Hb H Interference on Measurement Of HbA1c With Ion-Exchange HPLC

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Case report

ABSTRACT
In this article, an interference caused by hemoglobin H (Hb H), during the measurement of hemoglobin A1c (HbA1c) with ion exchange high pressure liquid chromatography (HPLC) method, was presented in blood sample of a 20-year-old male patient. HbA1c measurement was performed with Agilent 1200 HPLC system using a commercial Recipe HbA1c ion-exchange column. Hemoglobin electrophoresis was performed with Interlab G26 agarose electrophoresis automated compact system. HbA1c level was 18.2% and HbA0 level was 81.5% with ion-exchange HPLC method. Patient’s fasting serum glucose was assessed before HbA1c measurement and the result was 165 mg/dL (9.16 mmol/L). On the other hand, the result of HbA0 was 87.9%, Hb H was 10.8% and Hb A2 was 1.3% with electrophoresis. Whole blood test values were within reference ranges except MCV. MCV value was 79.6 fL. It is important to keep in mind that HbA1c level might be considered falsely high with ion-exchange HPLC method because of Hb H containing sample.

Key words: Hemoglobin H; Hemoglobin A1c, High Pressure Liquid Chromatography.

1. INTRODUCTION
According to International Federation of Clinical Chemistry (IFCC), hemoglobin A1c (HbA1c) is defined as hemoglobin that is irreversibly glycated at one or both N-terminal valines of the beta chains (1). HbA1c has been the most widely used and accepted test for diagnosing and monitoring the glycemic control in individuals with diabetes (2).

Several methods are used to determine HbA1c levels; based on chemical, structural and charge characteristics of the molecule; such as electrophoresis, isoelectric focusing, high pressure liquid chromatography (HPLC), affinity chromatography, immune measurements. Because of the variety of measurement methods and the factors causing interference; standardization has not been achieved yet (3).

Hemoglobinopathy is one of the factors that causes interference. Cayci et al. has shown that increased Hb F levels, which is the major hemoglobin of fetal life, cause falsely high HbA1c results (4). It was reported that Hb F, Hb S, Hb C and Hb D also caused false HbA1c results when used HPLC method (5). National Glycohemoglobin Standardization Program (NGSP) was established by American Assossiation for Clinical Chemistry (AACC) to study for standardization of HbA1c measurement in 1996. NGSP has published several factors that cause interference in HbA1c measurement (6).

Alpha thalassemia is a common genetic disorder that is characterized by deficient or absent synthesis of alpha globin chains of the hemoglobin molecule. The α-thalassemias usually result from deletions involving the α-globin genes, less commonly they are due to point (nondeletion) mutations. The incidence of alpha thalassemia is about 3 per cent in the Çukurova region at Southern Turkey (7).

There can be differences between several HPLC methods. Little et al. reported that, higher HbA1c results were obtained with affinity chromatography method than other HPLC methods. Little Tiran et al. compared boronate affinity chromatography method and ion-exchange HPLC method for measurement of HbA1c and found good correlation with each other and acceptable coefficient of variation (CV%) values for each method of HbA1c assay performance (11).

In our laboratory, HbA1c measurement performed with ion-exchange chromatography method using the Agilent 1200 instrument (Agilent technologies, USA) and HbA1c commercially kit was Recipe HbA1c (Recipe Chemicals – Instruments GmbH, Munich, Germany). This HPLC technique was certified by NGSP. Hemoglobin electrophoresis was performed with Interlab G26 agarose electrophoresis automated compact system (Via Rina Monti, Rome, Italy).

In this article, we wanted to draw attention the interference caused by Hb H, a variant hemoglobin, during the measurement of HbA1c with ion-exchange HPLC method in a sample from a 20-year-old patient whose fasting plasma glucose level was 165 mg/dL (9.16 mmol/L).
2. CASE REPORT

HbA1c assay was performed for a 20-year-old patient follow-up because of his elevated fasting plasma glucose level (165 mg/dL; 9.16 mmol/L). In our laboratory, we use HPLC device equipped with UV-1000 visible detector (Shimadzu Class-VP, Kyoto, Japan) and Recipe HbA1c assay kit (Recipe Chemicals–Instruments GmbH, Munich, Germany) for HbA1c measurement. The standart and control materials were appropriate for IFCC standards. Intraassay and interassay coefficient of variations (CVs) were 1.6% and 2.4%, respectively. Hemoglobin electrophoresis was performed with Interlab G26 (Via Rina Monti, Rome, Italy) device with Interlab SRE604K (Via Rina Monti, Rome, Italy) assay kit.

HbA1c and HbA₀ were found 18.2% and 81.5%, respectively (Figure 1).

The very high level of HbA1c was disputable and we decided to do hemoglobin electrophoresis. Consequently, we established that Hb A₀ result was 87.9%; Hb H result was 10.8% and Hb A₂ result was 1.3% (Figure 2).

3. DISCUSSION

There are more than 30 methods for measurement of HbA1c and these methods could be interfered by many factors. There are several studies about interferences on measurement of HbA1c with HPLC. In this case report, it has been shown that Hb H and HbA1c peaks are overlapped on the chromatogram and caused falsely high HbA1c level. Pravatmuang et al. reported that they found HbA1c levels were falsely low with the presence of Hb H (12). They thought that the possible explanation of these observations might be the amount of glycated β chain which polymerized as β₄ or Hb H, and it was evaluated as the first peak of the non-quantitating area of the chromatogram which caused the HbA1c level by HPLC to be very low. Conversely, Lee at al. found HbA1c levels were falsely higher with the presence of Hb H (13). Lee at al. reported Hb H and HbA1c peaks were overlapped on the chromatogram and this caused falsely high HbA1c levels.

However, there are some methods for HbA1c assay which are not affected by hemoglobin variants (14), ion-exchange HPLC method which is widely used all over the world and in our laboratory, is prone to interference with these hemoglobin variants. In this case, we did not compare HbA1c assay methods.

Patient’s HbA1c level was found 18.2% and it was assessed to be discordant with fasting plasma glucose level. Then hemoglobin electrophoresis was performed to evaluate this discordance. After electrophoresis, Hb H result was 10.8%. Because of the overlapping of HbA1c and Hb H peaks, the original HbA1c level was thought to be approximately 7.4%. However, 7.4% value is compatible with fasting plasma glucose level, this value was not the exact value of course. In this regard, HbA1c levels could be measured with immunoturbidimetric method, so this kind of interferences could be overcome.

As a result, HbA1c levels may falsely be found elevated, in the presence of Hb H, with ion-exchange HPLC. It is important to bear in mind not to confirm wrong results.

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