



AN ASSESMENT OF UNEXPECTEDLY HIGH HbA1c LEVEL IN A CASE WITH TYPE 1 DIABETES

Tuba Çandar¹, Sedat Özdemir¹, Ayça Törel Ergür², Selda Demirtaş¹

¹University of Ufuk, School of Medicine, Department of Biochemistry, Ankara

²University of Kırıkkale, School of Medicine, Department of Pediatric Endocrinology, Kırıkkale

ABSTRACT

The HbA1c test provides information about blood glucose levels of previous months depending on the erythrocyte lifetime when monitoring diabetic patients. However, various factors such as HbF and other hemoglobin variants can interfere with the measurement of HbA1c.

In this study, an unexpectedly high HbA1c level was observed in a patient with type 1 diabetes. In the hemoglobin chain analysis, which explained the reason for the high value, high fetal hemoglobin levels were detected and interfered with

HbA1c measurement with the HPLC method. This finding was in concordance with the literature. As a conclusion, it should be considered that hemoglobinopathy might be found in the patients who have higher levels of HbA1c which is not in agreement with the blood glucose. Thus, it can be said that HbA1c test is not a good marker for monitoring such diabetic patients. In such cases, fructosamine or other glycated end products may be a more reliable marker.

Keywords: HbA1c assay, HbF, type 1 diabetes. *Nobel Med* 2016; 12(1): 97-100

OLAĞANDIŞI HbA1c DÜZEYİ GÖZLENEN TİP 1 DİYABETLİ BİR OLGUNUN DEĞERLENDİRİLMESİ

ÖZET

HbA1c testi, diyabetik hastalarda eritrosit yaşam süresine bağlı olarak son birkaç aylık kan şekeri düzeyi hakkında bilgi veren bir testtir. Çeşitli yöntemlerle ölçülen HbF ya da diğer hemoglobin tipleri HbA1c ölçüm sonuçlarını etkileyebilir.

Bu çalışmada tip 1 diyabetli bir hastada beklenmedik yüksek HbA1c düzeyi gözlemlendi. Nedenini açıklamak

için gerçekleştirilen Hb zinciri analizinde, yüksek HbF düzeyinin HPLC metodu ile yapılan HbA1c ölçümünü etkilediği tespit edildi. Bu bulgu literatür ile de uyum göstermekteydi. Sonuç olarak, kan şekeri ile uyumu düşünüldüğünde yüksek bulunan HbA1c düzeyleri için hastalarda hemoglobino-pati bulunabileceği göz önüne alınmalıdır. Bu durumda HbA1c testinin böyle diyabetik hastaların takibi için iyi bir rehber olmadığı söylenebilir. Bu gibi vakalarda, fruktozamin veya diğer glukozile son ürünler daha güvenilir izlem belirteci olabilir.

Anahtar kelimeler: HbA1c ölçümü, HbF, tip 1 diyabet. Nobel Med 2016; 12(1): 97-100

INTRODUCTION

Hemoglobin (Hb) consists of four globin chains. Adult hemoglobin (HbA) is the most abundant form in adults and consists of two α and two β chains. Fetal hemoglobin (HbF), the predominant type of Hb at birth, consists of two α and two γ chains. At the end of the second year after birth, it decreases to <2% becoming a minor form in healthy adults. HbA2 is also a minor Hb in adults and consists of two α and two δ chains.¹

HbA1c is the product of irreversible, nonenzymatic glycation of one or both N-terminals of the Hb β chain. It is the marker that is most widely used to monitor long-term glycemic control in diabetic patients. Physiologically, HbA1c is the main glycosylated Hb (GHb) that consists of 80% of HbA1, and it is 4-6 % of total Hb.^{2,3} The four basic methods most commonly used to measure HbA1c are as follows: immunoassay, ion-exchange high-performance liquid chromatography (HPLC), boronate affinity HPLC, and enzymatic assays. Most immunoassays measure HbA1c using antibodies specific to the N-terminal glycosylated amino acids (usually the first 4–10 amino acids) of the Hb β chain. Ion-exchange HPLC separates Hb species based on the charge differences between HbA1c and the other hemoglobins. With boronate affinity methods, aminophenylboronic acid reacts specifically with the glucose bound to Hb. This method measures total glycosylated Hb, including HbA1c. This technique has been known to cause the least interference with the Hb variants and derivatives. The currently available enzymatic methods measure HbA1c using an enzyme that specifically cleaves the N-terminal.¹ In ion-exchange HPLC methods, clinically silent Hb variants may show additional peaks in chromatograms, resulting in a false positive HbA1c value. In the case of erroneous results caused by the Hb

variants, affinity chromatography may provide a more accurate measurement in HbA1c.^{4,5}

In this study, we sought to elucidate the unexpectedly high HbA1c level measured by HPLC in one diabetic case.

CASE

A four-year-old male patient diagnosed with type 1 diabetes was investigated. All of the biochemical parameters, including blood glucose and fructosamine, were measured using the Abbott Architect c 8000. CBC tests were measured using the Abbott Cell-Dyn 3700. HbA1c measurements and Hb chain analysis were performed using ion-exchange HPLC (Chromosystem, Agilent 1100 series). The urine analysis was performed automatically using a urine analyzer (IRIS-IQ 200). For manual HbF analysis, spectrophotometry was performed using the Humalyzer 2000.

The patient, diagnosed with type 1 diabetes and receiving insulin treatment, was admitted to our clinic for further research. Clinical evaluation was normal. The patient was first admitted to our outpatient clinic three months ago. Initial blood work showed that Fast Blood Glucose (FBG) was 290 mg/dl and the HbA1c level was 12.9%, and urine analysis showed that ketone was (+) and glucose was (+++). When admitted to our clinic for control evaluation after three months, FBG was 220 mg/dl and the HbA1c level was 15.02%, and urine analysis showed that ketone was (-) and glucose was (++) (Table).

When the biochemical parameters were evaluated, all of them improved except HbA1c with a value of 15.02%, which did not correlate with the blood glucose value of 220 mg/dl. As similar results were obtained when the HbA1c was repeated, we decided to perform Hb

chain analysis for detection of hemoglobinopathy. The results showed that HbA2: 2.134%, HbF: 4.850%, and HbA1: 93.08% (Figure). Hemoglobin F was measured by another technique, the manual method (alkali denaturation according to Betke).⁶ The results of the manual HbA1c technique were similar to the HPLC results. Fructosamine was measured in the sera of the patient, as we thought that the increased HbF level interfered with the HbA1c assay. The value of 600 mmol/L of fructosamine was in greater agreement with the results of the blood glucose and urine analysis.

DISCUSSION

Depending on the method of determination, the concentration of HbA1c is approximately 4-6% in healthy patients without diabetes. However, the presence of Hb variants may falsely produce low or spuriously increased HbA1c values.¹ This condition is especially important for diabetic patients who have extremely high HbA1c values.⁷

One of the most reliable methods for measuring Hb chain analysis is HPLC. In this technique, the subgroups of the Hb are separated with respect to their charge. Any pathology in the synthesis of Hb chains would cause their electrical charge to deteriorate.

In our case, we think that any difference in charge might be caused by damage during the elution of the Hb subgroups. In the ion exchange procedure, when the Hb content moves through the column, Hb is separated as fractions with respect to differences in charge, which is recognized by the stationary phase. Samples were eluted using 3 different buffers. In buffer 1, only HbA1a and HbA1b are eluted while in buffer 2, HbF and HbA1c are eluted. Buffer 3 has the highest ionic charge and is capable of eluting HbA0. Afterwards, HbF and HbA1c were sensed by the detector system, and we saw 2 different peaks in close proximity. The peak of the HbF is always next to the HbA1c peak. Thus, any increase in the peak of HbF would directly affect the analysis of HbA1c due to the change in the electrical charge of the Hb chains. These factors can explain the results of our case.

In literature, high HbF levels have been reported to interfere with the results of several HbA1c methods, including the boronate-affinity method.⁸ A study that used the reversed-phase HPLC procedure proved the technique to be a reliable method for the quantitative determination of Hb globin chains in newborns.⁹ It has been suggested that in pediatric patients, in whom are most often detected incongruent results, specific methods for HbA1c, such as HPLC, should be used. In a study of healthy children over 5 years of age, HbA1 values were within the reference limits similar to adults.

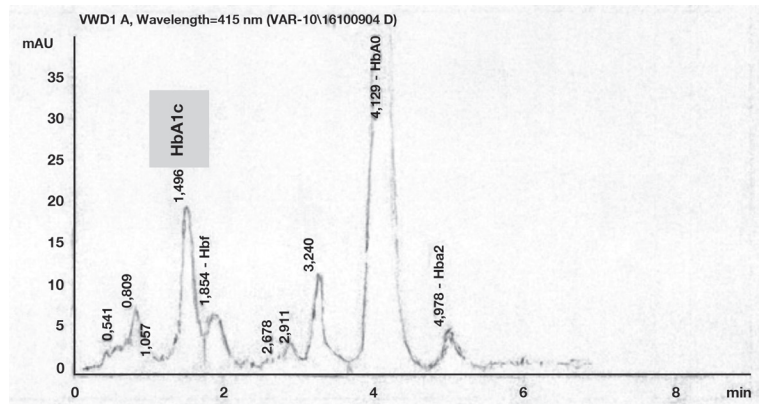


Figure: Hb chain analyze in our case

Table: The levels of some biochemical parameters in our case		
Biochemical Parameters	3 Months Ago	Now
Urine glucose	(+++)	(++)
Urine ketones	(+)	(-)
FBG	290 mg/dl	220 mg/dl
The mean of seven blood glucose profile	225 mg/dl	195 mg/dl
HbA1c	12.9%	15.02%
Fructosamine	-	600 mmol/L

FBG: Fasting blood glucose.

However, 69% of the children younger than 1 year of age and 7% within 1-5 years of age had HbA1 levels above 10%. Their HbA1c values were in the reference range, but the HbF values were higher. The discrepancy was also related to the raised concentrations of HbF. It was concluded that fetal hemoglobinemia is more prevalent than was previously known, especially among children younger than 5 years of age.¹⁰ This observation might have resulted from the increasing use of HPLC technique recently, which is the most successful way to determine Hb variants.

High HbF was found in 13 of 43 diabetic patients in the analysis of HbA1c by HPLC method, and increased HbF values were reported in HbA1c measurements.³ In another study, it was noted that not only HbF but also hemoglobin variants, such as HbS, HbE, HbC and HbD, might affect HbA1c levels.¹ Bard et al. emphasized that HbF persistence may occur in infants who suffer from hypoxemia.¹¹

Our results show that, as the HbF increases, it contributes to the HbA1c peak due to elution time during hemoglobin chain analysis on HPLC. Therefore, the peak of Hemoglobin F can form a shoulder on the HbA1c peak. Thus, the unexpected higher HbA1c levels should be regarded as a misleading factor, especially for diabetic children, and HbF subgroup analysis should be performed in these cases.

CONCLUSION

As a result, we saw that HbA1c is not a reliable marker for monitoring diabetic children. Fructosamine or other glycated end products assay may be a more

reliable marker for this type cases. In such cases these assays should be performed for the final decision.

* The authors declare that there are no conflicts of interest.

C	CORRESPONDING AUTHOR: Tuba ÇANDAR Ufuk University, Faculty of Medicine, Department of Biochemistry, 06520 Ankara, Türkiye tubacandar@msn.com
✓	DELIVERING DATE: 09 / 10 / 2014 • ACCEPTED DATE: 26 / 03 / 2015

REFERENCES

1. Little RR, Roberts WL. A Review of Variant Hemoglobins Interfering with Hemoglobin A1c Measurement. *J Diabetes Sci Technol* 2009; 3: 446-451.
2. Miedema K. Electrospray mass spectrometry for measurement of glycohaemoglobin. *Clin Chem* 1997; 43: 705-707.
3. Nasir NM, Thevarajah M, Yean CY. Hemoglobin variants detected by hemoglobin A1c (HbA1c) analysis and the effects on HbA1c measurements. *Int J Diabetes Dev Ctries* 2010; 30: 86-90.
4. Nagisa Y, Kato K, Watanabe K, et al. Changes in glycated haemoglobin levels in diabetic rats measured with an automatic affinity HPLC. *Clin Exp Pharmacol Physiol* 2003; 30: 752-758.
5. Lee ST, Weykamp CW, Lee YW, Kim JW, Ki CS. Effects of 7 hemoglobin variants on the measurement of glycohemoglobin by 14 analytical methods. *Clin Chem* 2007; 53: 2202-2205.
6. Betke K, Marti HR, Schlicht I. Estimation of small percentages of foetal haemoglobin. *Nature (Lond.)*, 1959; 184: 1877-1878.
7. Schnedl WJ, Reisinger EC, Lipp RW, Krejs GJ, Hopmeier P. Hemoglobin variants recently detected in Austria. *Ann Hematol* 1995; 71: 185-187.
8. Rohlfing CL, Connolly SM, England JD, et al. The effect of elevated fetal hemoglobin on hemoglobin A1c results: five common hemoglobin A1c methods compared with the IFCC reference method. *Am J Clin Pathol* 2008; 129: 811-814.
9. Huisman THJ, Altay C. The chemical heterogeneity of the fetal hemoglobin of black newborn babies and adults: a reevaluation. *Blood* 1981; 58: 491-495.
10. Rajantie J, Weber T, Stenman UH, Silvast A, Mäenpää J. Elevated HbA1 due to foetal haemoglobinaemia in children. A common artifact of certain ion-exchange chromatography methods. *Scand J Clin Lab Invest* 1992; 52: 317-320.
11. Bard H, Prosmanne J. Elevated levels of fetal hemoglobin synthesis in infants with bronchopulmonary dysplasia. *Pediatrics* 1990; 86: 193-196.