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Research Article

The effect of hemoglobin variants on high-performance liquid chromatography measurements of glycated hemoglobin

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Abstract

Objectives: Glycated hemoglobin (HbA1c) is routinely utilized to monitor long-term glycemic control. The presence of hemoglobin (Hb) variants may lead to a false HbA1c measurement. This study was an investigation of the effects of both common and rare Hb variants on the level of HbA1c measured with high-performance liquid chromatography (HPLC).

Methods: : The HbA1c level of a total of 391 patients without Hb variants (HbAA, n=44) and with Hb variants (HbAS, HbSS, HbSS(A), HbSS(F), HbAD, HbAE, HbAF, HbD-Iran/D-Iran, HbD-Los Angeles/A, HbE-Saskatoon/E-Saskatoon, HbEE, HbG-Coushatta/A, HbOArab/OArab, HbSE, and Hb Stanleyville II, n=347) was measured using an HPLC analyzer.

Results: The HbA1c level of all of the Hb variants but HbStanleyville II and HbG-Coushatta/A was extremely low. However, when the Hb variants were considered as a single group, a statistically significant difference was seen in comparison with the group that had no Hb variants ($p<0.001$).

Conclusion: It was determined that the measurement of HbA1c can be adversely influenced by the presence of some Hb variants. Hemoglobin variants should be investigated when the HbA1c level is incompatible with blood glucose.

Keywords: HbA1c, hemoglobin variants, high-performance liquid chromatography

Aglycated hemoglobin (HbA1c) test is used to monitor the glycemic control of patients with diabetes mellitus and to assess the risk of development of diabetic complications [1-3]. HbA1c is the major component of the hemoglobin protein hemoglobin alpha 1 (HbA1) formed by the irreversible binding of glucose to N-terminal valine of the hemoglobin beta chain as a result of the non-enzymatic glycation reaction [4]. Various methods are used to measure HbA1c, which indicates the average blood glucose level during the previous 2 to 3 months, based on the charge difference (high-performance liquid chromatography, electrophoresis, and isoelectric focusing), the structural difference (boronate affinity chromatography, immunoassays), or chemical difference [5]. HbA1c measurement should be effective, reliable, reproducible, and highly accurate to determine a diabetes diagnosis and achieve accurate monitoring. However, the results may not reflect the correct value as a result of several factors that can interfere in the measurement, such as hemoglobinopathies, iron deficiency anemia, or vitamin B12 deficiency [6-9].

Hemoglobinopathies are a common public health problem worldwide, and notably present in the southern region of Turkey [10]. The literature has documented inaccurate HbA1c results based on the measurement method used and the characteristics of each Hb variant [11, 12]. The aim of this study was to determine the HbA1c level of patients with Hb variants observed in the Çukurova region of Turkey using an Agilent 1100 analyzer (Agilent Inc., Santa Clara, CA USA).

Materials and Methods

Sample selection

This retrospective study was performed in the medical biochemistry laboratory of the Çukurova University Faculty of

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Medicine and was conducted with the approval of the Clinical Research Ethics Committee of Çukurova University on 04.03.2016 (number: 46). The HbA1c level of a total of 391 patients both without an Hb variant (HbAA: n=44) and with Hb variants (HbAS: n=266, HbSS: n=32, HbSS[A]: n=9, HbSS(F): n=6, HbAD: n=11, HbAE: n=6, HbAF: n=5, HbD-Iran/D-Iran: n=1, HbD-Los Angeles/A: n=1, HbE-Saskatoon/E-Saskatoon: n=1, HbEE: n=1, HbG-Coushatta/A: n=2, HbO-Arab/HbO-Arab: n=2, HbSE; n=1, Hb Stanleyville II: n=3) were evaluated.

Assay method: Whole blood samples were taken from individuals without Hb variants (HbAA) and with Hb variants and collected in tubes containing ethylenediaminetetraacetic acid as an anticoagulant. HbA1c values were measured using an Agilent 1100 analyzer according to the manufacturer's instructions. This method identifies hemoglobin types based on the electrical charge. A negatively charged cation-exchange column was utilized to measure positively charged Hb in ion exchange chromatography. Hemoglobin can be detected at a specific absorption wavelength of 415 nm.

Statistical analysis

All of the analyses were conducted using IBM SPSS Statistics for Windows, Version 23.0 software (IBM Corp., Armonk, NY, USA). Categorical variables were presented as numbers and percentages, while continuous variables were summarized as median and minimum-maximum, as appropriate. The Mann-Whitney U test was used to compare continuous variables of the 2 groups. The accepted level of statistical significance was p=0.05.

Results

HbA1c samples of 391 patients were analyzed. Of the group, 44 (11.3%) had no Hb variants (HbAA), while Hb variants were present in 347 (88.7%). Among those without Hb variants, the mean HbA1c level was 4.61% while the mean HbA1c level for those with Hb variants was 1.79%. A statistically significant difference was determined in a comparison between those with and without Hb variants (p<0.001; Table 1). The HbA1c level was determined to be abnormally low (306 cases) or 0% (37 cases) in the 347 cases with Hb variants, which was much lower than that of the HbAA patients. Those who presented with abnormally low HbA1c levels were found to be carriers of HbAS (n=266), HbSS (n=5), HbSS(A) (n=7), HbSS(F) (n=1), HbAD (n=11), HbAE (n=6), HbAF (n=5), HbE-Saskatoon/E-Saskatoon (n=1), HbG-Coushatta/A (n=2), and HbO-Arab/O-Arab (n=2). The HbA1c level was reported to be 0% in 37 patients who were carriers of HbSS (n=27), HbSS(A) (n=2), HbSS(F) (n=5), HbSE (n=1), HbEE (n=1), and HbD-Iran/D-Iran (n=1). The HbA1c level of Hb Stanleyville II (n=3) and Hb D-Los Angeles/A (n=1) carriers was close to that of the HbAA patients (Table 2).

Table 1. Frequency and HbA1c results

	Frequency	Percentage	Median	Min	Max	p
Without hemoglobin variants	44	11.3	4.61	4.00	5.46	0.000
With hemoglobin variants	347	88.7	1.79	0.00	5.80	
Total	391	100.0				

Data are expressed as median (min-max). HbA1c: Glycated hemoglobin

Discussion

The measurement of HbA1c is the gold standard for long-term monitoring of glycemic control in diabetic patients [1-13]. There are now various methods to measure HbA1c in clinical biochemistry laboratories based on different principles [5, 14]. International standardization studies of HbA1c measurement methods are ongoing. A worldwide standard for measurement and testing is needed. HPLC has been accepted as the primary reference method to measure HbA1c by the National Glycohemoglobin Standardization Program of the International Federation of Clinical Chemistry Working Group. They have established interlaboratory compatibility, which involves reference methods such as mass spectroscopy and capillary electrophoresis [13, 15-17]. Yet, despite advances in the standardization of analysis methods, it has been reported that certain hemoglobinopathies interfere with HbA1c measurement, leading to results that are falsely low or high [18].

Many screening studies of hemoglobinopathies have been performed in Turkey, and especially in the Çukurova region.

Table 2. Frequency and HbA1c results of hemoglobin variant patients

	Frequency	Percentage	Median	Min	Max
HbAS	266	76.7	1.82	1.12	2.86
HbAD	11	3.2	2.11	1.57	2.77
HbAE	6	1.7	2.46	2.03	2.68
HbEE	1	0.3	0.00	0.00	0.00
D-Iran/D-Iran	1	0.3	0.00	0.00	0.00
HbSS	32	9.2	0.00	0.00	1.44
HbSS(A)	9	2.6	0.77	0.00	2.09
HbSS (F)	6	1.7	0.00	0.00	0.19
HbSE	1	0.3	0.00	0.00	0.00
HbAF	5	1.4	1.35	0.77	1.82
HbO Arab/O Arab	2	0.6	0.29	0.25	0.33
HbE-Saskatoon/ E-Saskatoon	1	0.3	0.84	0.84	0.84
HbG Coushatta/A	2	0.6	2.19	2.19	2.20
HbD-Los Angeles/A	1	0.3	3.80	3.80	3.80
HbStanleyville II	3	0.9	4.60	3.90	5.80
Total	347	100.0			

Data are expressed as median (min-max). HbA1c: Glycated hemoglobin

Several hemoglobin variants that are rarely seen and clinically silent have been detected, in addition to commonly observed hemoglobin variants. There is a high prevalence of hemoglobinopathies and population migration in the Çukurova region, and therefore, accurate understanding of the effect of Hb variants on the measurement of HbA1c is essential [8, 19]. The objective of the present study was to determine the effect of Hb variants observed in the Çukurova region on HbA1c levels and it was observed that the results were extremely low in the presence of some Hb variants.

A literature review revealed that accurate measurement interference has been evident when the HbA1c values of patients with the 4 most common heterozygous Hb variants (HbAS, HbAE, HbAC, and HbAD) in the world were analyzed using certain measurement methods [17, 20]. Little et al. [21] conducted a more extensive evaluation of the HbA1c values of 49 different rare Hb variants using 8 different techniques and determined that certain Hb variants influenced HbA1c measurement. Tran et al. [22] found that both immunoassay methods and HPLC systems were acceptable techniques, though the HPLC method demonstrated greater analytical performance. Interference in HbA1c measurement has also been reported with the HPLC method [5, 12, 23, 24]. Chu et al. [5] used the ion exchange HPLC method (HLC-723 G7; Tosoh Corp., Tokyo, Japan) in order to determine the effects on HbA1c results of Hb variants widely observed in the south of Taiwan. They reported that various Hb variants caused false HbA1c results in 11 cases and that the presence of an Hb variant should be taken into consideration when using the HPLC method. Camargo et al. [24] performed an analysis with the HPLC method using a Merck-Hitachi L-9100 analyzer and found that the HbA1c results were below the reference range in 130 of 29.657 diabetic patients and that 73 of these patients (56%) had an Hb variant (HbAS: 69, HbAD: 2, HbAC: 1, HbSC: 1). In addition, in 5 non-diabetic cases who were homozygous for HbS, the HbA1c values were 0%. Lorenzo et al. [12] analyzed the HbA1c level of 134 patients and reported that it was abnormally low in 42 cases and produced no result in 92 cases. In our study, the HbA1c level of the patients with HbSS (27 cases), HbSS(A) (2 cases), HbSS(F) (5 cases), HbSE (1 case), HbEE (1 case), and HbD-Iran/D-Iran (1 case) was 0%, and very low HbA1c levels were found in cases with heterozygous HbAS, HbAD, and HbAE types, which is similar to the findings of these earlier studies.

Laboratories use many different methods to measure HbA1c based on different methods and inaccurate results can occur if patients have certain Hb variants. It must be considered that in methods that separate by migration based on molecular charge, a hemoglobin variant molecule can migrate as HbA1c does [23, 25]. Wei et al. [26] observed that a patient who had an HbA1c value of 0 was found to have the Hb Long Island variant. Yay et al. [27] examined the ion exchange HPLC method, and found that a case with a result below the HbA1c reference range (2.1%) was positive for HbD-Los Angeles Los Angeles and noted that low HbA1c levels measured with the ion exchange HPLC method might be the result of Hb variants lead to negative interference. Sheme et al. [7] analyzed clinically silent Hb variants using the HPLC variant window (Variant-II Turbo; Bio Rad Laboratories, Hercules, CA, USA), and reported that the HbA1c values were lower than the reference range.

Kurt et al. [2] used the cation-exchange HPLC method and found that an abnormal chromatogram accompanied low HbA1c values (2.2%), and they determined that the patient was an HbS carrier. Zhang et al. [28] investigated the effect of HbJ-Bangkok, HbE, HbG-Taipei, and α-thalassemia HbH on HbA1c results using the ion exchange HPLC method and they suggested that interference occurs at different levels depending on the Hb variant. Rohlfing et al. [29] also reported that certain methods demonstrated significant interference in the presence of one or a few Hb variants and that these results may negatively affect the follow-up of diabetic patients. Inaccurate measurements can lead to a diagnosis of diabetes in nondiabetic patients. It is clear that analysis methods have limitations and the presence of Hb variants must be a consideration to prevent misdiagnosis and provide the appropriate treatment.

Conclusion

Our analyses determined that the HbA1c level can be affected by certain Hb variants with the Agilent 1100 HPLC analyzer. Interference in HbA1c level measurement can lead to misdiagnosis and incorrect treatment. Hb variants must be considered when results are lower than the reference value or when HbA1c cannot be measured. In order to avoid incorrect measurements, it is necessary to consider the measurement method and factors such as hemoglobinopathies that can affect the measurement.

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