

Spuriously Elevated HbA1c Result

Mrinal Gupta^{*}, Krishnananda Prabhu^{**}, Pragna Rao^{***}

^{*} MBBS, MD postgraduate student, Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal, Karnataka, India

^{**} MBBS, MD., Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal, Karnataka, India

^{***} MBBS, MD., Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal, Karnataka, India

Abstract- Glycosylated haemoglobin (HbA1c) is routinely used test to monitor long term glycaemic control in diabetic patients. It is well known that genetic variants and chemically modified derivatives of haemoglobin can profoundly affect the accuracy of HbA1c measurements. The degree of interference depends on the HbA1c assay method, type & quantum of haemoglobin variant present in a given sample. Here, we report a rare case showing disproportionately high HbA1c value in a 69 years old female patient with carcinoma breast (adenocarcinoma) with hypertension.

Index Terms- Diabetes, Genetic variants, Haemoglobin, HbA1c.

I. INTRODUCTION

Glycated haemoglobin (HbA1c) is a biochemical test that has been routinely used in the management of diabetes mellitus to monitor long-term glycaemic control¹. While boronate affinity chromatography & immunoassays estimate HbA1c based on structure of haemoglobins others like HPLC & electrophoresis are based on the charge of haemoglobin. The gold standard method today for measuring HbA1c for most clinical laboratories is a "mini-column" utilizing ion exchange chromatography (HPLC).²⁻³ In our lab we use Biorad D – 10 and Biorad variant turbo, both of which in addition to estimation HbA1c can also detect haemoglobin variants and chemical modifications of haemoglobin that commonly interfere with estimation of glycated haemoglobin fractions.

In addition, pathologic conditions affecting red cell half-life, drugs like aspirin and penicillin, metabolites of alcohol can also positively bias the results of HbA1c generated by HPLC.³

II. CASE REPORT

A 69 years old female patient, known case of ca breast (adenocarcinoma) right side T₃N₀M₀, taking regular chemotherapy with letrozole and radiotherapy, came for regular follow up. She was a known case of dyslipidemia and hypertension since 2 years; recently diagnosed with type II diabetes. Patient was on regular treatment with metformin, telmisartan and rosuvastatin.

Fasting blood sugar (FBS) –122 mg/dl & Post prandial blood sugar (PPBS) was 215. Serum urea- 23mg/dl, serum creatinine- 0.6mg/dl, serum sodium- 138mmol/l and serum potassium- 4.2mmol/l.

On estimating the glycated haemoglobin in this patient by Bio-Rad D-10 & Variant turbo for estimating glycated haemoglobin, it showed a value of 60.9 gm % which was

unusually high and was disproportionate to patient's blood sugar levels. Both of these machines also are designed to identify most of haemoglobin variants known to interfere with glycated haemoglobin. As this sample did not show any known variants/chemical modifications it was reanalyzed by immunoturbidimetry using Cobas 6000 which showed the HbA1c value of 8.25 gm%.

III. DISCUSSION

HbA1c was originally a term for an ion exchange chromatographic peak and is now defined as irreversibly glycated haemoglobin molecules at one or both N-terminal valines of the β chains. Biorad D-10 & Variant turbo utilizes the principle of cation exchange chromatography to estimate glycated haemoglobin. The overestimation of glycated haemoglobin can be because of haemoglobin variants with amino acid substitutions on globin chains leading to their charge differences. These charge differences can alter retention time of the non glycated variants causing them to co-elute with glycated fraction leading to overestimation of HbA1c. Examples for such positive interferences include silent variants like Hb Raleigh (b1Val3Ala), Hb Graz (b2His3Leu), Hb Sherwood Forest (b104Arg3Thr), Hb South Florida (b1Val3Met) and Hb Niigata [bN-Methionyl-1(NA)Val3Leu]. In cases of Hb Raleigh, Hb South Florida, and Hb Niigata, the substitution at the NH₂ terminus leads to the formation of acetyl-Hb in vivo, providing a basis for falsely elevated HbA1c.⁴ The presence of haemoglobin (Hb) C or S trait has been shown to affect the HbA1c assays leading to overestimation of HbA1c. Cation-exchange chromatography for HbA1c is also subject to interference by labile HbA1 and HbF as both of them can co elute with HbA1c.⁵ HbF has γ chains, for which the N-terminus is a glycine residue which acetylates readily.^{5,6} In addition to genetic variants, glycated haemoglobin results can be affected by chemical modifications of Hb. These modifications may resemble glycated haemoglobin physically and chemically, which lead to inaccurate determinations of glycated Hb, particularly when charge differences are used for separation of haemoglobin. Carbamylated Hb, increased in uremic patients, is the most commonly encountered derivative. High concentrations of acetylated Hb are seen with rare mutations at the amino terminus of the beta-globin chain that enhances the formation of acetyl-Hb in vivo.⁷

These variant haemoglobins thus interfere only in the assays that are based on charge but not the assays which are based on structure of haemoglobin/antigenic characteristics thus we thought of estimating haemoglobin using immunoturbidimetry

which is not a charge based and uses antibodies that target N-terminal glycosylated amino acids on the β chain to quantify Hb A1c, and the Hb A1c percentage is calculated from the Hb A1c and Hb concentrations.⁸

HPLC can separate actual HbA1c fraction present in the sample from some haemoglobin variants and chemically modified haemoglobins unlike other methods which also measures these as “glycosylated” fractions; thus overestimating the glycosylated haemoglobin. Hemoglobinopathies, such as β -thalassemia, sickle cell disease, homozygous HbC disease & HbSC disease, frequently show increased amounts of minor Hb species, i.e., HbA₂ and HbF, which are known to interfere with estimation of glycosylated haemoglobin by chromatographic methods (HPLC).²

HPLC BIORAD variant turbo program can indicate the presence of haemoglobin variants under variant window or as separate additional peaks and alarms against reporting of spuriously high results.

As this patient had normal urea levels, no clinical features or lab reports suggestive of abnormal HbF presence he might be harbouring a rare silent Hb variant which might have interfered with HbA1c estimation. In such circumstances, estimation by immunoturbidimetry method may be more valid for estimation of HbA1c.

IV. TAKE HOME MESSAGES

1. HbA1c assays can be divided into methods that use molecular charge (CE-HPLC and electrophoresis) and methods that use molecular structure (immunoassays, boronate & affinity chromatography).

2. Hb variants (or their glycosylated forms) may interfere with HbA1c assays based on cation exchange-HPLC but D-10/Variant turbo has been designed to identify the presence of variants under variant window or as additional peaks and warns against reporting of spuriously high values.

3. If spurious HbA1c result is seen on HPLC, the interference by Hb variants should be suspected, and the interpretation of Hb A1c results should be based on the patient's medical history and other laboratory results. Additionally, efforts should be made to identify the Hb variant, and alternative Hb A1c methods that do not show interference by variants like immunoturbidimetry should be used to report the results.

V. LIMITATIONS

In this case Hb electrophoresis was not performed separately for confirmation assuming that machines used are capable of identifying common variables that interfere with HbA1c estimations.

ACKNOWLEDGMENTS

We are grateful to the Dean of Kasturba Medical College & Hospital for providing the facilities for conducting this study. All the authors solemnly affirm that there is no financial/conflict of interest whatsoever.

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AUTHORS

First Author – Mrinal Gupta, MBBS, MD postgraduate student, Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal, Karnataka, India, Pin Code: 576104, Telephone no.: 9036251217, Fax: (91 0820) 2570062, 2570061, E mail id: drmrinalbiochem@gmail.com

Second Author – Krishnananda Prabhu, MBBS, MD., Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal, Karnataka, India, Pin Code: 576104, Telephone no.: 9844380157, Fax: (91 0820) 2570062, 2570061, E mail id: krishnakunj2000@yahoo.com

Third Author – Pragna Rao, MBBS, MD., Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal, Karnataka, India, Pin Code: 576104, Email: drpragnarao@gmail.com