대한진단검사의학회지 제29권 제6호 2009
Korean J Lab Med 2009;29:536-40
DOI 10.3343/kjlm.2009.29.6.536

Hemoglobin Yamagata: HbAlc 검사 중 검출된 혈색소 변이

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Hemoglobin Yamagata: Hemoglobin Variant Detected by HbA1c Test

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Hemoglobin (Hb) Yamagata is a rare Hb variant, which has been reported only twice-one case each in Japan and Korea. This variant arises from a Lys → Asn substitution due to a mutation of AAA to AAC or AAT at codon 133 of the beta-globin gene. This study reports the third case of a patient detected with Hb Yamagata [*HBB*: c.399A>T; p.Lys133Asn] and discusses the effect of this variant on HbA1c measurement. This variant was detected in a 70-yr-old Korean man with diabetes mellitus during a routine follow-up. The HbA1c concentration determined using Variant II Turbo (Bio-Rad, USA) was abnormally high at 47.9%. It was impossible to measure the HbA1c level accurately using Variant II Thalassemia Mode (Bio-Rad, USA). However, the HbA1c levels analyzed by HLC-723 G7 (Tosoh, Japan), Cobas Integra (Roche, Switzerland) and NycoCard (Axis-Shield, Norway) were 5.0%, 8.0%, and 7.9%, respectively. This study shows that Hb Yamagata interferes with the accurate measurement of HbA1c levels in a diabetic patient. Taking these findings into consideration, we think that an immunoassay or affinity chromatography can be used as an alternate method for measuring the HbA1c level in a patient with this variant. In conclusion, a patient can be inferred to have an Hb variant if the HbA1c concentration is abnormally high or low or if there is a discrepancy between the results obtained using different methods, and if the clinical status of the patient suggests the presence of abnormal Hb. Subsequently, the HbA1c values can be determined by methods based on different principles. *(Korean J Lab Med 2009;29:536-40)*

Key Words : *Hb Yamagata, Hb variant, HbA1c, HPLC*

INTRODUCTION

More than 900 hemoglobin (Hb) variants have been reported, and most variants arise from point mutations in genes encoding the α, β, γ, or δ chains. Most Hb variants produce no clinical manifestations, but they can interfere with HbA1c measurements [1, 2]. In the present study, an Hb variant was detected during an HbA1c test performed using the ion-exchange HPLC method. This variant was confirmed to be Hb Yamagata by DNA sequence analysis. Hb Yamagata is a rare Hb variant and has been reported once each in Japan and Korea. It arises from Lys → Asn

Received : June 26, 2009 Manuscript No : KJLM09-080
Revision received : August 21, 2009
Accepted : October 30, 2009
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substitution due to a mutation of AAA to AAC or AAT at codon 133 of the beta-globin gene [3, 4]. This study reports the third case of a patient with Hb Yamagata and discusses the effect of this variant on HbA1c measurement.

CASE REPORT

An Hb variant was detected in a 70-yr-old Korean man with diabetes mellitus (DM) during a routine follow-up. He was diagnosed with DM in 1995 and had undergone periodic examinations at our hospital since 1999. In September 1999, the HbA1c value measured using Variant (Bio-Rad Laboratories Inc., Hercules, CA, USA) was 41.6%, which was extremely high. In March 2002, the HbA1c value measured using Variant ll (Bio-Rad Laboratories Inc., Hercules, CA, USA) was 42.1%, while that measured using IMx (Abbott, Chicago, IL, USA) was 6.7%. Since then, the HbA1c values have remained constant at 7-8%. In July 2008, the HbA1c level measured using Variant ll Turbo (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was abnormally high at 47.9% (Fig. 1A). It was impossible to measure HbA1c levels accurately using Variant II Thalassemia Mode (Bio-Rad Laboratories, Inc., Hercules, CA, USA) because of the interference between the variant and A1c peaks (Fig. 1B). However, the HbA1c levels analyzed using HLC-723 G7 (Tosoh Corporation, Tokyo, Japan), Cobas Integra 800 (Roche Diagnostics, Basel, Switzerland), and NycoCard (Axis-Shield, Oslo, Norway) were 5.0%, 8.0%, and 7.9%, respectively (Fig. 1C). These results are summarized in Table 1. At the time of this examination, the patient's fasting blood sugar was 177 mg/dL, which was in the diabetic range. His peripheral blood indices showed an Hb level of 12.9 g/dL, hematocrit (Hct) value of 37.2%, mean corpuscular volume (MCV) of 87.3 fL, mean corpuscular hemoglobin (MCH) of 30.3 pg, and red blood cell distribution width (RDW) of 12.7%. The peripheral blood smear showed normocytic and normochromic red blood cells. The levels of AST, ALT, blood urea nitrogen, and creatinine were 25 IU/mL, 31 IU/mL, 23 mg/dL, and 1.3 mg/dL, respectively. The DNA sequence analysis revealed the presence of Hb Yamagata (*HBB* c.399A>T; p.Lys133Asn), in which the Lys → Asn substitution was due to a mutation of AAA to AAT at codon 133 of the beta-globin gene (Fig. 2). The fractions of Hb Yamagata, HbA, and HbA2 obtained from Capillary EP (Sebia, France) were 40.9%,

Table 1. The HbA1c results of several methods based on different principles

Instruments	Principle of the method	HbA1c (%)*
Variant II turbo (Bio-Rad, USA)	Ion-exchange HPLC	Not calculated (47.9)
Variant II thalassemia mode (Bio-Rad, USA)	Ion-exchange HPLC -long elution time	Not calculated
HLC-723 G7 (Tosoh Co., Japan)	Ion-exchange HPLC	5.0
Cobas Integra (Roche, Switzerland)	Immunoassay	8.0
NycoCard (Axis-Shield, Norway)	Affinity chromatography	7.9

*These results were obtained in July 2008.

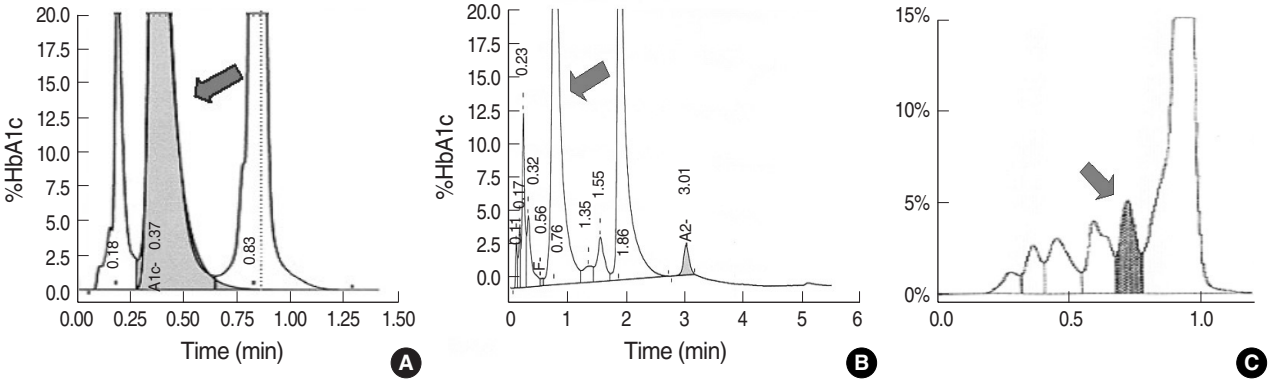

Fig. 1. Chromatograms for HbA1c level of the patient with Hb Yamagata obtained using (A) Variant ll Turbo (Bio-Rad, USA), (B) Variant ll Thalassemia mode (Bio-Rad, USA), and (C) HLC-723 G7 (Tosoh Corporation, Japan). The arrows indicate the retention time of Hb Yamagata.

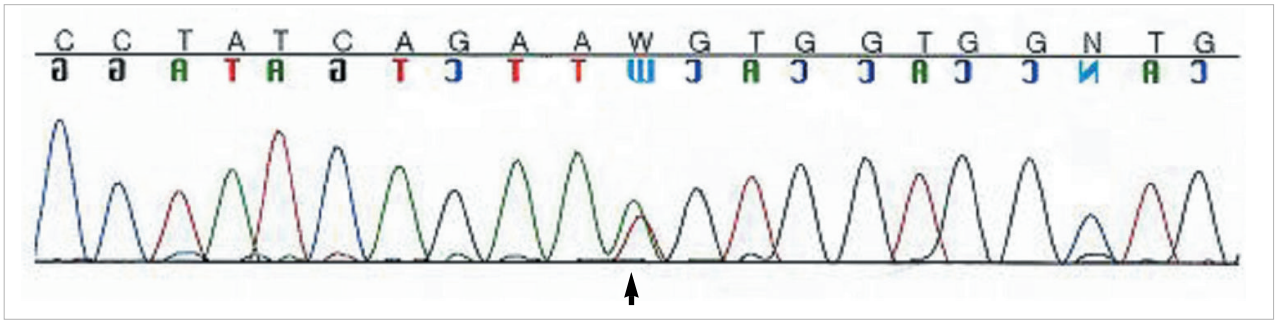

Fig. 2. Globin gene analysis: Hb Yamagata (*HBB* c.399A>T; p.Lys133Asn) (arrow). The DNA sequence analysis showed the presence of Hb Yamagata, in which the Lys → Asn substitution was due to a mutation of AAA to AAT at codon 133 of the beta-globin gene.

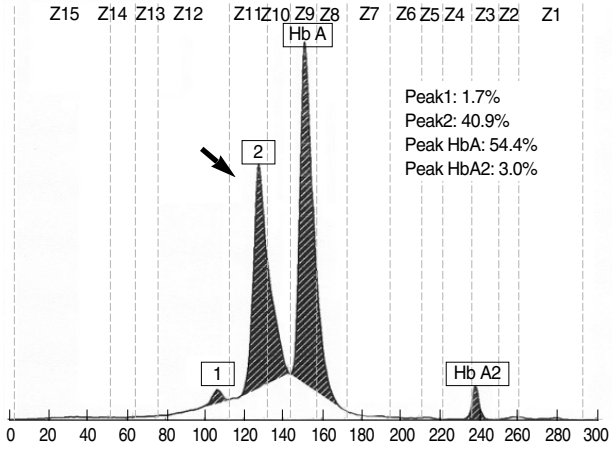

Fig. 3. Electrophoregrams of Hb Yamagata as obtained by the Sebia capillary EP system. The arrow indicates Hb Yamagata. The fractions of Hb Yamagata, HbA, and HbA2 were 40.9%, 54.4%, and 3.0%, respectively.

54.4%, and 3.0%, respectively (Fig. 3).

DISCUSSION

Since determination of HbA1c reflects glycemic control in the past 2–3 months most precisely, this is an established procedure for the diagnosis and treatment of diabetic patients. It is important to measure the HbA1c level accurately because it is an indicator of treatment results and helps in establishing the therapeutic strategy. However, an appropriate method should be used to determine the HbA1c value in the presence of Hb variants because the Hb variants can cause interference with certain methods. Currently, more than 30 methods are available for the estimation of HbA1c [1, 5, 6].

In Korea, the incidence of Hb variants has been estimated to be 1 in 2,700 on the basis of a survey that identified 10 patients with Hb variants among 27,000 patients tested for HbA1c at a tertiary hospital [7]. The most common variants were Hb G Coushatta (β22Glu → Ala; 5 patients) and Hb Queens (α134Leu → Arg; 4 patients). In addition to these, Hb Hoshida (β43Glu → Gln) was detected in 1 patient.

We found an extremely high level of HbA1c by using the ion-exchange HPLC method and confirmed that the patient had Hb Yamagata (*HBB* c.399A>T; p.Lys133Asn) by performing DNA analysis. Hb Yamagata is a rare Hb variant which has been reported in only 2 patients–one in Japan and the other in Korea. Our case is the second case in Korea and the third case worldwide. In both earlier cases, the patients were heterozygous carriers of this rare mutation, there were no clinical manifestations, and the oxygen affinity of hemoglobin was slightly decreased. Our patient was also a heterozygous carrier but had no specific abnormalities, as in the above-mentioned cases. Since the amino acid residue at the 132nd position of the β chain is located at an external site and near the central cavity of the Hb molecule, thus, its substitution may not result in any abnormalities in the Hb function [3].

The HbA1c levels in this patient were also measured by immunoassay and affinity chromatography to evaluate the effect of Hb Yamagata on the HbA1c level. The HbA1c levels measured by Cobas integra 800 and NycoCard assays were 8.0% and 7.9%, respectively. The HbA1c level was found to be as low as 5.0% when measured using HLC-723 G7 although the ion-exchange method was the same as

that used with Variant ll Turbo. In patients with Hb Yamagata, the HbA1c levels measured using the ion-exchange HPLC methods were erroneously high. The HbA1c levels could not be detemined accurately using the Variant ll Thalassemia Mode because Hb Yamagata had a retention time similar to that of HbA1c; the HbA1c levels measured using Tosoh G7 were relatively low. The estimated average glucose (eAG) levels derived from the HbA1c levels determined using Cobas Integra 800, Nycocard, and HLC-723 G7 were 183 mg/dL, 180 mg/dL, and 97 mg/dL, respectively [8]. Hence, the results of HbA1c by immunoassay and affinity chromatography are considered to be accurate since the patient's fasting blood glucose level was 177 mg/dL at the time of this examination.

Hb variants have been known to influence HbA1c measurements by the ion-exchange HPLC assay. Many studies have discussed various situations, including false high or low values of HbA1c because of interference from Hb variant [1, 9, 10]. For example, Hb G-Coushatta levels were underestimated in the analysis using Variant ll Turbo and Tosoh G7 and the Hb Queens levels were underestimated in the analysis using Tosoh G7 [6]. In our present study, the HbA1c levels differed when measured using different analyzers even though the same HPLC methods were used. Detection of HbA1c by ion-exchange HPLC may be influenced by Hb variants; however, this method has an advantage in that it allows the detection of Hb variants and, in some cases, enables the identification of the Hb variant types from the characteristic retention patterns. In contrast, the methods using immunoassays and affinity chromatography are thought to help measure the HbA1c levels accurately but do not allow the identification of Hb variants [5, 11]. Therefore, if HPLC is used for determining HbA1c, the abnormal peaks produced by Hb variants can be detected in the chromatograms.

Hb predominantly consists of HbA (~95%), HbF (<2%), and HbA2 (<3.5%) in healthy individuals. Analysis of Hb by the Sebia capillary electrophoresis (EP) system showed that Hb Yamagata migrated faster than HbA. Several studies have revealed that Hb Yamagata migrates towards the anode faster than HbA in isoelectric focusing (IEF) and cellulose acetate electrophoresis. This may be because the amino acid substitution resulted in decreased positive charge in the central cavity of the Hb molecule [3, 4].

HbA1c is important for the evaluation and management of patients with DM and therefore should be estimated precisely. Hb variants can be suspected when HbA1c value is abnormally high (usually >15%) or below the nondiabetic reference interval or if there is a discrepancy between the results and the clinical status. In cases where the HbA1c value measured using different methods differs significantly, the Hb variant must be detected. In such cases, factors that can be responsible for the production of Hb variants should be identified by evaluating several conditions, including clinical status and history. Additional peaks of Hb variants can be identified in the ion-exchange chromatograms. The samples of patients with suspected Hb variants should be analyzed by methods based on different principles. Furthermore, these samples can be measured by alternative tests such as a fructosamine test or calculation of mean blood glucose levels [1, 5, 10, 11].

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