ORIGINAL ARTICLE

Effect of Elevated levels of Hemoglobin F on HbA1c Measurements: Evaluation of Three HbA1c Assays

Khajuria A[1], Scheibe B[2]

[1]Covance, Central Laboratory Services, 750 Walnut Avenue, Cranford, NJ, 07065, USA, [2]Laboratory Incharge, Marshfield Clinic, 1000N Oak Avenue, Marshfield, WI, 54449, USA

ABSTRACT

Introduction: Accurate measurement of HbA1c is crucial in the diabetic control and diagnosis. Elevated levels of HbF are reported to falsely decrease the HbA1c result and effect is very much method dependent. **Material & Methods:** Commercial assay methods G8 HPLC analyzer and DCA 2000 were evaluated. G8 is an ion exchange, high performance liquid chromatography (HPLC) method that measures the HbA1c as a percentage of total amounts of hemoglobin present in the sample. Two whole blood EDTA patient pools were prepared with HbA1c concentrations in the normal (5% to 7%) and abnormal range (7% to 8%). All chromatograms from G8 were reviewed for any change in the peak resolution time due HbF concentrations. **Results:** The mean value for normal and abnormal pool was 5.8% and 7.5% resp. HbF showed no interference on Tosoh HbA1c results up to 30% in normal pool and up to 25% in abnormal pool. Observed difference between G8 and both Dimension EXL and DCA 2000 was clinically significant beyond 10% HbF. **Conclusion:** Accurate measurement of HbA1c is crucial for the decision making for diabetic control and diagnosis. The allowable error proposed by College of American Pathologist (CAP) is 6% therefore, appropriate knowledge about factors interfering with HbA1c results is absolutely important.

Keywords: HbA1c, diabetes, HbF

INTRODUCTION

HbA1c is a key indicator for the mean glycemic status and an important biomarker in the diagnosis, evaluation and management of diabetes mellitus and its relationship with the clinical outcome and complications of diabetes mellitus is well demonstrated for both type I and type II diabetes.[1,2] HbA1c is a major form of glycated hemoglobin which results from the non-enzymatic glycation of N-terminal valine residues of the beta chain of HbA. The American Diabetes Association (ADA) recommends ≥6.5% HbA1c as the diagnostic criteria for diabetes mellitus.[3] The criteria and cut-off values for treatment decisions have become more stringent and measurements need to comply with increased accuracy and precision. At a glycemic control of 6.5% the critical difference between two results with in a subject should not exceed ~ 0.4%.

Access this article online	
Website: www.actamedicainternational.com	Quick Response code
DOI: 10.5530/ami.2015.3.7	

Hemoglobin F (HBF; $\alpha 2Y2$) is a major hemoglobin component in the fetus; however it is progressively replaced by adult hemoglobin A (HbA; $\alpha 2\beta 2$) during the first five years of life. Adults have <1% of HbF. Elevated levels of HbF are reported to interfere with the HbA1c measurements by falsely decreasing the HbA1c results. Several inherited and acquired conditions cause elevated HbF, such as β-thalassemia, hereditary persistence of fetal Hb (≤30%), sickle cell anemia, congenital aplastic anemia, leukemia (3-17%), pregnancy (3-5%), starvation ketoacidosis, certain drugs as erythropoietin, sodium valproate, zidovudine (HIV) etc.[4,5] The prevalence of elevated HbF can be as high as 7-8% in diabetic population and some of these conditions are asymptomatic and clinicians may not be aware of existence of conditions and potential of interference when interpreting HbA1c results.[6,7]

All HbA1c methods are National Glycohemoglobin Standardization Program (NGSP) certified, but does not include evaluation of interferences as a part of certification program and different methods have variable degree of interference.[8]

This study evaluates the effect of elevated HbF levels on HbA1c results in the normal and abnormal value

Corresponding Author:
Annu Khajuria, Technical Director, Central Laboratory North Americal, Covance Central Lab Services. Phone: +1-908-709-5856, Mobile: +1-732-428-9094, E-mail: khajura@labcorp.com

range from three commercial HbA1c assay method based on different principles and commonly used in Laboratories and to examine if HbF has same effect at different levels of HbA1c.

MATERIAL AND METHODS

The following commercial assay methods G8 HPLC analyzer (TOSOH, Biosciences), Dimension EXL and DCA 2000 (Siemens Diagnostics) were evaluated. G8 is an ion exchange, high performance liquid chromatography (HPLC) method that measures the HbA1c as a percentage of total amounts of hemoglobin present in the sample. Dimension EXL measurement is based on turbidimetric inhibition immunoassay (TINIA) principle, measures both total hemoglobin and HbA1c and relative proportion of total glycated hemoglobin is calculated and reported. DCA 2000 is a POCT analyzer and measurement is based on latex immune-agglutination inhibition methodology and it measures both total hemoglobin and HbA1c and ratio is reported as percent HbA1c.

Two whole blood EDTA patient pools were prepared with HbA1c concentrations in the normal (5% to 7%) and abnormal range (7% to 8%). Umbilical cord blood was used as a representative specimen for HbF and levels quantitated by G8 analyzer. EDTA patient pools were then incubated with varying concentrations of HbF (5% to 40%) by mixing by mixing umbilical cord blood with known HbF levels.

The effect of HbF was evaluated relative to normal and abnormal pools without HbF incubation (Normal HbF levels <2%). All chromatograms from G8 were reviewed for any change in the peak resolution time due HbF concentrations. Mean values were derived from five replicates of each concentration. A decrease in HbA1c results greater than 0.4% was considered a significant change. Friedman test was used to test the difference between control pools (without HbF) and HbF incubated pools.

Manufacturers Claims

The manufacturer stated levels below which there is no significant interference from HbF and HbA1c results not affected; for G8 being 15% and 10% for DCA 200 and Dimension EXL.

RESULTS

Figure 1 shows the change in HbA1c results with varying concentration of HbF (5 – 40%) on G8, Dimension EXL and DCA 2000 methods. The mean value for normal and abnormal pool was 5.8% and 7.5% resp. HbF showed no interference on Tosoh HbA1c results up to 30% in normal pool and up to 25% in abnormal pool. Chromatogram inspection did not show any overlap of HbF peak with labile A1c between 25-30% HbF. Dimension EXL and DCA methods did not show interference at 5% of HbF in normal pool however; in abnormal pool 5% of HbF had slight negative interference on HbA1c results and clinically significant and dose dependent negative bias (interference) on HbA1c results ($p<0.001$) Figure 2. Figure 3 shows difference in HbA1c results between the methods with and without the presence of HbF. There was no clinically significant difference in normal and abnormal pools with normal HbF levels (<2%) between G8 and Dimension and DCA methods, however observed difference between G8 and both Dimension EXL and DCA 2000 was clinically significant beyond 10% HbF.

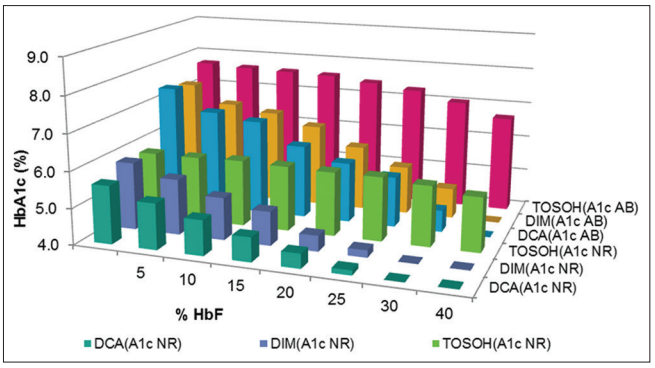

Figure 1: HbA1c results with varying concentration of HbF (5 – 40%) on G8 (Tosoh), Dimension EXL and DCA 2000 (Siemens Diagnostics) methods

DISCUSSION

Our results show that that ion-exchange HPLC method (G8, Tosoh, Biosciences) had minimal interference from elevated levels of HbF up to 25%. Dimension EXL and DCA 2000 (Siemens Diagnostics) HbA1c results were falsely low and interference was clinically significant at 10%. The interference observed within normal and abnormal levels was not identical with immunoassay based methods. The effect appears to be related to antibody and not binding to glycated fraction of HbF as total hemoglobin levels measurement includes HbF.

Few studies have examined the effect of elevated levels of HbF on HbA1c results with variable results[9-12] due to differences in study design and assay methods compared. With ion-exchange method the presence of elevated HbF is evident in the chromatograms and it is important to inspect the chromatogram to avoid any mis-identification of peaks, whereas with immunoassays methods (Dimension EXL and DCA 2000) there is no way to identify the effect of elevated HbF. Falsely low HbA1c results will therefore be reported for samples with HbF greater than 10%.

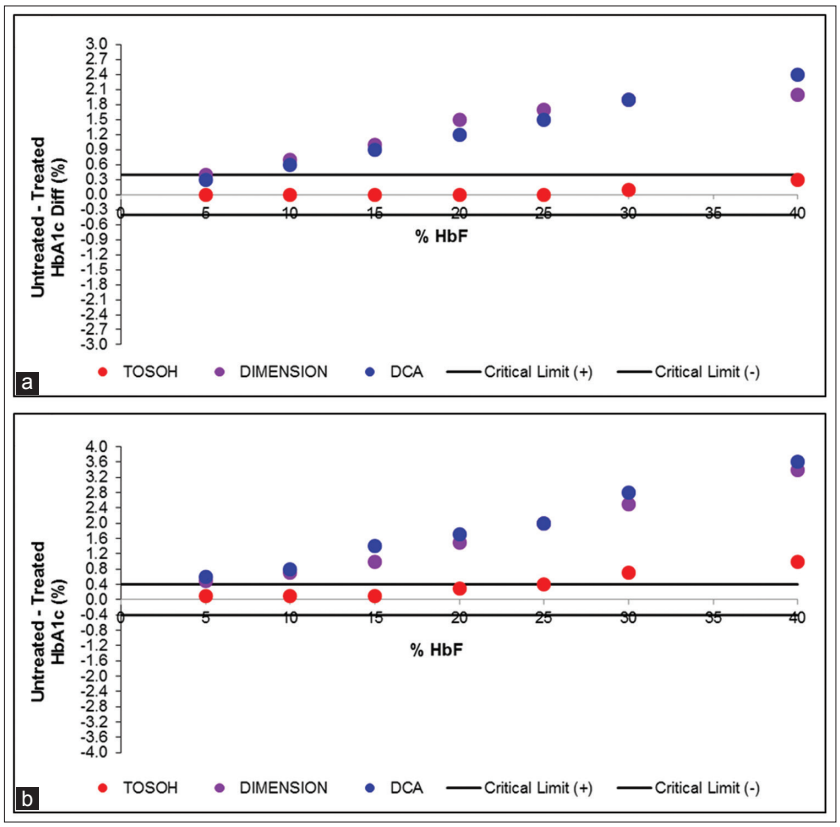

Figure 2: Effect of HbF HbA1c results with varying concentration of HbF (5 – 40%) on G8 (Tosoh), Dimension EXL and DCA 2000 (Siemens Diagnostics) methods; a: Normal Pool; b: Abnormal Pool

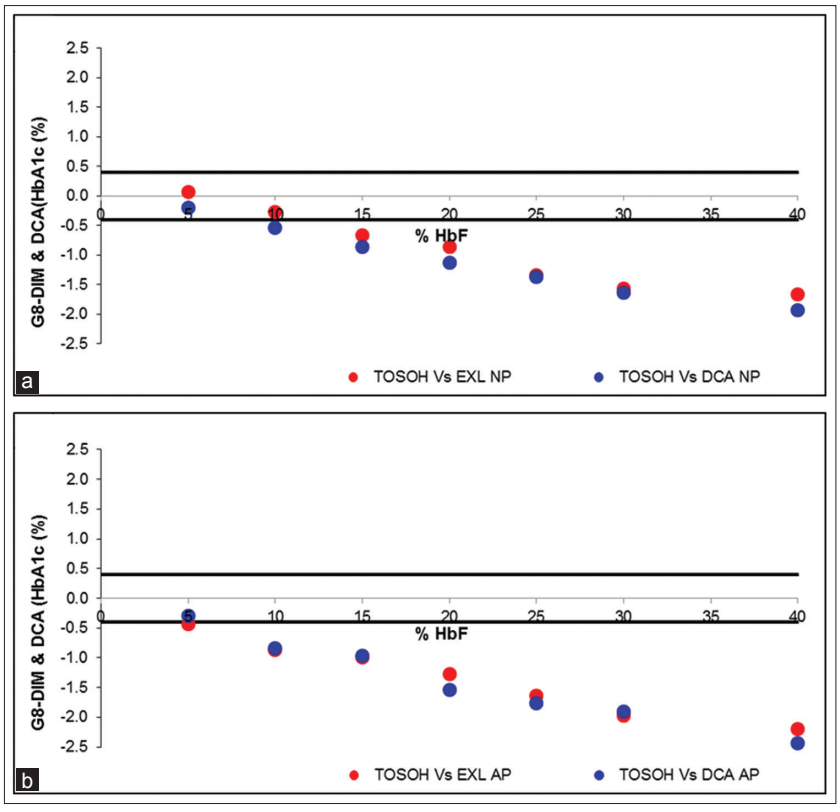

Figure 3: Difference in HbA1c results between G8 (ion exchange method) and Dimension and DCA 2000 (Immunoassay methods) with and without the presence of HbF. a: G8 and Dimension EXL and DCA in Normal Pool; b: G8 and Dimension EXL and DCA in Abnormal Pool

Accurate measurement of HbA1c is crucial for the decision making for diabetic control and diagnosis. The allowable error proposed by College of American Pathologist (CAP) is 6% therefore, appropriate knowledge about factors interfering with HbA1c results is absolutely important. From clinical perspective falsely decreased results of HbA1c even by 1% will have significant difference in monitoring and diagnosis of patients with diabetes mellitus leading to under treatment and increased risk to complications.

Laboratory professionals should make clinicians aware of potential interference from elevated levels of HbF on a particular method assay affecting HbA1c results. Clinicians will be able to make an informed decision if HbA1c results appear discrepant related to patient history and glucose hemostasis.

REFERENCES

1. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. New England Journal of Medicine, 1993; 329:977-986.
2. UK Prospective Diabetes Study Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet, 1998; 352: 837-853.
3. Classification and Diagnosis of Diabetes. Diabetes Care, 2015; 38(Suppl.1): S8-S16.
4. Bain BJ. Hemoglobinopathy Diagnosis. 2nd Edition, Blackwell Publishing Ltd. USA. 2006; 120, 239.
5. Felner EI, McGrath M. Inaccurate hemoglobin A1c levels in patients with Type I diabetes and Hereditary Persistence of Hemoglobin F. The Journal of Pediatrics, 2008; 153: 137-139.
6. Koskinen LK, Lahtela JT. Fetal hemoglobin in diabetic patients. Diabetes Care, 1994; 17(8): 828-831.
7. Mullis P, Schuler J, Zuppinger K. Increased prevalence of fetal hemoglobin levels in type I (insulin-dependent) diabetes mellitus. Diabetologia, 1989; 32: 227-230.
8. Little RR, Roberts WL. A review of Variant hemoglobins interfering with Hemoglobin A1c measurements. Journal of Diabetes Science and Technology, 2009; 3(3): 446-451.
9. Rohfing CL, Connolly SM, England JD, Hanson SE, Moellering CM, Bachelder JR and Little RR. The effect of elevated Fetal hemoglobin on hemoglobin A1c results. American Journal of Clinical Pathology, 2008; 129: 811-814.
10. Shu I, Devraj S, Hanson SE, Wang P. Comparison of hemoglobin A1c measurements of samples with elevated fetal hemoglobin by three commercial assays. Clinica Chimica Acta, 2012; 413: 1712-1713.
11. Little RR, Rohfing CL, Hanson SE, Schmidt RL, Lin Chia-Ni, Madsen RW, Roberts WL. The effect of increased Fetal hemoglobin on 7 common HbA1c assay methods. Clinical Chemistry, 2012; 58(5): 945-947.
12. Nitta T, Yamashiro Y, Hattori Y, Ezumi T, Nishioka M, Nakamura J. The interference by HbF on HbA1c (BM test HbA1c) measurements in enzymatic method. Annals of Clinical Biochemistry, 2015; Jan 13. pii: 0004563214568872. [Epub ahead of print].

How to cite this article: Khajuria A, Scheibe B. Effect of elevated levels of hemoglobin F on HbA1c measurements: Evaluation of three HbA1c assays. Acta Medica International. 2015;2(2):85-88.

Source of Support: Nil, **Conflict of Interest:** None declared.

Handling Editor: Nidhi Sharma India