ACTA INFORM MED. 2013 Sep; 21(3): 216-218

doi: 10.5455/aim.2013.21.216-218
Received: 05 May 2013 • Accepted: 02 July 2013
CONFLICT OF INTEREST: NONE DECLARED

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

Mehmet Agilli[1], Halil Yaman[1], Fevzi Nuri Aydin[1], Erdinc Cakir[1], Tuncer Cayci[1], Yasemin Gulcan Kurt[1], Emin Ozgur Akgul[1], Ibrahim Aydin[1]
Department of Medical Biochemistry, Gulhane Military Medical Academy, Ankara, Turkey[1]

Correspondence Author: Mehmet Agilli, M.D. Department of Medical Biochemistry, Gulhane Military Medical Academy. 06018 Etlik, Ankara, Turkey. mehmetagilli@yahoo.com Phone: +90 505 6829819 Fax: +90 312 3043300

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 glycaemic 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 in the presence of Hb E (8). This finding was supported with another study (9). Moiz et al. thought affinity chromatography was superior than other HPLC methods for measurement of HbA1c (10). However 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).

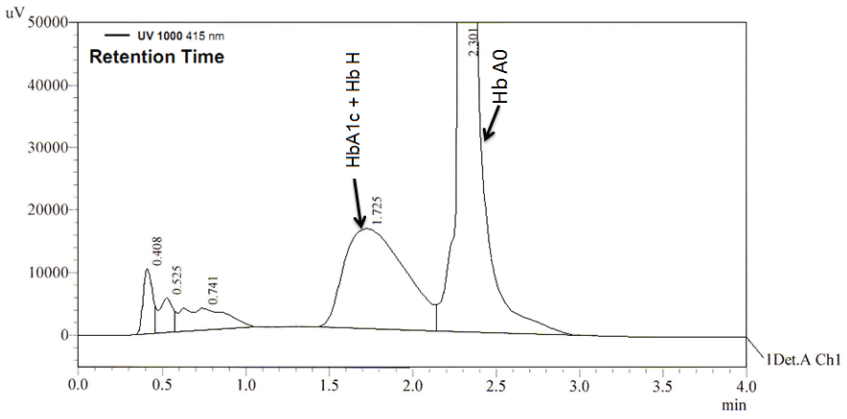

Figure 1. Chromatogram of HbA1c with HPLC. HbA1c and Hb H peaks were thought to be overlapped.

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 HbA_0 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 A_0 result was 87.9%; Hb H result was 10.8% and Hb A_2 result was 1.3% (Figure 2).

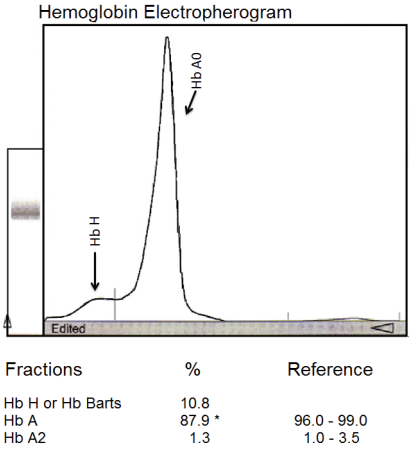

Figure 2. Electropherogram of patient's blood specimen.

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.

REFERENCES

1. Genc S, Omer B, Aycan-Ustyol E, Ince N, Bal F, Gurdol F. Evaluation of Turbidimetric Inhibition Immunoassay (TINIA) and HPLC Methods for Glycated Haemoglobin Determination. Journal of Clinical Laboratory Analysis. 2012, 26(6): 481-485.
2. Organization WH. Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus. Abbreviated report of a WHO consultation. Geneva, WHO, 2011: 1-25.
3. Stein DS, Lyles RH, Graham NM, Tassoni CJ, Margolick JB, Phair JP, Rinaldo C, Detels R, Saah A, Bilello J. Predicting clinical progression or death in subjects with early-stage human immunodeficiency virus (HIV) infection: a comparative analysis of quantification of HIV RNA, soluble tumor necrosis factor type II receptors, neopterin, and - 2-microglobulin. Journal of Infectious Diseases. 1997; 176(5): 1161-1167.
4. Cayci T, Kurt YG, Aydin I, Yaman H, Cakir E. Interference of HbF in HbA1c measurement by high performance liquid chromatography method: a case report. Gulhane Med J. 2011; 53(3): 211-213.
5. Nadzimah N, Thevarajah M, Chew Y. Hemoglobin variants detected by hemoglobin A1c (HbA1c) analysis and the effects on HbA1c measurements. Int J Diabetes Dev Ctries. 2010, 30(2): 86-90.
6. Little RR, Sacks DB. HbA1c: how do we measure it and what does it mean? Current Opinion in Endocri-

nology. Diabetes and Obesity. 2009; 16(2): 113-118.
7. Curuk MA, Genc A, Huseynova P, Zeren F, Aksoy K. Genotypes of alpha thalassemia and Hb H disease in Çukurova. Turkiye Klinikleri J Pediatr Science. 2007; 3(10): 17-20.
8. Little RRR, CL, Hanson S, Connolly S, Higgins T, Weykamp CW, D'Costa M, Luzzi V, Owen WE, Roberts WL. Effects of hemoglobin (Hb) E and HbD traits on measurements of glycated Hb (HbA1c) by 23 methods. Clinical chemistry. 2008; 54(8): 1277-1282.
9. Tsai LY, Tsai SM, Lin MN, Liu SF. Effect of hemoglobin variants (Hb J, Hb G, and Hb E) on HbA1c values as measured by cation-exchange HPLC (Diamat). Clinical chemistry. 2001, 47(4): 756-758.
10. Moiz B, Rasool Hashmi M, Sadaf S. Performance evaluation of ion exchange and affinity chromatography for HbA1c estimation in diabetic patients with HbD: A study of 129 samples. Clinical biochemistry. 2008; 41(14-15): 1204-1210.
11. Tiran AP, Tiran T, Halwachs-Baumann B, Dobnig G, Grubelnig H, Wilders-Truschnig H, MM. Automated determination of glycated hemoglobin: comparative evaluation of five assay systems. Journal of Clinical Laboratory Analysis. 1994; 8(3): 128-134.
12. Pravatmuang P, Sae-Ngow B, Whanpuch T, Leowattana W. Effect of HbE and HbH on HbA1C level by ionic exchange HPLC comparing to immunoturbidimetry. Clinica Chimica Acta. 2001; 313(1-2): 171-178.
13. Lee SC, Wang LH, Tsai SM, Fang HY, Tsai LY. Effects of the Hb E, Hb H and Hb G-Taichung variants on HbA1c values by the Bio-Rad Variant (TM) II Turbo analyzer. Clin Biochem. 2011; 44(16): 1338-1342.
14. Chang J, Hoke C, Ettinger B, Penerian G. Evaluation and interference study of hemoglobin A1c measured by turbidimetric inhibition immunoassay. American journal of clinical pathology. 1998; 109(3): 274-278.

INSTRUCTIONS FOR THE AUTHORS

ALL PAPERS NEED to be sent to: Editorial board of the journal "Acta Informatica Medica", electronically over the web site www.scopemed.org and www.avicenapublisher.org. Every article sent to Acta Inform Med gets its number, and author(s) will be notified if their paper is accepted and what is the number of paper. Original paper could not contains more than 3,000 words, Review article more than 4,500 and Case report more than 1,500 words, including References.

EVERY CORRESPONDENCE WILL use that number. The paper has to be typed on a standard format (A4), leaving left margins to be at least 3 cm. All materials, including tables and references, have to be typed double-spaced, so that one page has no more than 2000 alphanumerical characters (30 lines) and total number of used words must not to be more than 3,500. Presenting paper depends on its content, but usually it consists of a title page, summary, tex t references, legends for pictures and pictures. type your paper in MS Word and send it on a diskette or a CD-ROM, so that the editing of your paper will be easier.

EVERY ARTICLE HAS to have a title page with a title of no more than 10 words: name(s), last and first of the author(s), name of the institution the author(s) belongs to, abstract with maximum of 45 letters (including space), footnote(s) with acknowledgments, name of the first author or another person with whom correspondence will be maintained.

THE PAPER NEEDS to contain structured summary, 200 words at the most. Summary needs to hold title, full name(s) and surname(s) of the author(s) and coauthor(s), work institution, and all essential facts of the work, introduction, formulation of problems, purpose of work, used methods, (with specific data, if possible) and basic facts. Summary must contain the re- view of underlined data, ideas and conclusions from text. Summary must have no quoted references. Four key words, at the most, need to be placed below the text.

AUTHENTIC PAPERS CONTAIN these parts: introduction, goal, methods, results, discussion and conclusion. Introduction is brief and clear review of the problem. Methods are shown, so that interested reader is able to repeat described research. Known methods don't need to be identified, they are cited (referenced). If drugs are listed, their generic name is used, (brand name can be written in brackets). Results need to be shown clearly and logically, and their significance must be proven by statistical analysis. In discussion, results are interpreted and compared to the existing and previously published findings in the same field. Conclusions have to give an answer to author 's goals.

QUOTING REFERENCES MUST be on a scale, in which they are really used. Quoting most recent literature is recommended. Only published articles, (or articles accepted for publishing), can be used as references. Not published observations and personal notifications need to be in text in brackets. Showing references must be as how they appear in the text. References cited in tables or pictures are also numbered according to the quoting order. All references should be compiled at the end of the article in the Vancouver style or pubMed style (i.c. www.scopemed.org).

TESTS USED FOR statistical analysis need to be shown in text and in tables or pictures containing statistical analysis.

TABLES HAVE TO be numbered and shown by their order, so they can be understood without having to read the paper. Every column needs to have a title, every measuring unit (SI) has to be clearly marked (i.e. preferably in footnotes below the table, in Arabic numbers or symbols). Pictures also have to be numbered as they appear in the text. drawings need to be enclosed on a white or tracing paper, while black and white photos have to be printed on a radiant paper. Legends (e.g. next to pictures and photos), have to be written on a separate A4 format paper. All illustrations, (pictures, drawings, diagrams), have to be original, and on their backs contain, illustration number, first author 's last name, abbreviated title of the paper and picture at the top. It is appreciated, if author marks the place for the table or picture. Papers could not be submitted with included more of 4 Tables, Figures or Graphs. Every additional must be paid 20 euros each.

USE OF ABBREVIATIONS have to be reduced to a minimum. Conventional units can be used without their definitions. Supplement. If paper contains original contribution to a statistical method or author believes, without quoting original computer program, that paper 's value will be reduced. Editorial staff will consider possibility of publishing mathematics /statistic analysis in extension.

ANY PRACTICE OF plagiarism will not be tolerated regarding submitted articles. Non-identifiable quoted parts of the articles from other authors are known act of plagiarism if it is not cited or referencing in appropriate places in the article. Advertent practice of plagiarism will abort reviewing process or article submission. Author(s) may suggest or exclude peer-re-viewers for their articles but Editorial Board has the right to reject their(s) opinions or suggestions according to copyright Assignment form signed by authors before reviewing process. Authors must respect guidelines and rules of IcMjE, WAME, cOpE, E A SE, linked on www.avicenapublisher.org.

ALL INDIVIDUALS LISTED as authors should qualify for authorship and should have participated sufficiently in the work to take public responsibility for appropriate portions of the content and follow the next conditions: a) substantial contributions to the conceptions and design, acquisition of data, or anal- lysis and interpretation of data; b) drafting the article or revising it critically for important intellectual content; c) final approval of the version to be published (all co-authors must sign copyright Assignment form downloaded from www.avicenapublisher.org). All other contributors to the article's subject who does not qualify for authorship should be listed in acknowledgement section. for all relevant information about authorship follow IcMjE guidelines.

ALL AUTHORS MUST make a formal statement at the time of submission indicating any potential conflict of interest that might constitute an embarrassment to any of the authors if it were not to be declared and were to emerge after publication. Such conflict of interest might include, but not limited to, share holding in or receipt of grant or consultancy free form a company whose product features in the submitted manuscript or which manufactures a competing product. All authors must submit a statement of conflict of Interest to be published at the end of their article (conflict of Interest: non declared).