “return to health phenomenon” and certain ART medications increase the risk for the development of diabetes. The prevalence of abnormal glucose metabolism in HIV-infected patients has increased with the widespread use of antiretroviral therapy (ART) and resulting improved longevity. The prevalence of diabetes is 4.6 times higher in HIV-infected patients than in the general population, and more than 35% of HIV-positive patients have impaired glucose tolerance (compared to 5% in the general population).

HbA1c may underestimate glycaemia in HIV-positive individuals. Greater HbA1c-glucose discordance has been shown in patients with higher mean corpuscular volume (MCV), use of ART (especially abacavir), and lower CD4 counts. This may be due to HIV-infected individuals having a faster turnover of red blood cells. Studies have also determined that fructosamine is not a more accurate measure of glycaemic control than HbA1c in HIV-infected patients.

Current recommendations are therefore not to use HbA1c for the diagnosis of diabetes in HIV-infected patients. Fasting serum glucose should be used for the diagnosis of diabetes in these patients. An oral glucose tolerance test (OGTT) is recommended for patients with impaired glucose tolerance or impaired fasting glucose with additional risk factors. Fasting serum glucose and lipid levels should be measured in HIV-positive patients before starting ART, and repeated every 6 – 12 months. The use of HbA1c for monitoring purposes in diabetic HIV-positive patients is also problematic due to the reasons stated above.

Haemoglobin variants

It is estimated that approximately 7% of the world's population have a haemoglobin variant, and although many are innocuous, some are not. In South East Asia, about 30% of the population may manifest a heterozygote haemoglobin variant, often S, E, D or F. About 10% of the African American population has a haemoglobin variant. Haemoglobins S and E are prevalent variants in people of African, Mediterranean or South East Asian descent. Sickle cell anaemia (also called HbSS disease) occurs when sickled red blood cells interfere with normal circulation and decrease the lifespan of red blood cells. The condition can result in splenic sequestration, aplastic crises and multiple complications. Other genetic variants (e.g. HbS trait or HbC trait) or elevated foetal haemoglobin (HbF) can affect the accuracy of HbA1c measurement.

In general, HbA1c measurement is not reliable in patients with homozygous haemoglobin variants (i.e. HbSS or HbCC). It can be used in patients with heterozygous variants (i.e. HbAS or HbAC), as long as the appropriate assay is used. In the absence of specific method data, it can generally be assumed that immunoassay methods do not have clinically significant interference from HbE and HbD. It can also be assumed that both immunoassay and borate affinity methods show interference from HbF levels above 10 – 15%. Please contact a chemical pathologist where there are interpretative difficulties.

Alternatives to using HbA1c

1. *Fructosamine* – is a measure of all glycated proteins. It reflects a much shorter period of glycaemic control, typically the preceding 2 to 3 weeks. Falsely low levels may occur in patients with hypoalbuminaemia, commonly seen in nephrotic syndrome, or severe liver disease. Other interferences include uraemia, lipaemia and ascorbic acid.
2. *Glycated* albumin – typically reported as a percentage of total albumin, and also reflects glycaemic control over the preceding 2 to 3 weeks. It is particularly advocated in patients with end stage renal failure.
3. *Continuous glucose monitoring* – data obtained from wearing a sensor for up to 5 days has been shown to correlate well with HbA1c levels in devices that are approved for use.

References are available on request

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