The Effect of Increased Fetal Hemoglobin on 7 Common Hb A₁c Assay Methods

To the Editor:

Hemoglobin variants and increases in circulating fetal hemoglobin (Hb F),¹ (defined as an Hb F level >2%) have been reported to interfere with some assay methods for Hb A₁c (1–3). In view of the relatively common occurrence of such Hb F increases (approximately 1.5% of the US population) and the fact that individuals with an increased Hb F level are usually asymptomatic, it is important to know whether Hb A₁c methods show Hb F interference and, if so, at what level of Hb F. In this report, we evaluate the presence of Hb F interference with several HPLC methods.

This study was approved by the Institutional Review Board of the University of Utah and Johns Hopkins Medical Institutional Review Board, where the samples originated. The following commercial HPLC methods were evaluated: the G7 and G8 Variant Mode (Tosoh Bioscience); the D-10ᵀᴹ Hemoglobin A₁c Program (D-10), the VARIANTᵀᴹ II Hemoglobin A₁c Program (VII NU), the VARIANTᵀᴹ II TURBO Hemoglobin A₁c Program (VII Turbo), and the VARIANTᵀᴹ II TURBO HbA₁c Kit – 2.0 (VII Turbo 2.0) (all Bio-Rad Laboratories); and the ultra²ᵀᴹ A₁c boronate affinity method (ultra²) (Trinity Biotech). The Hb F level was estimated from the G7 or G8 Hb F percentage.

A small number of samples (n = 24) with and without an increased Hb F level were analyzed by both the IFCC capillary electrophoresis (CE) method (4) and the G7 method to verify that the G7 method did not have interference from increased Hb F levels (3). We analyzed 88 to 123 samples (depending on the method) with each of the commercial HPLC methods. Results that were considered acceptable according to manufacturer guidelines for each method were compared with the G7 results. A multiple regression model (Y = α + βX + γZ + e, where Y is the test method Hb A₁c percentage, X is the G7 Hb A₁c percentage, Z is the Hb F percentage, and e is random error) was used to determine if the relationship between Hb A₁c results obtained with each test method and the IFCC CE (in the case of the G7) or G7 method were significantly (P < 0.05) affected by Hb F. Methods shown to have statistically significant interference were then evaluated for clinical importance by dividing the samples into 3 groups (0% to 5% Hb F, >5% to 15% Hb F, and >15% Hb F) and by conducting a Deming regression comparison of the results for each method and the G7 results. We then calculated the bias between the groups attributable to the presence of an increased Hb F level. For a given method, a relative percentage difference between groups of >7% at a G7 Hb A₁c level of 6% or 9% was defined as clinically important.

Hb A₁c results for the Tosoh G7 and the IFCC CE methods showed excellent agreement for samples with normal Hb F levels (r² = 0.99; y = 1.048x – 0.172%) and for those with Hb F levels >2% (r² = 0.99; y = 1.038x – 0.007%). There was no statistically significant difference between the IFCC CE and G7 methods in the relationships (P = 0.16). Fig. 1 shows the bias in Hb A₁c percentage for 6 assay methods vs the Hb F percentage (as measured by the G7 method) for samples within the manufacturer recommendations and, for 2 methods, additional results outside the manufacturer’s limits. For the D-10 method using only samples considered to be within the manufacturer specifications (D-10 Hb F levels ≤10%), there was no statistically significant effect of increasing Hb F (P = 0.47). For the G8 method, there was no statistically significant effect of Hb F on Hb A₁c results (P = 0.54) within the manufacturer’s claimed limit of 15% Hb F. In addition, when all samples were included (up to approximately 30% Hb F), the effect of Hb F was not found to be clinically important. For the VII NU method, although there was a marginally statistically significant effect of Hb F on Hb A₁c results (VII NU Hb F levels ≤10%; P = 0.03), this interference was not clinically important. When all Hb F levels were included, there was still no clinically important effect of Hb F. For the VII Turbo method, no statistically significant effect of Hb F was found (VII Turbo Hb F levels ≤5%; P = 0.23). For the VII Turbo 2.0 method, although there was a statistically significant effect of Hb F on Hb A₁c results (VII Turbo 2.0 Hb F levels ≤25%; P < 0.0001), the differ-

¹ Nonstandard abbreviations: Hb F, fetal hemoglobin; Hb A₁c, hemoglobin A₁c; D-10, D-10ᵀᴹ Hemoglobin A₁c Program; VII NU, VARIANTᵀᴹ II Hemoglobin A₁c Program; VII Turbo, VARIANTᵀᴹ II TURBO Hemoglobin A₁c Program; VII Turbo 2.0, VARIANTᵀᴹ II TURBO HbA₁c Kit – 2.0; ultra², ultra²ᵀᴹ A₁c boronate affinity method; CE, capillary electrophoresis.
ences were not clinically important. The current results for the Trinity Biotech HPLC method support previous findings: The differences are both statistically significant ($P < 0.0001$) and clinically important for samples with increased Hb F levels owing to lower glycation of Hb F. For samples with Hb F values $>15\%$, however, the effect of Hb F on Hb A1c results was not clinically important.

Our Hb A1c results for the ion-exchange HPLC methods evaluated thus far show that if the manufacturer’s instructions are followed, clinically appropriate results will be reported. Results for the boronate affinity method showed both statistically significant and clinically important effects from increased Hb F levels $>15\%$. There are no manufacturer claims of Hb F interference for this method. Moreover, as with immunoassay methods for which the same Hb F interference applies (1), there is no indication in the reported result that an increased Hb F level is present (unlike with ion-exchange HPLC methods); therefore, artificially low results will be reported for samples with Hb F values $>15\%$. Physicians and laboratory professionals need to be aware of potential interference from increased Hb F levels that could adversely affect Hb A1c results.

**Fig. 1. Differences in Hb A1c percentage ($\%$Hb A1c) between each test method (panels A–F) and the Tosoh Bioscience G7 method vs Hb F percentage.**

Data are for samples within manufacturers’ acceptable Hb F intervals (●), as well as for samples outside manufacturers’ Hb F upper limit (∙) for 2 of the methods.

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Letters to the Editor

Measurement of Late-Night Salivary Cortisol and Cortisone by LC-MS/MS to Assess Preanalytical Sample Contamination with Topical Hydrocortisone

To the Editor:

Measurement of increased late-night salivary cortisol (LNSC) is now a mainstay in the diagnosis of endogenous Cushing syndrome (1). Patients typically sample their saliva at home and are carefully instructed to avoid using potential contaminants, such as topical creams or ointments containing hydrocortisone. Using these creams or ointments can cause preanalytical contamination of the saliva sample that cannot be distinguished from endogenous cortisol as measured by either immunoassay or liquid chromatography–tandem mass spectrometry (LC-MS/MS). With endogenous cortisol production, the cortisone concentration in the saliva is usually higher than the cortisol concentration, and typically the cortisol-to-cortisone ratio is <1, owing to the transfer of cortisone from the plasma and the conversion of cortisol to cortisone by 11-β-hydroxysteroid dehydrogenase type 2 (11-β-HSD2) in the salivary gland (2, 3). Contamination of the saliva with topical or oral hydrocortisone (cortisol) during sample collection would not be expected to increase salivary cortisone because there is no 11-β-HSD2 activity in collected saliva (3). Furthermore, topical hydrocortisone is usually not absorbed into the plasma compartment in substantial quantities. Therefore, topical hydrocortisone constitutes true contamination of the saliva and is not converted to cortisone. We hypothesized that a very high cortisol-to-cortisone ratio would be strong evidence that an increased salivary cortisol value was due to contamination with topical or oral hydrocortisone. Such a finding is prima facie evidence of contamination and is justification for the clinician to reinterview the patient.

We evaluated samples referred to the Endocrine Research Laboratory at Aurora St. Luke’s Medical Center for standard LNSC analysis. These samples yielded very high values by ELISA, raising the suspicion of contamination (4) (Table 1). ELISA was performed as previously described (5) with both undiluted and diluted (1 volume of sample in 19 volumes of diluent) LNSC samples (reference interval for LNSC by ELISA, <4.2 nmol/L). We used LC-MS/MS analysis (2) and our previously published algorithm (4) to evaluate whether the patient might have contaminated samples with topical hydrocortisone during collection. The LC-MS/MS reference interval is <2.8 nmol/L for LNSC and <28 nmol/L for late-night salivary cortisone. The reference interval for the salivary cortisol-to-cortisone ratio is 0.2–1.1 at awakening (when the cortisol concentration is typically at its circadian peak) and 0.1–1.2 for late-night samples (the circadian nadir).

References


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