

A Rare Hemoglobin Variant Which Interfered Hemoglobin A1C Result: Hemoglobin South Florida [β 1(NA1)Val>Met, GTG>ATG; HBB: c.4G>A]

[Hemoglobin A1c Sonucunu Etkileyen Nadir Hemoglobin Varyantı: Hemoglobin South Florida (β 1(NA1)Val>Met, GTG>ATG; HBB: c.4G>A)]

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ABSTRACT

Hb South Florida is a rare hemoglobin beta chain variant that is not associated with any clinical disorder. We report a heterozygous Hb South Florida [β 1(NA1)Val>Met, GTG>ATG; HBB:c.4G>A] case determined during a premarital screening program. This hemoglobin variant can be identified with high performance liquid chromatography analysis confirmed with DNA sequencing. We emphasize in our study the importance of an interdisciplinary collaborative study at the provincial basis for the success of the hemoglobinopathy control program.

Key Words: Hb South Florida, hemoglobinopathy, abnormal hemoglobin

Conflict of Interest: The authors report no conflicts of interest.

ÖZET

Hb South Florida, herhangi bir klinik hastalık ile birlikte olmayan ender hemoglobin beta zincir varyantıdır. Biz evlilik öncesi tarama programı sırasında saptanan heterozigot Hb South Florida olgusunu rapor ediyoruz. Bu hemoglobin varyantı yüksek performans sıvı kromatografisi ile belirlendi ve DNA dizi analizi ile doğrulandı. Bu çalışma hemoglobino-pati kontrol programının başarısı için bölgesel düzeyde disiplinler arası işbirliğinin önemini vurgulamaktadır.

Anahtar Kelimeler: Hb South Florida, hemoglobino-pati, anormal hemoglobin

Çıkar Çatışması : Yazarlar arasında çıkar çatışması bulunmamaktadır.

Introduction

Hemoglobin (Hb) South Florida [β 1(NA1) Val>Met] is a rare beta hemoglobin variant which may interfere with the measurement of HbA_{1c}. So far only one case has been identified in Turkey [1].

A base substitution mutation as adenine (A) is replaced by guanine (G) results in a change at the second residue of β -globin chain Val to Met as a result of the retention of the initiator methionine on the mutant polypeptide. Hb South Florida makes up 40-45 % of the total hemoglobin and cannot be detected by Hb electrophoresis as acetylation of the N-terminal methionine residue occurs less easily than in the other amino acids. Also, the initiator methionine residue was preserved. If the initiator methionine is preserved, this residue is often acetylated. Approximately 20% of the protein was acetylated at the NH₂ terminus of the β chain [2]. The acetylated form of this hemoglobin co-eluted with hemoglobin A_{1c} (HbA_{1c}) by cation-exchange methods of hemoglobin separation. It has interfered with the assessment of diabetes in carriers of this variant. Hb South Florida does not cause any significant clinical problems and other hematologic abnormality in affected individuals [1-2].

We report here, for the second time in Turkey, clinical and laboratory data of a 24 years old woman with Hb South Florida.

Materials and Methods

Case Presentation

The propositus was detected during a premarital screening program. Verbal and written consent was obtained from the 24 years old woman prior to blood collection. Hematological data were obtained with automated cell counters (Advia 2120i, Siemens Healthcare Diagnostics, Monaco, Germania), while other routine biochemical

parameters were determined by standard methods (Dimension RxL Max, Siemens Healthcare Diagnostics). Ion exchange high performance liquid chromatography (HPLC) was performed with the Primus Ultra² system (Primus Corporation, Kansas City, USA) for the chromatographic identification of the hemoglobins according to the manufacturer's instructions. HbA_{1c} value was evaluated by the boronate affinity (Primus Diagnostics, a Trinity Biotech Company, Kansas City, USA) HPLC method.

DNA was isolated from whole blood samples in EDTA containing vacutainers, using a commercially available DNA extraction kit (RTA Lab, Ltd., Sti, Turkey). For characterization of the abnormal Hb by DNA sequencing, genomic DNA was isolated from leukocytes. Regions of the β -globin gene were sequenced bi-directionally using an ABI PRISM™ BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions, and the sequence reaction was analyzed using an ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems).

Results

Hematological and biochemical parameters of the propositus were within the normal range (Table 1). The propositus had no history of diabetes.

The HPLC profile showed a retention time of 3.86 min. for Hb A, 3.593 min. for an abnormal Hb, 1.067 min acetylated form of this hemoglobin and 0.628 min for Hb F (Figure 1). In cation exchange chromatography, the acetylated form of this hemoglobin coelutes with Hb A_{1c}. Hb A_{1c} value was 5 % within the non-diabetic reference range with the boronate affinity method (Figure 2).

DNA sequencing shows that the mutation (G>A) at the β -globin gene in codon 2 gives rise to the heterozygous Hb South Florida mutation (Figure 3).

Table 1. Hematological Data of the Hb South Florida Carriers

Parameters Subject	Patient	Women Reference Values
RBC (10¹²/L)	4.00	4.00 – 5.00
MCV(fL)	90	82 - 98
MCH (pg)	30	26 - 34
MCHC (g/dL)	34	32 - 36
Hb (g/dL)	12	12 - 16
Glucose (mg/dL)	72	70 - 100
Urea (mg/dL)	21	15 – 40
Creatinine (mg/dL)	0.57	0.57 - 1.11
Total bilirubin (mg/dL)	0.65	0.2 - 1.2
Direct bilirubin (mg/dL)	0.13	0 - 0.5

RBC (red blood cell), (MCV) mean corpuscular volume , (MCH) mean corpuscular hemoglobin, MCHC (mean corpuscular hemoglobin concentration), (Hb) hemoglobin

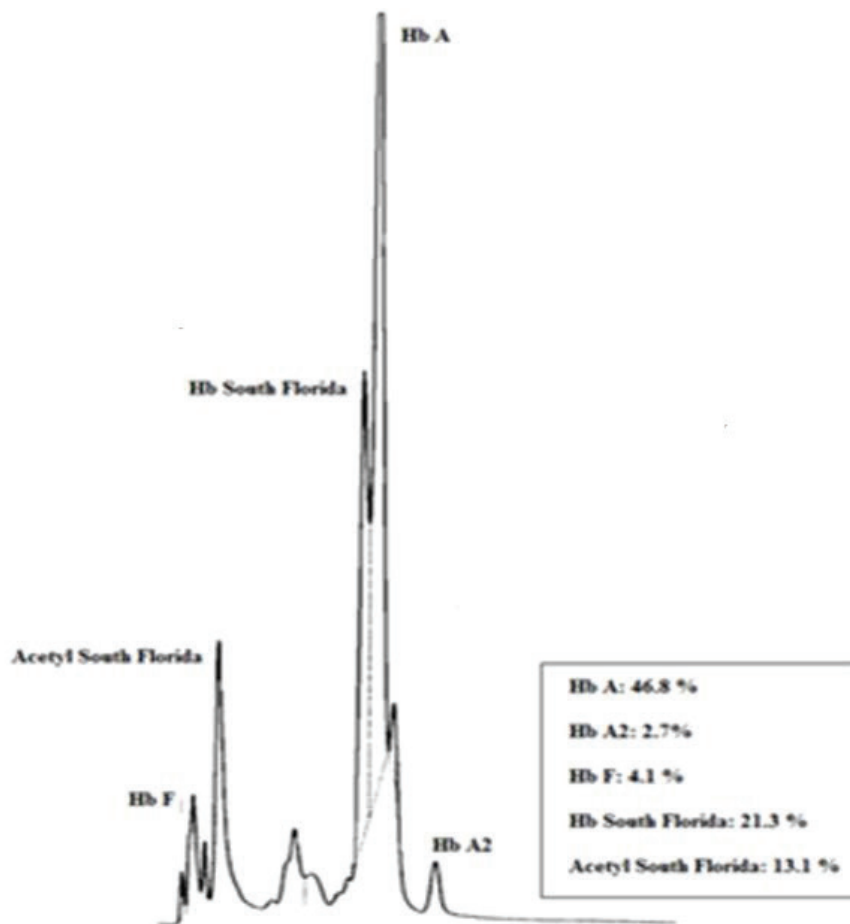


Figure 1. High performance liquid chromatography profile of Hb South Florida trait

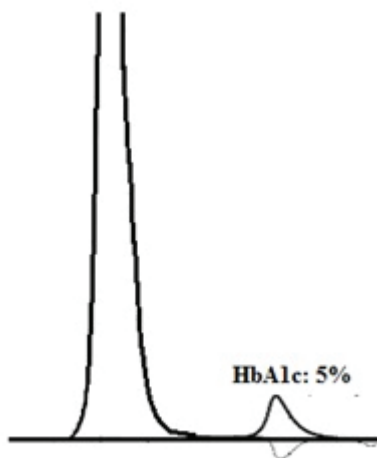


Figure 2. High performance liquid chromatography profile of HbA1c

Conclusion

Abnormal hemoglobins are the second most common hemoglobinopathies after β -thalassemia in Turkey. In this case, the Hb South Florida for the second time in Turkey was found during a premarital screening program. Therefore, the Ministry of Health initiated a control and prevention program in regions of Turkey where these anomalies are known to be prevalent. The Aegean region is one of the target areas for premarital screening. Rare, or new Hb variants, are regularly found during systematic programs of neonatal or premarital screening for the main hemoglobinopathies [3].

HbA1c is used routinely to monitor long-term glyce-mic control in people with diabetes mellitus, as HbA1c is related directly to risks for diabetic complications. HbA1c analysis methods are dependent on the molecular charge and structure. In ion exchange HPLC, Hb A_{1c} can be separated from Hb A because glycation of the N-terminal valine decreases the positive charge. Therefore, this method may be affected by posttrans-

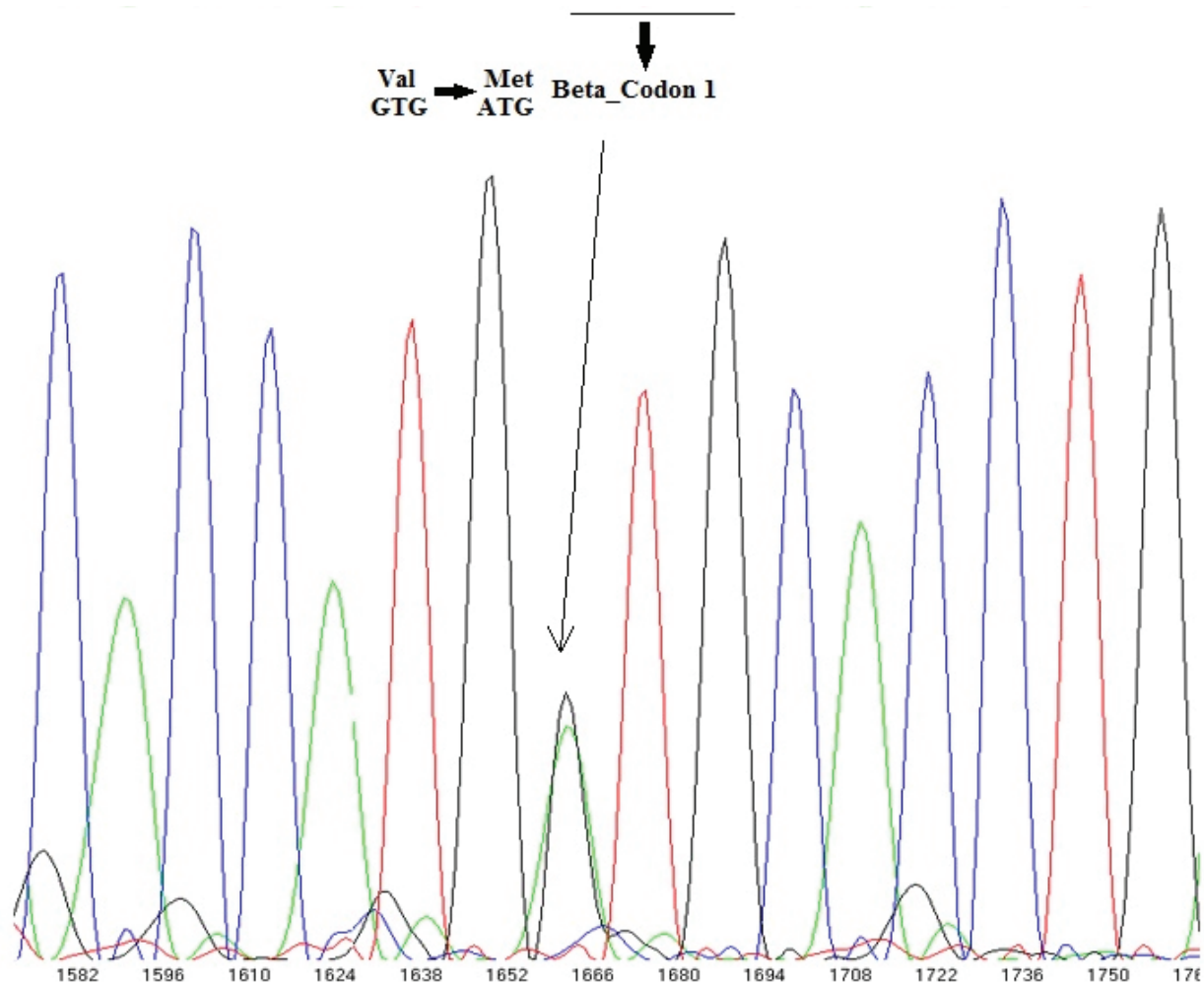


Figure 3. DNA sequencing of Hb South Florida mutation [β 1 (F1) Val>Met, GTG>ATG; HBB: c.4G>A]

lational modifications (e.g., carbamylation and acetylation) [4] or by Hb mutations [5] that alter the charge. In the boronate affinity chromatographic assay, boronic acid reacts with the cis diol groups created by glycation, thereby allowing glycohemoglobins such as Hb A_{1c} to be separated from Hb A [5]. On the other hand, Hb variants with excessive glycation, can interfere with boronate affinity chromatography [6]. Immunoassays use antibodies that target N-terminal glycosylated amino acids on the β chain to quantify Hb A_{1c}, and the Hb A_{1c} percentage is calculated from the Hb A_{1c} and Hb concentrations [5]. Thus, any factor that prevents glycation or any mutation in the epitope of the N-terminal amino acids that affects antibody recognition will produce erroneous results. Additionally, patients with increased Hb F (>10%) will have a falsely low Hb A_{1c} value by immunoassay because the γ chain shares only 4 of the first 10 amino acids with the β chain of Hb A and has little to no immunoreactivity with most antibodies used in Hb A_{1c} assays [5].

Previous studies examined the influence of hemoglo-

binopathies and Hb variants on the A1c test results [6]. In regions where populations have a high prevalence of variant hemoglobins, methods for the determination of HbA1c must be carefully selected to allow accurate determination of HbA1c in these individuals. While the prevalence of Hb South Florida appears to be very low, this report serves to remind laboratory workers and clinicians that caution is required when interpreting HbA1c results in individuals with hemoglobinopathies even when the hemoglobinopathy itself may be of little clinical significance. The management of patients with diabetes must be aware of the possibility of hemoglobin variants interfering in their patient's results particularly in those areas of the world where the prevalence of hemoglobin variants is high.

Acknowledgements

The authors are grateful to the staff of the Ismail Cosar (BiocanTip Laboratory, Istanbul, Turkey) and medical supplies (Primus Ultra2 system; Trinity Biotech, Kansas City, MO, USA) for their valuable contributions.

Conflict of Interest Statement: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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