

Significantly High Hba1c in Diabetic Patient with HbJ: Case Report

Wan Nor Fazila Hafizan Wan Nik¹, Noorazliyana Shafii^{1*}, Noor Azlin Azraini Che Soh Yusof¹ and Rosnah Bahar²

¹Department of Chemical Pathology, School Of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan

²Department of Hematology, School Of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan

Received: 23 July 2020

Accepted: 9 January 2021

**Corresponding author: noorazliyana@usm.my*

DOI 10.5001/omj.2022.14

ABSTRACT

Glycated haemoglobin (HbA1c) is used to monitor the long term management of diabetes and reflects the average blood glucose level over the past 3 months. HbJ is an alpha globin gene variant that occurs less commonly but can interfere with HbA1c result. This case report presents two cases of abnormally high HbA1c in diabetic patient with HbJ using High Performance Liquid Chromatography (HPLC) method and repeated value using capillary electrophoresis (CE) method. The first case was a 26 years old, female Malay patient, presenting at the gestational age of 25 weeks with diabetes mellitus (DM). Her HbA1c results from HPLC showed persistently high level (>18.5% (>179mmol/mol) despite optimum diabetic control (fasting blood sugar (FBS) range 4.0 to 6.1mmol/L). The second case was a 62 years old female Malay with Type 2 DM. Her HbA1c results from HPLC also was

persistently high (>18.5% (>179mmol/mol) despite good diabetic control (FBS average 5.0 to 7.0mmol/L). Both patients' haemoglobin (Hb) analysis report were suggestive of HbJ. Repeated HbA1c using CE principle were 6% (42mmol/mol) and 8.1% (65mmol/mol) respectively and supported the presence of HbJ variant peak. HbA1C measurement in patients with variant should be interpreted with caution to avoid misdiagnosis and mismanagement in these kinds of patients.

Keywords: HbA1c, diabetes mellitus, haemoglobin variant, haemoglobin J, HPLC, CE

CASE REPORT

First case

A-26-year-old, female Malay, at 25 weeks period of gestation was first diagnosed with DM during antenatal booking at 6 weeks of pregnancy. Her initial diabetic work up was: glycosuria: 3+, random blood sugar (RBS): 15.5mmol/L and HbA1c: 18.5% (>179 mmol/mol). Insulin treatment was commenced and she was able to achieve good glycaemic control with fasting blood sugar (FBS) range 4 to 6.1mmol/L. However, despite optimum diabetic control based on FBS, the HbA1c result using HPLC showed persistently high level with >18.5% (>179mmol/mol). Other biochemical laboratory results were as follows (reference range in parentheses); sodium 135mmo/L (135-145), potassium 4.1mmol/L (3.5-5.0), urea 2.1mmol/L (1.7-8.3), creatinine 59umol/L(70-130), albumin 46U/L (38-44), aspartate aminotransferase (AST) 18U/L (5-34) and alanine aminotransferase (ALT) 11U/L (<34). Her full blood count (FBC) results were red blood cells (RBC) $5.00 \times 10^{12}/L$ (3.52-5.16), Hb 14.8g/dL (9.81-13.85), mean cell volume (MCV) 84.6fL (77.5-94.5) and mean corpuscular Hb (MCH) 29.6pg (24.8-31.2). This raises the suspicion of the presence of Hb variant. Full blood picture (FBP) and Hb analysis was sent. FBP showed normal RBCs morphology and count with no features of thalassemia or haemoglobinopathy. Hb analysis by

HPLC with beta thalassaemia short program revealed 29% abnormal peak at P3 with the retention time (RT) of 1.47min (Figure 1) and there was presence of additional band anodal to Hb A on alkaline gel electrophoresis which suggestive for HbJ. HbA1c test was rerun with different platform using CE principle. The finding of Hb analysis is supported by the presence of HbJ variant peak and the result was in good diabetic control with HbA1c of 6% (42mmol/mol) with the use of CE.

Second case

A-62-year-old female Malay was diagnosed with Type 2 DM for the past 2 years on oral hypoglycaemic agent. During first year of treatment, the diabetes was not well controlled with FBS ranging from 8.3 to 11.1mmol/L and HbA1c was persistently >18.5% (>179 mmol/mol). Subsequently, the treatment was optimised and good glycaemic control was achieved with average FBS around 5 to 7mmol/L. Despite good diabetic control based on FBS, yet the HbA1c remains high with the level >18.5% (>179mmol/mol). Other biochemical laboratory results were as follows; sodium 140mmo/L, potassium 4.2mmol/L, urea 3.9mmol/L, creatinine 55umol/L, albumin 43U/L, AST 19U/L and ALT 21U/L. Her FBC results were RBC $5.75 \times 10^{12}/L$, Hb 16.3g/dL, MCV 89.0fL, and MCH 28.3pg. Due to this condition, interference from Hb variant was taken into consideration. The results from FBP and Hb analysis were similar to the previous patient; there was 29.8% abnormal peak at P3 with the RT of 1.44min (Figure 2). Analysis of HbA1c using CE supports the presence of HbJ variant in this patient with normalising level of HbA1c of 8.1% (65mmol/mol).

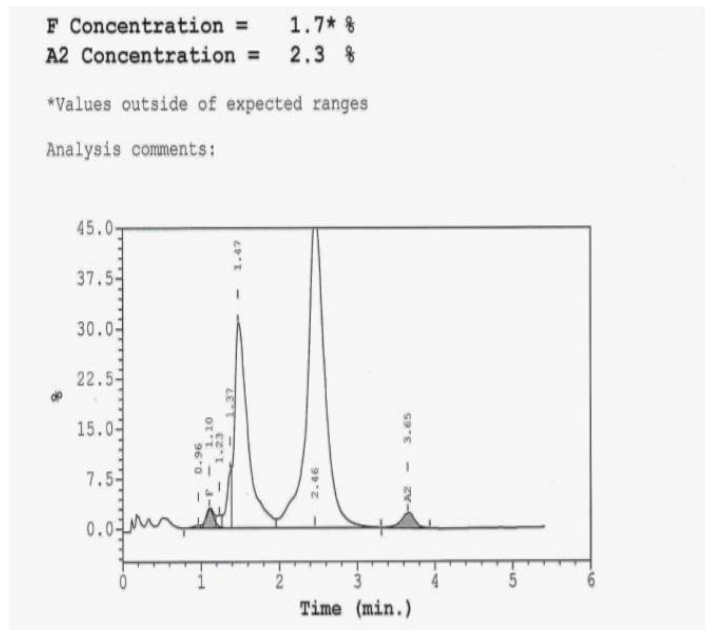


Figure 1: HPLC analysis of the first case with abnormal peak (RT 1.47) that suggests the presence of HbJ.

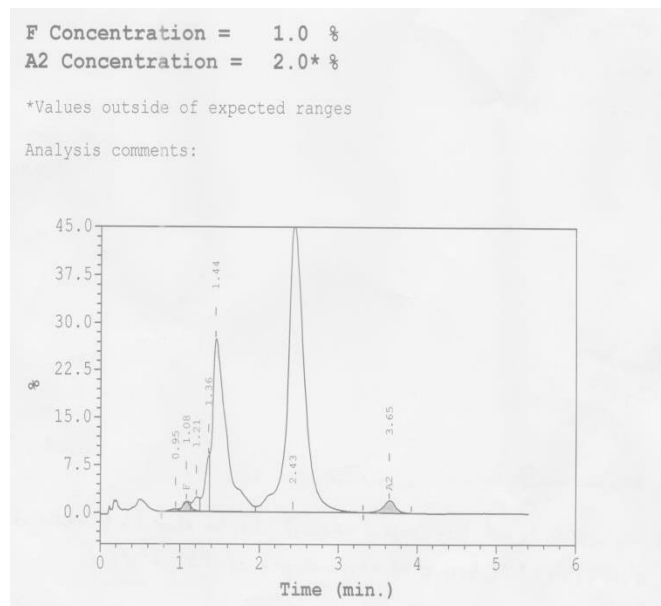


Figure 2: HPLC analysis of the second case with abnormal peak (RT 1.44) that suggests the presence of HbJ.

DISCUSSION

Glycated haemoglobin (HbA1c) is used in the monitoring and diagnosis of DM.¹ HPLC is the most common method used where HbA1c is separated from other Hb molecules using charge differences.^{2,3} However, many factors such as Hb variants, carbamylated Hb, and haematological diseases interfere to varying degrees with this method.^{4,5} There are few causes that can produce significantly high HbA1c such as haemoglobinopathies, iron or vitamin B12 deficiencies and chronic renal failure (CRF).⁶ The cause of high HbA1c in both cases was due to the presence of HbJ evidenced from 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/macrocyclic anaemia. CE in HbA1c assessment has said to give a more accurate result in those patient with Hb variant.⁷ Hb variant is defined as Hb that having substitution of single amino acid resulting in alteration in Hb structure and biochemical functions.⁸ In Southeast Asia, HbE is the most prevalence accounting 50-60% near Thailand, Cambodia and Laos, and 1-8% are Hb Constant Spring (CS).⁹ HbJ is an alpha globin gene variant with rare incidence worldwide.¹⁰ Depending on its variant, HbJ has particular characteristics and functions. It can range from a completely normal clinical features such as in HbJ Sardegna up to severe presentation like in heterozygous HbJ Capetown which it is associated with increased oxygen affinity and polycythaemia. Other HbJ variant such as HbJ Bangkok and Baltimore are associated with sickle Hb.¹¹ In both cases, HbJ 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 1.44min respectively, HbJ Bangkok and HbJ Singapore need to be considered as well. Being the less common occurrence of Hb disorders as compared to HbE trait (19.3%) and thalassemia¹², impact of HbJ in HbA1c measurement interference may be undervalued. Incidental detection of HbJ

during routine HbA1c test may add a value in identifying this rare Hb variant and other appropriate measures for glycaemic control should be taken into consideration.

The mechanism of Hb variant interference in HbA1c measurement is method dependent and it can be classified into physiological or analytical factors. Physiologically, Hb variant may disrupt the process of HbA1c formation in which will cause underestimation of HbA1c.⁵ For instance, in the case of homozygous Hb variant, there will be no HbA1c formation because only glycated form of the Hb variant can be found. Similarly, heterozygous Hb variant will produce lesser amount of HbA1c in addition to glycated form of Hb variant.⁹

In more detail, Hb variant may interfere during testing process by co-eluting with HbA1c peak if it carries similar charge with HbA1c. This is valid especially for HPLC method.⁵ Depending on mutation point in the variant and the assay used for measurement of HbA1c, each variant may cause a clinically misleading value of HbA1c, affecting the interpretation of the result.^{4,13} In the case of HbJ, there were a few studies reported lower HbA1c values in patients with this Hb variant^{7,11,14,15} in contrast to our patients who showed abnormally high HbA1c using HPLC method.

The repeated HbA1c using CE showed results within normal range. Separation of HbA1c 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 HbA1c value and at the same time detection of Hb variant and other Hb adducts. CE is able to display the various peak of Hb components at different interval and give better resolution compared to HPLC.¹⁶⁻¹⁹ This feature is being utilised in order to give more exact assessment of HbA1c in relation to glycaemic control with minimization of interference from other factors. In addition, CE is also preferred than 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 HbA1c results is essential in populations with a relatively high prevalence of Hb variants such as in Malaysia. Interference from an Hb variant should be suspicious when the HbA1c results do not correlate clinically or with blood glucose levels. This is very crucial to prevent wrong diagnosis and mismanagement of the patients.

Disclosure

Both patients have given consent for their data to be published.

REFERENCES

1. Sikaris K. The correlation of hemoglobin A1c to blood glucose: SAGE Publications; 2009.
2. Gupta S, Jain U, Chauhan N. Laboratory diagnosis of HbA1c: a review. *J Nanomed Res.* 2017;5(4):00120.
3. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomarker insights.* 2016;11:BMI. S38440.
4. Radin MS. Pitfalls in hemoglobin A1c measurement: when results may be misleading. *J Gen Intern Med.* 2014;29(2):388-394.
5. Sacks DB, Arnold M, Bakris GL, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Clin chem.* 2011;57(6):e1-e47.
6. Amin Mohsin Z, Paul A, Devendra S. Pitfalls of using HbA1c in the diagnosis and monitoring of diabetes. *London J Prim Care.* 2015;7(4):66-69.
7. Chu C-H, Lam H-C, Lee J-K, et al. Common hemoglobin variants in southern Taiwan and their effect on the determination of HbA1c by ion-exchange high-performance liquid chromatography. *J Chin Med Assoc.* 2009;72(7):362-367.
8. Thom CS, Dickson CF, Gell DA, Weiss MJ. Hemoglobin variants: biochemical properties and clinical correlates. *Cold Spring Harb Perspect Med.* 2013;3(3):a011858.
9. Fucharoen S, Winichagoon P. Haemoglobinopathies in southeast Asia. *Indian J Med Res.* 2011;134(4):498.
10. Deopa B, Chordiya K, Parakh M, Dara P. Rare association of Hemoglobin variant Hb J (α mutation) with haemophilia A: case report. *Int J Contemp Pediatrics.* 2017;4(6):2226.
11. Ares BJ, Bernardo GA, Martin-Nieto A, Gonzalez-Martinez S, Delgado-Alvarez E, Menendez-Torre E. Effect of hemoglobin J variant on HbA1c values as measured by

- HPLC (high-performance liquid chromatography). Paper presented at: 20th European Congress of Endocrinology 2018.
12. George E. HbE β -thalassaemia in Malaysia: revisited. *J Hematol Thromb Dis.* 2013;1(101):2.
 13. Florida S. A Rare Hemoglobin Variant Which Interfered Hemoglobin A1C Result: Hemoglobin South Florida [β 1 (NA1) Val> Met, GTG> ATG; HBB: c. 4G> A]. *Türk Biyokimya Dergisi [Turk J Biochem]*. 2014;39(2):226-230.
 14. Tsai L-Y, Tsai S-M, Lin M-N, Liu S-F. Effect of hemoglobin variants (Hb J, Hb G, and Hb E) on HbA1c values as measured by cation-exchange HPLC (Diamat). *Clin chem.* 2001;47(4):756-758.
 15. Zhang XM, Wen DM, Xu SN, Suo MH, Chen YQ. Effects of hemoglobin variants HbJ Bangkok, HbE, HbG Taipei, and HbH on analysis of glycated hemoglobin via ion-exchange high-performance liquid chromatography. *J Clin Lab Anal.* 2018;32(1):e22214.
 16. Ji L, Yu J, Zhou Y, et al. Erroneous HbA1c measurements in the presence of β -thalassemia and common Chinese hemoglobin variants. *Clin Chem Lab Med (CCLM)*. 2015;53(9):1451-1458.
 17. Suo M, Wen D, Wang W, et al. False measurement of glycated hemoglobin in patients without hemoglobin A. *Biosci rep.* 2019;39(1).
 18. Dessi M, Pieri M, Pignalosa S, Martino FG, Zenobi R. Performances of Capillary Electrophoresis and HPLC Methods in HbA1c Determination: Diagnostic Accuracy in HbS and HbD-Iran Variants' Presence. *J Clin Lab Anal.* 2015;29(1):57-60.
 19. Urrechaga E. High-resolution HbA1c separation and hemoglobinopathy detection with capillary electrophoresis. *Am J Clin Pathol.* 2012;138(3):448-456.
 20. Warade J. Comparison of glycated hemoglobin with HPLC and capillary electrophoresis. *Int J Res Med Sci.* 2017;5(5):1976-1979.