

Interference in the measurement of Hemoglobin A1c: Presence of Hemoglobin D

Hemoglobin A1c ölçümünde interferans: Hemoglobin D Varlığı

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ABSTRACT

HbA1c measurement was performed by the high-performance liquid chromatography (HPLC) method, and the result was measured as 3.5% (14.75 mmol/mol). Since the HbA1c result was inconsistent with the other clinical values of the patient, the same sample was run in the variant mode of the instrument and Hb variant warning was received. The HbD variant was detected in the analysis of the sample and the HbA1c result was reported as 5.1% (32 mmol/mol). In the presence of a low HbA1c value that is not suitable for the patient's clinic, the sample should be re-examined considering the presence of Hb variant.

Key words: Glycated Hemoglobins, Hemoglobinopathies, HPLC

ÖZET

Olgumuzda HbA1c ölçümü yüksek performanslı sıvı kromatografisi (HPLC) yöntemiyle gerçekleştirildi ve sonuç %3.5 (14.75 mmol/mol) olarak ölçüldü. HbA1c sonucunun hastanın diğer klinik değerleriyle uyumsuz olması nedeniyle, aynı örnek cihazın varyant modunda çalışıldı ve Hb varyantı uyarısı alındı. Örneğin varyant analizi sonucunda HbD varyantı varlığı tespit edildi ve HbA1c sonucu %5.1 (32 mmol/mol) olarak rapor edildi. Hastanın kliniğine uygun olmayan düşük HbA1c değerinin varlığında Hb varyant varlığı düşünülerek örnek tekrar incelenmelidir.

Anahtar Kelimeler: Glikozillenmiş hemoglobin, Hemoglobinopatiler, HPLC

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INTRODUCTION

The terms glycosylated hemoglobin and HbA1c (A1C test, A1C) are the common expressions used to describe hemoglobin with added glucose residue as "nonenzymatically". The formation of glycosylated hemoglobin is non-enzymatic, that is, a spontaneous event that does not involve the enzyme. The HbA1c value reflects blood glucose levels over the past two to three months. An HbA1c value of $\geq 6.5\%$ or ≥ 48 mmol/mol, measured using a method standardized according to the Diabetes Control and Complications Trial (DCCT) and certified by the National Glycohemoglobin Standardization Program (NGSP), is one of the diagnostic criteria for diabetes mellitus according to the American Diabetes Association (ADA) criteria (1). It is stipulated that the HbA1c analysis method used by all the laboratories in the United States must be calibrated according to the high-performance liquid chromatography (HPLC) method, which is the gold standard for HbA1c (2). HbA1c is used not only to guide the diagnosis and treatment of diabetes but also to evaluate the quality of patient care and to predict the risk of developing diabetes complications (3).

In the presence of factors that may affect the results such as hemoglobinopathies, age, race, iron deficiency, and vitamin B12 deficiency, the result of HbA1c measurement may not reflect the true value (4). High or low HbA1c values due to Hb variants, which are the subject of this case report, may cause errors in the diagnosis and treatment of patients (5).

In this case report, we intended to show the interference triggered by the presence of the hemoglobin variant causing a low HbA1c result, which is inconsistent with the laboratory results and clinical status of the patient, with the ion-exchange HPLC-based method used in our laboratory.

CASE PRESENTATION

A 29-year-old male patient applied to our laboratory for routine biochemistry,

hemogram, and HbA1c test measurement during a routine general health check-up. The routine biochemistry and hemogram values of the patient are presented in Table 1. The patient's HbA1c test was performed with a cation-exchange HPLC-based Adams A1C HA-8180V (Arkray, Inc., Kyoto Japan) instrument. The patient's HbA1c value was measured as 3.5% (14.75 mmol/mol) in our instrument (Figure 1). Since the patient's HbA1c result was inconsistent with the fasting blood glucose and HOMA index values, the same sample was reevaluated in the instrument's variant mode with the suspicion of a possible Hb variant (Figure 2).

Upon receiving a Hb variant warning in the sample evaluated in the variant mode, the sample was sent to the Provincial Public Health laboratory for detailed analysis. Hb variant analysis was performed on the Adams A1C HA-8180T (Arkray, Inc., Kyoto Japan) instrument and HbD was detected (Figure 3).

Table 1. Laboratory parameters of the patient

Parameters	Result	Reference value
WBC ($10^9/L$)	9.18	3.91 - 10.9
RBC ($10^{12}/L$)	5.35	4.44 - 5.61
HGB (g/dL)	15.9	13.5 - 16.9
HCT (%)	46.8	40 - 49.4
MCV (fL)	87.5	81.8 - 95.5
MCH (pg)	29.7	27.0 - 32.3
MCHC (g/dL)	34.0	32 - 36
PLT ($10^9/L$)	234	150 - 450
MPV (fL)	9.6	9.3 - 12.1
Glucose (mg/dL)	82	70 - 100
Blood Urea Nitrogen (mg/dL)	8,8	5 - 18
Creatinine (mg/dL)	0.89	0.70 - 1.20
ALT (IU/L)	41	0 - 41
AST (IU/L)	26	0 - 40
Calcium (mg/dL)	9,76	8.6 - 10.2
Folate (ng/mL)	13,0	4.6 - 34.8
Ferritin ($\mu g/L$)	251	30 - 400
Vitamin B12 ($\mu g/L$)	331	191 - 663
TSH (mIU/L)	2,04	0.270 - 4.20
Free T4 (ng/dL)	1,49	0.93 - 1.70
Free T3 (ng/dL)	3,94	2.0 - 4.4
Insulin (mIU/L)	3,81	2.6 - 24.9
HOMA Index	0,77	0 - 2.5

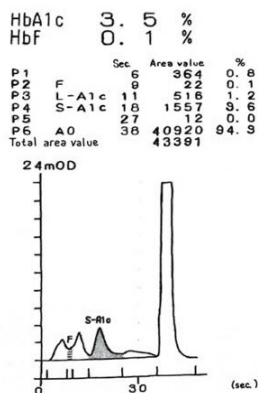


Figure 1. HbA1c chromatogram: The fast mod result of Adams A1C HA-8180V in our laboratory

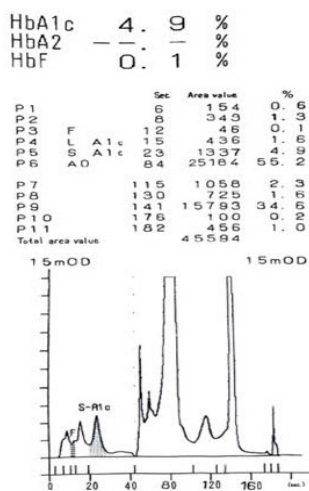


Figure 2. HbA1c chromatogram: The variant mod result of Adams A1C HA-8180V in our laboratory

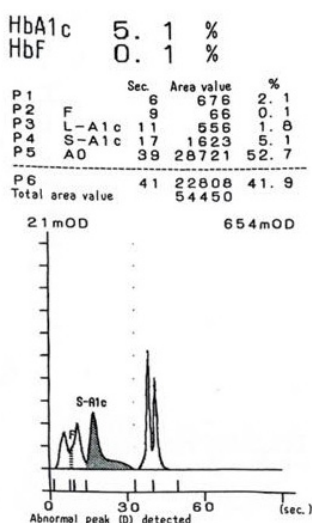


Figure 3. HbA1c chromatogram: The variant mod result of Adams A1C HA-8180T in the public health laboratory

DISCUSSION

More than 1000 variants of hemoglobin have been identified worldwide, many of which are clinically asymptomatic. The most common Hb variants observed in our country are Hb S, Hb D, Hb E, and Hb O Arab (6). Apart from those, 42 abnormal Hb variants have been reported to be observed in our country. While the incidence of the Hb D-Los Angeles variant in Kahramanmaraş region was reported as 0.3%, the total incidence in our country was reported as 0.2%. The Hb D-Punjab variant is the most common abnormal hemoglobin variant in the population of the Chinese Uyghur region (7).

The effect of hemoglobinopathies and the presence of chemically modified Hb derivatives (carbonylated Hb) on glycosylated hemoglobin varies depending on the method. It is difficult to generalize the effect of hemoglobinopathies on HbA1c measurement results; however, low, or high values for HbA1c have been reported in the presence of some of these variants, depending on the method (HPLC, immunoassay, and immunoagglutination). In the presence of hemoglobinopathies, HbA1c results measured by the boronate affinity method have been reported to be acceptable (8).

Considering that the 1% deviation of the HbA1c value causes a variation in the mean blood glucose concentration of 1.4 – 1.9 mmol/L, the HbA1c values, which are reported incorrectly below or above the true value, may cause important misconceptions in interpreting the patient results (9).

Cation exchange HPLC separates hemoglobin types according to their charge differences. Inaccurate HbA1c values may occur when hemoglobin variants or glycosylated derivatives can not be separated from Hb A or HbA1c. Co-elution of hemoglobin variants with HbA1c results in an overestimation of HbA1c from the true value. On the other hand, the co-dissolution of the glycosylated hemoglobin variant from HbA1c because of the co-elution of the hemoglobin variant with

HbA leads to an underestimation of HbA1c results less than it is (5).

Hb D is characterized by the substitution of glutamine for glutamic acid at position 121 of the beta-globin chain. In the presence of the HbD variant, it has been reported that the HbA1c measurement results obtained by the chromatographic method give lower values than the immunoassay measurement results (10).

With ion-exchange HPLC incorrect HbA1c values may occur due to electrical charge differences in Hb variants (6). No data has been reported on the NGSP website that the presence of the HbD variant for the Adams A1C HA-8180V causes an interference with the HbA1c measurement. The result of this case is important in terms of showing that the HbD variant causes a decrease in the HbA1c results.

Biochemists need to evaluate patient results collectively during result validation to prevent analytical errors that may occur. In the presence of an HbA1c result inconsistent with the patient's clinic, HbA1c chromatograms and warnings should be examined with caution and the Hb variant should be considered. As in this case report, in the presence of a low HbA1c value inconsistent with the patient's clinic, the instrument should be switched to variant mode and sample should be reevaluated; however, in the presence of Hb variant warning, the variant measurement should be performed on an instrument that performs variant analysis. In addition, if possible, HbA1c measurement should be performed with another method. It should be considered that variant hemoglobin D interference may cause a significant analytical error. Thus, this situation can play an important role in reducing the errors that may occur.

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