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In-house algorithm for reporting discrepant HbA1c result and troubleshooting a case of false low HbA1c

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ABSTRACT

We report an unusual case of a patient having low glycosylated hemoglobin (HbA1c) below the reportable range, despite having borderline fasting blood glucose. The patient had decreased erythrocytes count and elevated reticulocyte count, with no evidence of hemoglobinopathy.

He reported taking multidrug therapy for borderline lepromatous leprosy. Dapsone induced hemolysis was identified as the cause for the discordant HbA1c. Thus, it is important to be aware of medications and conditions that may lead to a falsely low HbA1c level so that incorrect treatment decisions are not made. In such situations, alternative measure of glycemic control, such as fructosamine is recommended. Further

it is also recommended that clinical laboratories have standard protocol to troubleshoot any discrepant HbA1c result.

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INTRODUCTION

The application of hemoglobin A1C (HbA1c) result as a glycemic control indicator relies on glycation efficiency, which is determined by the integrity of globin chains of adult hemoglobin and life span of the erythrocytes. There are several clinical situations where globin chain or erythrocyte life span are affected and HbA1c is spuriously high or low and thus is not reflective of the true glycemic control. The most encountered situation in our laboratory is a low HbA1c result discrepant with blood glucose measurement. Our laboratory has established and implemented a reporting algorithm to approach any cases of discrepant HbA1c in relation to blood glucose measurement. (Figure 1)

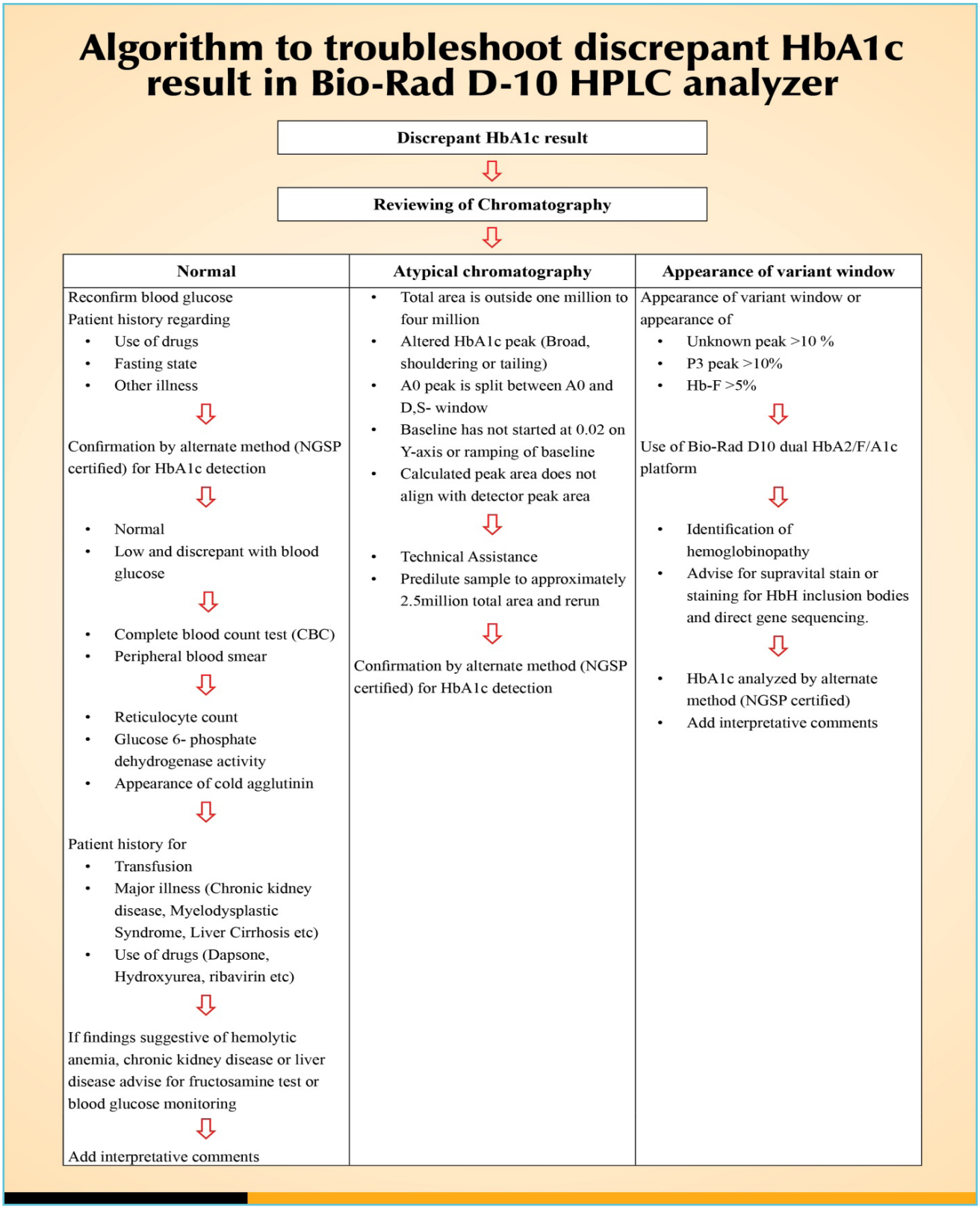
Our laboratory uses BioRad D-10 high performance liquid chromatography (HPLC) analyzer (*BioRad Laboratories Inc., Hercules, CA, USA*) with manufacturers provided retention time system for correct HbA1c and adult hemoglobin (HbAo) peaks identification. The reportable range for HbA1c is 3.7% - 18.4%. (17-178 mmol/mol). Variant hemoglobin and hemolytic disorders comprises the majority of cases with false HbA1c results.

Nepalese population harbors significant numbers of individuals with hemoglobinopathy, with the most common being sickle cell anemia and beta thalassemia. (1) In case of variant window detected in a chromatogram, the laboratory uses Bio-Rad D-10 HPLC system in short program mode (Variant II Beta Thalassemia Short Program, *BioRad Laboratories Inc., Hercules, CA, USA*) for the percent determination of hemoglobin A2, F and A1c and detection of any abnormal hemoglobin under the conditions specified by the manufacturer. HbA1c result is reportable if hemoglobin S trait and hemoglobin C trait is identified when sample is run in a Bio-Rad D-10 variant II beta thalassemia short program mode. (2) Similarly, HbA1c result is reportable if hemoglobin E trait and D trait is detected, unless a degradation peak (Unknown peak) is identified between HbA1c and P3 peaks in a chromatogram. (2) P3 denotes the level of degraded hemoglobin and should be less than 10% for the HbA1c result to be reported. In conditions with S and C window in a chromatogram, HbA1c is not reportable. (2) In such cases, HbA1c is analyzed by alternate method using boronate affinity chromatography (NycoCard™ HbA1c test, *Abbott Diagnostic technologies AS, Oslo, Norway*) or nephelometry (MISPA i3, *Agappe Diagnostic Switzerland*). These are National Glycohemoglobin Standardization Program (NGSP) certified methods traceable to the Diabetes Control and Complications Trial (DCCT) reference method used as back up at our laboratory. (3)

Complete blood count (CBC) testing is done in cases where technical error or variant window is not detected but HbA1c is spuriously low or high, to rule out anemia as a potential cause of discrepant HbA1c result. Serum iron chemistry and testing for vitamin B12 is advised if CBC findings are suggestive. Based on the CBC findings, peripheral smear (PS) examination and reticulocyte count is done in selected cases to rule out possibility of hemolytic disorder. If features suggestive of hemolysis are seen then, Glucose-6-phosphate dehydrogenase (G6PD) activity is determined by CareStart TM G6PD Biosensor Analyzer (*Access Bio Korea, Inc*) at our laboratory. The prevalence of G6PD deficiency in Nepal is 3.5% and this percentage varies according to the ethnicity. (4)

Apart from this, patient history is taken for any recent blood transfusion; presence of any

Figure 1 Algorithm to troubleshoot discrepant HbA1c result



hematological condition or other major illness and use of any drugs having potential to interfere with HbA1c report. Finally, the respective HbA1c result is reported with comment and additional laboratory findings, to facilitate physician's proper HbA1c result interpretation. The majority of cases that need troubleshooting consist of variant hemoglobin and hemolytic anemia where testing HbA1c by alternate method and monitoring glycemic status by alternate method such as fructosamine is advised respectively.

This report highlights the limitations of using HbA1c as a diagnostic tool in a patient using Dapsone for leprosy treatment. Furthermore, this report emphasizes the careful interpretation of each measurement of HbA1c in context of a clinical situation.

CLINICAL DIAGNOSTIC CASE

We received a blood sample of a 28 years male for investigating HbA1c level. The patient's result was 3.4% (13.66 mmol/mol) which was below the reportable range. His fasting blood glucose was 5.6 mmol/L (3.89 – 5.55 mmol/L). Since his HbA1c result was discordant with the fasting blood glucose and was not in the reportable range, the possibility for presence of hemoglobinopathy was suspected. We used Bio-Rad D-10 HPLC system (Variant II Beta Thalassemia Short Program, *BioRad Laboratories Inc., Hercules, CA, USA*) for the percent determination of hemoglobin A2, F and A1c and detection of any abnormal hemoglobin under the conditions specified by the manufacturer. The chromatogram was normal. His blood sample was also investigated by alternative methods for HbA1c analysis. The result was still 3.1% (10.38 mmol/mol) and 3.3% (12.57 mmol/mol) by nephelometry (MISPA i3, *Agappe Diagnostic Switzerland*) and boronate affinity chromatography method (NycoCard™ HbA1c test, *Abbott Diagnostics Technologies AS, Oslo, Norway*).

Since his HbA1c was very low and was discordant with the plasma blood glucose in three different assay platforms, the cause for false low HbA1c was further investigated. The CBC test and examination of PS was done as per our established protocol. Patient had normal hemoglobin but reduced red blood cells count and increased mean corpuscular volume (MCV) (Table 1). Anisopoikilocytosis and normochromia along with occasional microspherocytes were observed in the peripheral smear test. Patient had high reticulocyte count.

Since he had a hemolytic picture upon hematological investigation, the biochemical markers for hemolysis were done. High lactate dehydrogenase, high total and indirect bilirubin and reduced haptoglobin further suggested the presence of hemolysis. (Table 1) His renal function test, Glucose-6-phosphate dehydrogenase (GPPD) activity and thyroid function test was normal. Considering the possibility of hemolysis, detail patient history was taken.

The patient was a known case of Borderline lepromatous Leprosy. He was taking Gabapentin 300 milligram, Cyanocobalamin 1500 microgram and Prednisolone 10 mg daily for peripheral ulnar neuritis along with medications for leprosy (Rifampicin 600 mg monthly, Dapsone 100 mg daily and Moxifloxacin 400 mg daily). There was no family history of any hematological conditions.

On reviewing the literature, we found various reports on dapsone leading to a falsely low HbA1c. The drug dapsone has been listed in a troubleshooting algorithm, due to a significant number of cases reported from our part of world. (5, 6)

Fructosamine level of the patient was assessed, and it was found to be high (Table 1). Interpretative comments about unreliability of

Table 1 Laboratory investigations

Parameters	Result	Reference range /Unit
Hemoglobin	144	135 - 169 g/L
Red blood cells (RBC) count	4300	4400 - 5600 G/L
Mean Corpuscular Volume (MCV)	106.3	81.8 - 95.5 fL
Mean Corpuscular Hemoglobin (MCH)	33.2	27 - 32.3 pg
MCV Concentration (MCHC)	31.5	32.4 – 35 g/dL
Platelet count	100	150 – 450 G/L
Reticulocyte count	4.8	Up to 2 %
Packed cell volume (PCV)	45.7	42 – 52 %
Erythrocyte Sedimentation Rate (ESR)	06	0 – 22 mm/hr
Total Bilirubin	39	5 –31 µmol/L
Direct bilirubin	5	0 – 5 µmol/L
Aspartate Transaminase (AST)	25	17 – 59 U/L
Alanine Transaminase (ALT)	32	10 – 45 U/L
Alkaline Phosphatase (ALP)	348	98 – 279 U/L
Lactate Dehydrogenase (LDH)	345	100 – 210 U/L
Haptoglobin	0.28	0.45–2.05 g/L
Fasting Blood Glucose (FBG)	5.6	3.89 – 5.55 mmol/L
Fructosamine	317.40	205 – 285 µmol/L
Cortisol (8 am)	22.62	137.95 – 634.57 nmol/L
Adrenocorticotropin Hormone (ACTH)	4.18	< 10.13 pmol/L

Vitamin B12	812.54	138.01 – 614.75 pmol/L
Vitamin D	56.41	74.88 – 249.6 nmol/L
Iron	17.3	13 – 31 µmol/L
Ferritin	270.2	21.81 – 274.66 µg/L

HbA1c for monitoring glycemic status in the index patient and advice on testing for plasma glucose or serum fructosamine level was added to the report. Informed consent has been taken from the patient for the publication of this case report.

DISCUSSION

Shortened erythrocytes life span and rapid red cell turnover as seen in patients using dapsone, might have low HbA1c level but not indicating good glycemic condition due to inefficient and lowered degree of glycation. There are several case reports of falsely low HbA1c levels in patients on dapsone, including reports of patients infected with the human immunodeficiency virus (7), patients with polychondritis (8), organ transplant patients (9) patients diagnosed with leprosy (10) and patients using dapsone for prophylaxis against *Pneumocystis jiroveci* pneumonia (11).

Dapsone, also known as diaminodiphenyl sulfone, is an antibiotic commonly used in combination with rifampicin and clofazimine for the treatment of leprosy. It is a second-line medication for the treatment and prevention of pneumocystis pneumonia and for the prevention of toxoplasmosis in those who have poor immune function. Additionally, it has been used for acne, dermatitis herpetiformis, and various other skin conditions. Dapsone leads to a false low HbA1c via various mechanisms such as by inducing hemolysis, promoting the oxidation of hemoglobin to methemoglobin which interferes with the HPLC assay and finally by reducing erythrocyte survival independent of its hemolytic effect (12,13,14). The index patient had elevated reticulocyte counts, decreased haptoglobin, elevated indirect bilirubin and elevated lactate dehydrogenase levels, which are indicative of hemolysis. He also had anisopoikilocytes and microspherocytes noted in the PS examination reflecting hemolytic disease. Hemolysis does not always result in anemia (15), as seen in our patient who had normal hemoglobin in spite of having decreased erythrocyte count. The high MCV in this patient is attributed to the high reticulocyte count since these cells are larger than mature erythrocytes. High level of vitamin B12 and low cortisol in the index patient is attributed to the chronic use of vitamin B12 and steroid.

Methemoglobinemia results in functional anemia where hemoglobin appears normal but the ability of hemoglobin to carry oxygen to the tissues is impaired thus resulting in severe symptoms such as headache, shortness of breath, fatigue, seizures and coma. Test for methemoglobin was not done for our patient; however, he was warned about the symptoms of severe methemoglobinemia. Thus, hemolysis and reduction of erythrocyte lifespan was a cause of misleadingly low HbA1c in the index case.

Since dapsone leads to a falsely low HbA1c, another measure of glycemic control is necessary in patients taking this medication. Fructosamine refers to proteins that are non-enzymatically

glycated via ketoamine linkages at the N-amino terminal. Since, albumin is a major plasma protein, fructosamine primarily reflects glycated albumin. Further, because the half-life of albumin is 2-3 weeks, fructosamine indicates recent glycemic status. The index patient had higher fructosamine level (Table 1). We present case of a patient who had very significant and spurious reduction in his HbA1c result occurring secondary to treatment with dapsone. Prompt recognition of the dapsone effect avoided inappropriate intervention in this patient.

LEARNING POINTS

HbA1c result that lies below the reportable range and is discrepant with the blood glucose result should alert laboratory physician about the conditions associated with shortened red blood cell survival which may result in an inaccurate HbA1c. Alternate methods to assess glycemic control, such as fructosamine or glucose monitoring, should be used in such cases. Clinical laboratory should prepare and follow the algorithm suitable to its assay design, to troubleshoot any discrepant HbA1c result. The addition of the interpretative comments when applicable for the ease of clinicians and patient safety is recommended.

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Conflict of interest

The authors declare that there is no conflict of interest in the publication of this manuscript.

Consent

Informed consent was taken from patient for the publication of this case report.

Authors' contribution

VP - Conceptualization and writing of the report

AS, DP, SP, KG - Scientific Content for the report

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