Significantly High HbA$_{1c}$ in Diabetic Patient with Hb J: Case Report

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ABSTRACT

Glycated hemoglobin (HbA$_{1c}$) is used to monitor the long-term management of diabetes and reflects the average blood glucose level over the past three months. Hb J is an alpha-globin gene variant that occurs less commonly but can interfere with the HbA$_{1c}$ result. This case report presents two cases of abnormally high HbA$_{1c}$ in patients with Hb J using the high-performance liquid chromatography (HPLC) method and repeated value using the capillary electrophoresis (CE) method. The first case was a 26 years old female Malay patient, presenting at 25 weeks gestation with diabetes mellitus (DM). Her HbA$_{1c}$ results from HPLC showed persistently high level (> 18.5%, > 179 mmol/mol) despite optimum diabetic control (fasting blood sugar (FBS) range 4.0–6.1 mmol/L). The second case was a 62-year-old female Malay with type 2 DM. Her HbA$_{1c}$ results from HPLC was also persistently high (> 18.5%, > 1;79 mmol/mol) despite good diabetic control (FBS average 5.0–7.0 mmol/L). Both patients' hemoglobin analysis reports were suggestive of Hb J. Repeated HbA$_{1c}$ using CE were 6.0% (42 mmol/mol) and 8.1% (65 mmol/mol), respectively, and supported the presence of the Hb J variant peak. HbA$_{1c}$ measurement in patients with a variant should be interpreted with caution to avoid misdiagnosis and mismanagement in these kinds of patients.

The peculiar feature of glycated hemoglobin (HbA$_{1c}$) as a measure of chronic glycemic control makes it compelling to be used as an estimation of the average blood glucose over the past 120 days in monitoring as well as diagnosis of diabetes mellitus (DM).[1] HbA$_{1c}$ is a hemoglobin (Hb) adduct resulting from irreversible glycation at either one or both of the N-terminal valines of beta chains.[2] It can be quantified by various methods in which the principle of the measurement can be divided into three major categories which are depending upon charge difference, structural characteristics of glycogroups on Hb, and chemical reactivity. High-performance liquid chromatography (HPLC) is the most common method being used where HbA$_{1c}$ is abstracted from other Hb molecules in a column using charge difference.[3,4] However, many factors such as Hb variants, carbamylated Hb, and hematological diseases interfere to varying degrees with these current analytical methods.[2,5] Introduction of capillary electrophoresis (CE) method in HbA$_{1c}$ assessment has said to give a more accurate result in those patients with Hb variant.[6]

Here, we report two cases of Hb J with abnormally high HbA$_{1c}$ levels using HPLC and comparison with HbA$_{1c}$ value given by CE method.

CASE REPORT

Case one

A 26-year-old female Malay patient at 25 weeks gestation was first diagnosed with DM during antenatal booking at six weeks pregnancy. Her initial diabetic workup were: glycosuria 3+, random blood sugar 15.5 mmol/L, and HbA$_{1c}$ 18.5% (> 179 mmol/mol). Insulin treatment was commenced, and she was able to achieve good glycemic control with a fasting blood sugar (FBS) range of 4–6.1 mmol/L. However, her HbA$_{1c}$ result using HPLC showed a persistently high level (> 18.5%, > 179 mmol/mol). Other biochemical laboratory results were as follows (reference range in parentheses); sodium 135 mmol/L (135–145), potassium 4.1 mmol/L (3.5–5.0), urea 2.1 mmol/L (1.7–8.3), creatinine 59 umol/L (70–130), albumin 46 U/L (38–44), aspartate aminotransferase (AST) 18 U/L (5–34),

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F Concentration =   1.7* %
A2 Concentration =   2.3  %

*Values outside of expected ranges

Analysis comments:
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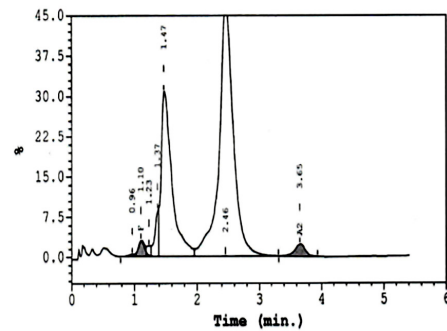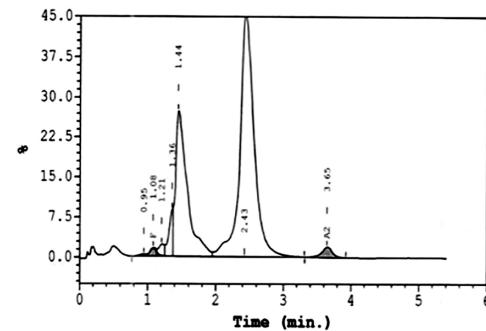

Figure 1: High-performance liquid chromatography analysis of the first case with an abnormal peak (retention time of 1.47) that suggests the presence of Hb J.

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F Concentration =   1.0  %
A2 Concentration =   2.0* %

*Values outside of expected ranges

Analysis comments:
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Figure 2: High-performance liquid chromatography analysis of the second case with abnormal peak (retention time of 1.44) that suggests the presence of Hb J.

and alanine aminotransferase (ALT) 11 U/L (< 34). Her full blood count (FBC) results were RBC 5.00 × 10^{12}/L (3.52–5.16), Hb 14.8 g/dL (9.81–13.85), mean cell volume (MCV) 84.6 fL (77.5–94.5), and mean corpuscular Hb (MCH) 29.6 pg (24.8–31.2). This raised suspicion of the presence of a Hb variant. A full blood picture (FBP) and Hb analysis were sent. FBP showed normal RBC morphology and count with no features of thalassemia or hemoglobinopathy. Hb analysis by HPLC with beta-thalassemia short program revealed a 29% abnormal peak at P3 with a retention time (RT) of 1.47 min [Figure 1]. There was presence of additional band anodal to HbA on alkaline gel electrophoresis suggestive of Hb J. HbA_{1c} test was rerun with a different platform using the CE principle. The presence of Hb J variant peak supports the finding of Hb analysis, and the result was good diabetic control with HbA_{1c} of 6% (42 mmol/mol) with the use of CE.

Case two

A 62-year-old female Malay was diagnosed with type 2 DM two years prior and on an oral hypoglycemic agent. During the first year of treatment, the diabetes was not well controlled, with FBS ranging from 8.3–11.1 mmol/L, and her HbA_{1c} was persistently > 18.5% (> 179 mmol/mol). Subsequently, the treatment was optimized and good glycemic control was achieved with average FBS of around 5–7 mmol/L. Despite good diabetic control based on FBS, the HbA_{1c} remains high with a level of

> 18.5% (> 179 mmol/mol). Other biochemical laboratory results were as follows: sodium 140 mmol/L, potassium 4.2 mmol/L, urea 3.9 mmol/L, creatinine 55 umol/L, albumin 43 U/L, AST 19 U/L, and ALT 21 U/L. Her FBC results were RBC 5.75 × 10^{12}/L, Hb 16.3 g/dL, MCV 89.0 fL, and MCH 28.3 pg. Due to this condition, interference from Hb variant was taken into consideration. The FBP and Hb analysis results were similar to the previous patient; there was 29.8% abnormal peak at P3 with RT of 1.44 min [Figure 2]. Analysis of HbA_{1c} using CE supports the presence of Hb J variant in this patient with a normalizing HbA_{1c} level of 8.1% (65 mmol/mol).

DISCUSSION

HbA_{1c} is used to monitor and diagnose DM.[1] HPLC is the most common method used where HbA_{1c} is separated from other Hb molecules using charge differences.[3,4] However, many factors such as Hb variants, carbamylated Hb, and hematological diseases interfere to varying degrees with this method.[2,5] There are few causes that can produce significantly high HbA_{1c}, such as hemoglobinopathies, iron or vitamin B12 deficiencies, and chronic renal failure (CRF).[7] The cause of high HbA_{1c} in both our cases was due to the presence of Hb J evidenced by Hb analysis and CE. CRF was excluded as the urea, electrolytes, and creatinine levels were normal. Iron or vitamin B12 deficiencies were also excluded since the FBC results showed no evidence of micro/

macrocytic anemia. CE in HbA_{1c} assessment has been said to give a more accurate result in patients with Hb variant.[6] Hb variant is defined as Hb that has a substitution of single amino acid resulting in alteration in Hb structure and biochemical functions.[8] In Southeast Asia, Hb E is the most prevalent accounting for 50%–60% near Thailand, Cambodia, and Laos, and 1%–8% are Hb constant spring.[9] Hb J is an alpha-globin gene variant with rare incidence worldwide.[10] Depending on its variant, Hb J has particular characteristics and functions. It can range from completely normal clinical features such as in Hb J Sardegna up to severe presentation like in heterozygous Hb J Capetown, which is associated with increased oxygen affinity and polycythemia. Other Hb J variants such as Hb J Bangkok and Baltimore are associated with sickle Hb.[11] In both cases, the Hb J variant could not be determined as the Hb genotypes were not carried out due to the logistic problem. With the RT of 1.47 and 1.44 min, respectively, Hb J Bangkok and Hb J Singapore need to be considered. Being the less common occurrence of Hb disorders than Hb E trait (19.3%) and thalassemia,[12] the impact of Hb J in HbA_{1c} measurement interference may be undervalued. Incidental detection of Hb J during routine HbA_{1c} test may add value in identifying this rare Hb variant, and other appropriate measures for glycemic control should be taken into consideration.

The mechanism of Hb variant interference in HbA_{1c} measurement is method-dependent, and it can be classified into physiological or analytical factors. Physiologically, the Hb variant may disrupt the process of HbA_{1c} formation, which will cause an underestimation of HbA_{1c}.[5] For instance, in the homozygous Hb variant, there will be no HbA_{1c} formation because only the glycated form of the Hb variant can be found. Similarly, the heterozygous Hb variant will produce a lesser amount of HbA_{1c} in addition to the glycated form of the Hb variant.[9]

In more detail, the Hb variant may interfere during the testing process by co-eluting with HbA_{1c} peak if it carries a similar charge with HbA_{1c}. This is valid, especially for the HLPC method.[5] Depending on the mutation point in the variant and the assay used for measurement of HbA_{1c}, each variant may cause a clinically misleading value of HbA_{1c}, affecting the interpretation of the result.[2,13] In the case of Hb J, there were few studies that reported lower HbA_{1c} values in patients with this Hb variant[6,11,14,15] in

contrast to our patients who showed abnormally high HbA_{1c} using HPLC method.

The repeated HbA_{1c} using CE showed results within the normal range. The separation of HbA_{1c} using CE is based on electrophoretic mobility and electroosmotic flow in an alkaline buffer with a specific pH. This segregation principle results in CE being more accurate in providing true HbA_{1c} value and, at the same time, detection of Hb variant and other Hb adducts. CE can display the various peak of Hb components at different intervals and give better resolution than HPLC.[16–19] This feature is being utilized to give more exact assessment of HbA_{1c} in relation to glycemic control with minimization of interference from other factors. In addition, CE is also preferred over HPLC in terms of easy experimental setup, faster time for analysis, and requires very low reagent and sample as well as cost effective.[20]

CONCLUSION

Careful interpretation of HbA_{1c} results is essential in populations with a relatively high prevalence of Hb variants, such as in Malaysia. Interference from a Hb variant should be suspicious when the HbA_{1c} results do not correlate clinically or with blood glucose levels. This is crucial to prevent wrong diagnosis and mismanagement of the patients.

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