Hb Ube-2 in a Diabetic Case with an Abnormally Low HbA_{1c} Value

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A 69-year-old male diabetic patient had an abnormally low HbA_{1c} value of 2.8%, which was inconsistent with his elevated fasting plasma glucose of 8.2 mmol/l. Hb analysis disclosed that the abnormal Hb was Hb Ube-2 [α 68 (E17) Asn $\rightarrow$ Asp] and it accounted for 21.5% of the total Hb. Since the glycated abnormal Hb emerged at the same position as did HbF on high performance liquid chromatography, the HbA_{1c} value was falsely low. The present case demonstrates that Hb Ube-2 is one of the abnormal Hbs in which caution should be exercised when monitoring diabetic control.

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Key words: diabetes monitoring, high performance liquid chromatography (HPLC), plasma glucose

Introduction

The means of analysis of three important parameters, *i.e.*, plasma glucose, HbA_{1c} and fructosamine (glycoalbumin), is now available for monitoring the control of plasma glucose in diabetic patients. Out of these 3 parameters, HbA_{1c} is notably advantageous for evaluating the plasma glucose level of the previous month, hence HbA_{1c} is indispensable for the management of diabetes, particularly in outpatient clinics. With the increased frequency of HbA_{1c} tests performed due to the increasing number of diabetic patients in Japan, cases with abnormal Hb have accumulated recently (1–6).

Here, we report a case with Hb Ube-2 (7–11) found in a diabetic patient. Hb Ube-2 has not been extensively described because of its minimal effect on hematological functions (7, 9). It has not been previously reported in relation to abnormal HbA_{1c} level in diabetic patients. In the present report, we demonstrate a patient with Hb Ube-2 who shows an abnormally low HbA_{1c} value compared to the corresponding fasting plasma glucose levels. Moreover, we report the geographic distribution of 72 cases of Hb Ube-2 registered in Japan. We also summarize the cases of abnormal Hb found by the discrepancy between the values of plasma glucose and HbA_{1c} in Japan.

Case Report

A 69-year-old male patient was diagnosed as having impaired glucose tolerance when he was 60-year-old, but he did not pay much attention to the diagnosis. Upon referring to an

orthopedist for the treatment of neck pain, high plasma glucose (12.7 mmol/l) was pointed out and he was referred to Abe Diabetes Clinic for further examination. There was no remarkable family history. He had been drinking 350 ml/day of beer, and smoked 10 cigarettes daily. He had no past history of liver damage or hemolytic anemia. His height was 169.8 cm, body weight 77.6 kg, body temperature 35.7°C, pulse rate 68/min, respiratory rate 20/min, and blood pressure 141 mmHg systolic and 83 mmHg diastolic. His consciousness was clear with no signs of conjunctival anemia or jaundice. No abnormal sign was found on X-ray examination of the chest or the abdomen. Electrocardiogram revealed no abnormal findings.

The laboratory examination revealed a normal hemogram. Reticulocyte count and serum chemistry including haptoglobin, lactate dehydrogenase and iron were all within normal limits. Instability test with hemolysates by isopropanol method was negative. The fasting plasma glucose was elevated (6.7 mmol/l), but the serum chemistry was otherwise normal. HbA₁ (3.8%) and HbA_{1c} (2.9%) values were extremely low by high performance liquid chromatography (HPLC). A HbF value was below the level of assay sensitivity (<1%) by the alkali denaturation method. Stable HbA_{1c} value (5.3%) assayed by latex agglutination nephelometric immunoassay was within the normal range (4.3–5.8%). Glycoalbumin value (17.0%) was elevated (normal range: 11.1–15.3%). 75g oral glucose tolerance test (OGTT) showed a diabetic pattern by WHO criteria; 0 minute, 6.7 mmol/l; 30 minutes, 12.4 mmol/l; 60 minutes, 17.3 mmol/l; 90 minutes, 17.8 mmol/l; 120 minutes, 14.2 mmol/l.

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Biochemical Analysis of Abnormal Hb

Informed consent was obtained from the patient for the further analysis of abnormal Hb. Chromatographic pattern of Hb by HLC-723GHbII (TOSOH, Tokyo) showed the presence of an abnormal shoulder before the HbA peak (Fig. 1). Since the patient's HbF was below the assay sensitivity when analyzed by both the alkali denaturation method and isoelectrofocusing (IEF), the peak at the HbF position was assumed to be glycated HbX_{1c} (arrow in Fig. 1). Analysis of the abnormal Hb by IEF revealed the presence of fast-moving abnormal Hb observed in a slightly more anodal position of the HbA band (Fig. 2).

Figure 1. Elution pattern of hemoglobin in the present patient on HPLC (HLC-723GHbII). The abnormal Hb is shown in the figure as a notched peak at the front shoulder of a HbA peak. Since the patient's HbF is below the assay sensitivity when analyzed by both the alkali denaturation and IEF methods, a peak at the HbF position (arrow) is assumed to be glycated HbX_{1c}. The amount of HbA_{1c} peak (shaded area) is 2.8%.

Quantitative analysis of the abnormal Hb by DEAE-HPLC showed the abnormal peaks of HbX and HbX₂, the contents of which were 21.5% and 0.8%, respectively (Fig. 3). The appearance of both of these abnormal peaks strongly indicated that the abnormality of Hb was located in the α -chain. Globin obtained from whole hemolysate was analyzed by chromatography on a carboxymethyl cellulose column. The collected abnormal globin fraction was subjected to further analyses. Tryptic digest of the abnormal α -chain disclosed an almost normal pattern by reversed-phase HPLC analysis except that α T-8-9 (α 61- α 90) and α T-9 (α 62- α 90) peptides migrated to slightly later positions. Therefore, these peptides were subjected to amino acid sequencing, which revealed the substitution of asparagine by aspartic acid at amino acid position 68 of α -chain. Finally, the abnormal Hb was identified as Hb Ube-2 [α 68(E17)Asn $\rightarrow$ Asp].

Analysis of α -globin Gene

Genomic DNA was extracted from white blood cells of the peripheral blood by the standard method. DNA was amplified by polymerase chain reaction by use of the specific primer sets for the α 1- and α 2-genes, and subcloned into an M13mp19 vector. Single stranded DNAs (ssDNAs) were sequenced by the dideoxy method using a DNA Sequencing Kit [Sequenase Version 2.0; United States Biochemicals (USB), Cleveland, OH, USA]. The nucleotide sequences of the ssDNA demonstrated that the α 68 codon of the α 1-globin gene, which was distinguishable from a different sequence of the second intervening sequence (IVS-II), was heterozygous for the normal AAC (Asn) and abnormal GAC (Asp) base sequences (Fig. 4).

Discussion

Although Hb Ube-2 was first observed in a Japanese woman

Figure 2. Isoelectrofocusing of the patient's hemolysate. Note that fast-moving abnormal Hb (HbX) is observed in an anodal position of the HbA band. HbA_{1c} and HbX_{1c} are observed in a slightly anodal position to the HbA and HbX bands, respectively.

Figure 3. Elution pattern of the patient's hemolysate on DEAE-HPLC. The abnormal peaks of HbX and HbX₂, and their content (%) are indicated in the figure.

Figure 4. DNA sequencing of the mixed ssDNA from the α 1-globin gene. The α 68 codon of the α 1-globin gene was heterozygous for the normal AAC (Asn) and abnormal GAC (Asp) base sequences.

Figure 5. Geographic distribution of Hb Ube-2 in Japan. Names given are the prefecture names where Hb Ube-2 is registered. Numbers in the circles indicate the numbers of registered cases within the prefecture.

in 1960, an amino acid substitution of the Hb was identified as $\alpha_2^{68\text{Asp}}\beta_2$ in 1967 (8). From the first discovery until January 1998, 72 cases including the present case have been registered in Japan and 2 cases in Turkey (11, 12). The geographic distribution of this abnormal Hb in Japan showed a higher prevalence in the western part than that in the eastern part of Japan (Fig. 5). One possible reason for this may be due to extensive surveillance of the abnormal Hb in the west. It is worthwhile to discuss why the same mutation was found among the Turkish (10). Evidence may indicate a mutational hot spot at this nucleotide position. Another possibility may be some relationship between these two races from the view of population genetics. It would be valuable to know whether the 2 Turkish cases have a Japanese individual in their ancestral history. Irrespective of the nation-wide distribution of Hb Ube-2 in Japan,

few cases have been reported in the literature (7–9), probably because of the minimal effect of this abnormal Hb on hematological functions. Indeed, an instability test of the Hb was negative and no hematological symptoms were subjectively registered in the present case. Although the delicate difference in the shape of O₂-dissociation curve between Hb Ube-2 and normal adult Hb has been reported (7), the amino acid position 68 is far from the distal histidine at position 58 which is directory related to the function of heme.

Of the 500 variants of HbA reported in the world, about 180

Table 1. Abnormal Hb Found by the Discrepancy of Plasma Glucose and HbA_{1c} Values

Abnormal Hb	a. a.* ¹ substitution	FPG* ²	HbA ₁ (%)	HbA _{1c} (%)	Registered No.* ³	References
Hb Oita	α45 His → Pro	5.2	3.4	2.5	1	(13)
Hb Ube-2	α68 Asn → Asp	8.2	3.8	2.9	72	(7–9)
Hb J-Meerut	α120 Ala → Glu	9.5	4.7	3.7	3	(6)
Hb I-High Wycombe	β59 Lys → Glu	8.7	3.7	3.1	1	* ⁴
Hb Tsukumi	β117 His → Tyr	6.1	6.4	5.6	1	* ⁵
Hb Takamatsu	β120 Lys → Gln	9.0	9.2	3.6	62	(2)
Hb Riyadh	β120 Lys → Asn	6.7	11.3	3.1	109	(5)
Hb Camden	β131 Gln → Glu	19.0	22.4	7.6	9	(4)
Hb Himeji	β140 Ala → Asp	10.3	24.9	5.1	3	(1, 3)

Not only the diabetic cases but also the non-diabetic cases are included in the table. Values of FPG, HbA₁ and HbA_{1c} from the representative case in each abnormal Hb are listed. *¹: amino acid; *²: fasting plasma glucose (mmol/l); *³: number of registered cases in Japan; *⁴: Hamaguchi K, et al (manuscript in preparation); *⁵: Oribe Y, et al (manuscript in preparation).

have been found in Japan (12). In accordance with an increase in the prevalence of diabetes in Japan, measurement of HbA_{1c} by HPLC has become more popular not only in public hospitals but also in many private clinics where diabetes mellitus is a major disease to be treated. In addition, measurement of HbA_{1c} as well as plasma glucose has been used as a marker for the diagnosis of diabetes mellitus in mass screening. Consequently, cases with abnormal Hb have been found more frequently recently. The abnormal Hbs found in Japan due to a discrepancy in the values between plasma glucose and HbA_{1c} are listed in Table 1 (1–9, 13), together with the number of registered cases at Kawasaki Medical School, Kurashiki, Japan.

The precise analysis of Hb in patients who showed abnormal HbA_{1c} values and/or an abnormal elution pattern by HPLC is essential to avoid misunderstanding of the value as a marker for the control of diabetes. In this context, the turbidimetric inhibition immunoassay (14) or the latex agglutination nephelometric immunoassay (15) is valuable for obtaining the precise measurements of HbA_{1c} when the site of amino acids recognized by the antibodies is different from the one where the mutation exists (16–18). Since the HbA_{1c}-specific antibodies are developed with the glycated synthetic peptides (Fruc-Val-His-Leu) located at the N-terminal portion of the β-chain, the amino acid substitution of Hb Ube-2 does not interfere with these assays.

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