A Diabetic Case with Hemoglobin J-Meerut and Low HbA_{1C} Levels

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A diabetic patient with hemoglobin (Hb) J-Meerut and low HbA_{1C} levels is reported. An automatic glycohemoglobin analyzer used for the determination of HbA_{1C} revealed an abnormal peak of the peripheral blood obtained from a Japanese female with diabetes. She showed a lower HbA_{1C} level (3.7%) than expected from her fasting plasma glucose (172 mg/dl). High performance liquid chromatography and isoelectric focusing indicated that her abnormal hemoglobin was Hb J-Meerut [α 120(H3)Ala→Glu] and it accounted for 28.3% of the total hemoglobin. Abnormal hemoglobinemia should be considered when a major discrepancy between the levels of HbA_{1C} and fasting plasma glucose is observed.

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Introduction

Abnormal hemoglobin (Hb) which produces no hematological symptoms is rarely detected. However, diabetes mellitus provides an opportunity for the discovery of abnormal Hb because the determination of glycated hemoglobin (HbA_{1C}) is a good index of the blood glucose level over the previous few months (1–4). We report a diabetic patient with Hb J-Meerut (5–9), who showed a low level of HbA_{1C} in spite of a high concurrent level of plasma glucose.

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The purpose of this report is to describe this patient with abnormal Hb and to draw attention to the presence of abnormal Hb when a diabetic patient shows a discrepancy between levels of HbA_{1C} and plasma glucose.

Case Report

A 48-year-old female who was living in Kanagawa Prefecture was admitted to Tokai University Hospital because of a brain abscess in the left lateral lobe caused by α hemolytic streptococcus on October 13, 1994. She had been taking an oral

hypoglycemic drug from a local physician for non-insulin-dependent diabetes mellitus (NIDDM) since 1984, and was referred to us for evaluation and control of diabetes mellitus. She never complained of thirst, polyuria, blurred vision or numbness in the extremities. At the age of 20, she had an operation for pulmonary valve stenosis. Her father and paternal grandfather had diabetes mellitus, but there have been no diabetics on her mother's side. No consanguineous marriage was noted. Her height was 160 cm, body weight 50.0 kg, blood pressure 110/70 mmHg and pulse rate 64/min. On physical examination, she showed no particular findings except right hemiparesis with facial nerve palsy and dysarthria.

Laboratory findings

Laboratory findings on admission are shown in Table 1. A slightly elevated white blood cell count (9,200/ μ l; segment: 86.5%) and a slightly elevated C-reactive protein (0.31 mg/dl) value were noted. Fasting plasma glucose (FPG) was 180 mg/dl, HbA_{1C} 12.4%, total cholesterol 247 mg/dl and triglyceride 91 mg/dl. Urinalysis showed 3(+) glucose (159.3 g/day) and negative proteinuria. Urinary C-peptide level was 46.5 μ g/day. Based on these findings, she was diagnosed as having NIDDM.

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Table 1. Laboratory Data on Admission

Complete blood count		Serum test	
White blood cell	9,200/ μ l	IgG	944 mg/dl
	(Segment: 86.5%)	IgA	206 mg/dl
Red blood cell	423×10^4 / μ l	IgM	99 mg/dl
Hemoglobin	14.2 g/dl	C reactive protein	0.31 mg/dl
Hematocrit	39.6%		
Platelet	25.4×10^4 / μ l	Hemoglobin A _{1C}	12.4%
Reticulocyte	33%		
Blood chemistry		Urinalysis	
Fasting plasma glucose	180 mg/dl	Glucose	(+++)
Creatinine	0.6 mg/dl	Protein	(-)
Blood urea nitrogen	17 mg/dl		0.11 g/day
Uric acid	3.4 mg/dl	Occult blood	(-)
Total protein	6.7 g/dl	Urinary C-peptide	46.5 μ g/day
Albumin	3.9 g/dl	Urinary albumin	38.5 μ g/min
Aspartate aminotransferase	6 U/l		
Aranine aminotransferase	5 U/l	Glomerular filtration rate	97 ml/min
Total bilirubin	0.6 mg/dl		
Low density lipoprotein	227 U/l		
γ -glutamyl transpeptidase	12 U/l		
Alkaline phosphatase	168 U/l		
Na	134 mEq/l		
K	4.7 mEq/l		
Cl	93 mEq/l		
Total cholesterol	247 mg/dl		
Triglyceride	91 mg/dl

Figure 1. Clinical course of the patient from December 1994 to November 1995. "Glucose" means fasting plasma glucose and "1,5AG" indicates plasma 1,5 anhydroglucitol. The method for measurement of HbA_{1c} was changed from June 1, 1995 (arrow).

Clinical course

She had burr hole opening and irrigation of the brain abscess on October 26, 1994. After discharge on December 15, 1994, she was treated by calorie restriction (1,440 kcal/day) and an oral hypoglycemic drug (glibenclamide, 5 mg/day) for treatment of diabetes mellitus. The level of HbA_{1c} was 8.2 to 9.4% and that of FPG from 200 to 300 mg/dl in the outpatient clinic (Fig. 1). However, the levels of HbA_{1c} suddenly fell to 3.7% on June 20, 1995, with relatively improved glycemic control (FPG: 172 mg/dl), high level of fructosamine (365 μ mol/l, normal range: 205–285 μ mol/l) and a low level of plasma 1,5 anhydroglucitol (2.5 μ g/ml, normal range: 12–29 μ g/ml).

Measurement of HbA_{1c}

HbA_{1c} was measured by high performance liquid chromatography (HPLC) using HLC-723GHbIII (Tosoh, Tokyo) in our hospital until May 31, 1995. Samples were incubated with hemolytic solution C (pH 7.4) at 45°C for 3.5 minutes before analysis. Hemolytic solution C did not eliminate unstable HbA_{1c} from these samples. This method included unstable Hb fraction separation, so that the normal range of HbA_{1c} by this method was relatively high at 5.2 to 6.7%. For a precise analysis, we performed the HPLC for 5 minutes. The elution

pattern of hemoglobin of a control patient is shown in Fig. 2. The level of stable HbA_{1c} (peak A1) was 4.6% and that of peak A2 which was composed of unstable HbA_{1c} and other unknown substances was 2.2%.

The elution pattern of hemoglobin of this patient is shown in Fig. 3. The level of stable HbA_{1c} (peak A1) was 6.4%, and there were two peaks in the A2 position in Fig. 2. The first such peak (peak A2) was recognized as the unstable HbA_{1c} and other unknown substances because this peak was decreased from 2.0% to 1.2% in Fig. 4 (see below). The second peak was also recognized to be abnormal hemoglobin.

According to the recommendation of the Japan Diabetes Society, the measurement of HbA_{1c} was changed to HPLC using HLC-723GHbIII (Tosoh) from June 1, 1995. Samples were incubated with hemolytic solution E (pH 5.8) at 50°C for 1.5 minutes before analysis. Hemolytic solution E eliminated the unstable HbA_{1c} from the samples. The new method excluded unstable Hb fraction separation, and the normal range of HbA_{1c} was 4.3–5.8%. The elution pattern of hemoglobin of this patient using solution E is shown in Fig. 4. Peak J was not changed even after using solution E (from 2.5% to 2.6%).

Figure 2. Elution pattern of hemoglobin of a control patient on HPLC (HLC-723GHbIII) using hemolytic solution C for 5 minutes. The level of stable HbA_{1c} (peak A1) was estimated to be 4.6%. Peak A2 included unstable HbA_{1c} and was 2.2%.

Name	%	Time	Area
FP	0.0	0.3	20.70
A1A	0.5	0.6	33.23
A1B	0.6	0.7	38.31
P00	0.5	1.0	31.37
P01	0.4	1.4	26.65
F	1.3	1.7	78.38
LA1C	2.0	1.9	126.42
J	2.5	2.1	154.22
S-A1C	6.4	2.5	395.19
A0	85.7	3.3	5,303.06

Figure 3. Elution pattern of hemoglobin of the present patient on HPLC (HLC-723GHbIII) using hemolytic solution C for 5 minutes. The amount of stable HbA_{1C} (peak A1) was estimated to be 6.4%. Peak A2 included unstable HbA_{1C} and was 2.0%. The abnormal Hb (Hb J-Meerut) was found as peak J and it was estimated to be 2.5%. The blood sample was obtained in August, 1996.

Analysis of abnormal Hb

Quantitative analyses of abnormal Hb by isoelectric focusing (IEF), HPLC and the isopropanol precipitation test were performed at the Department of Biochemistry, Kawasaki Medical School. The results showed that the abnormal Hb made up 28.3% of the total Hb. Structural analysis of the abnormal Hb was carried out as described previously (10), and revealed that it was an α chain variant, namely Hb J-Meerut (or Hb J-Birmingham) [α 120(H3)Ala→Glu] (5–9). The results of an isopropanol precipitation test were negative, and this abnormal Hb did not show any instability or dysfunction.

Discussion

This study reported a diabetic patient with Hb J-Meerut (or Hb J-Birmingham) and a low HbA_{1C} value. This variant of hemoglobin has been described previously in two sisters from Meerut, India (5), in two brothers from Bangladesh living in Birmingham, England (6), in two unrelated Japanese families

(7, 8) and in one family from Ankara, Turkey (9).

Approximately 500 kinds of abnormal Hb have been reported all over the world (11). One-third of these patients showed some hematological symptoms, such as cyanosis or polycythemia, but the remaining two-thirds showed no hematological symptoms. In the latter group, the abnormal Hb revealed a deviation in electric charge caused by the replacement of amino acids, and thus it was discovered when determination of HbA_{1C} by HPLC was performed in diabetic patients. Several diabetic patients who showed lower levels of HbA_{1C} than expected from the level of fasting plasma glucose were reported (12–19) (Table 2). In contrast, some diabetic patients showing higher levels of HbA_{1C} than expected from the level of fasting plasma glucose were also reported (20–25).

In the present case, it appeared that the HbA_{1C} value was higher than expected from her diabetic condition before May 31, 1995. The levels of HbA_{1C} suddenly decreased by about 5% on June 20, 1995. The level of unstable HbA_{1C} was about 0.8% (11% of total HbA_{1C}) and that of abnormal Hb (Hb J-Meerut)

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Name	%	Time	Area
FP	0.0	0.4	26.68
A1A	1.3	0.6	70.95
A1B	0.4	0.9	22.15
P00	0.4	1.3	22.08
F	0.8	1.7	45.41
LA1C	1.2	1.9	66.76
J	2.6	2.1	141.78
S-A1C	6.3	2.5	347.67
A0	87.1	3.3	4,823.65

Figure 4. Elution pattern of hemoglobin of this patient on HPLC (HLC-723GHbIII) using hemolytic solution E after excluding unstable HbA_{1C} for 5 minutes. The level of stable HbA_{1C} (peak A1) was estimated to be 6.3% and peak A2 was 1.2%. The abnormal Hb (Hb J-Meerut) was found as peak J and was estimated to be 2.6%. The blood sample was obtained in August, 1996.

Table 2. Cases of Abnormal Hb with Lower HbA_{1C} Level

Abnormal Hb	Amino acid abnormality	Age	Sex	Diag.	FBS (mg/dl)	HbA ₁ (%)	HbA _{1C} (%)	1,5-AG (μg/ml)	FRA (μmol/l)	Ref. no.
Hb Himeji	β140 Ala→Asp	52	M	NIDDM	186	24.9	5.1			12
		31	F		106		2.0			13
Hb Takamatsu	β120 Lys→Gln	70	M	NIDDM	162	9.2	3.6			14
Hb Camden	β131 Gln→Glu	34	M	DM	379	17				15
		75	F	DM	343	22.4	7.6			16
Hb Riyadh	β120 Lys→Asn	52	F	DM	120	11.3	3.1			17
		55	M	NIDDM	136		3.5			18
		74	M	NIDDM	219		3.0			19
Hb J-Meerut	α120(H3)Ala→Glu	48	F	NIDDM	172	4.7	3.7	2.5	365	PC

FBS: fasting blood sugar, 1,5-AG: 1,5-anhydroglucitol, FRA: fructosamine, PC: present case.

was 2.5 to 2.6% in Figs. 3 and 4. The reasons for this 5% decrease were considered to be the removal of the unstable HbA_{1C} and Hb J-Meerut after using hemolytic solution E, and improved glycemic control (Fig. 1).

Automatic determination of HbA_{1C} using HPLC is becoming popular as a means of evaluation of metabolic control in diabetic patients and in health examinations. The presence of abnormal Hb should be suspected when the level of HbA_{1C} is higher or lower than expected from the level of plasma glucose.

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