Hemoglobin Bethesda Causing Polycythemia in a Japanese Family

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A family with Hemoglobin Bethesda is reported. A 23-year-old man was hospitalized for the evaluation of polycythemia. Analysis of hemoglobin using high pressure liquid chromatography showed the presence of hemoglobinopathy. Separation of globin into α and β chains revealed approximately 50% of the β chain to be abnormal. Analysis of the DNA sequence of the β chain gene identified Hb Bethesda. The family study disclosed that his father and sister also had the same hemoglobinopathy. This case is the first report of Hb Bethesda in Japan. (Internal Medicine 33: 242–247, 1994)

Key words: polycythemia, hemoglobinopathy

Introduction

Hemoglobin Bethesda is a variant of hemoglobin which presents with polycythemia. Although there are several reports on this type of variant with development of polycythemia (1), no case of Hb Bethesda in Japan has been.

Here, we present the first documented case of Hb Bethesda in Japan, and the results of amino acid analysis, DNA sequencing analysis, and the hemoglobin oxygen equilibrium curve.

Case Report

In 1993, a 23-year-old man visited our hospital for evaluation of general fatigue. He was hospitalized for analysis of abnormal results in liver function tests and erythrocytosis. Physical findings on admission revealed no abnormality. The results of diagnostic blood tests performed at the time of admission are presented in Table 1. In evaluation of the increased red cell count, the circulating blood volume as measured by sodium chromates (2), was found to be increased. These findings established the diagnosis of polycythemia. Serum erythropoietin level and arterial blood gas analysis were within normal limits. Neutrophil alkaline phosphatase score, serum vitamin B₁₂, total iron binding capacity and serum iron were also normal. However, an elevated value of 4.18 $\mu\text{mol/ml}$ RBC was measured for 2,3-diphosphoglyceric acid (2,3-DPG) (3). This patient had a normal myelogram. His serum was positive HBs and for HBe antigen.

Table 1. Laboratory Values on Admission

Hematology	
RBC	607 $\times 10^4/\text{mm}^3$
Hb	19.1 g/dl
Hct	57.7%
WBC	3,800/ mm^3
N. band	4%
N. segment	52%
Mono	3%
Lymph	41%
Retic	15%
PLTs	6.7 $\times 10^4/\text{mm}^3$
Coagulation	
Prothrombin time	72%
APTT	32.7 sec (30.4 sec)
Fibrinogen	150 mg/dl
Circulating blood volume	
Red cell volume	3,381 ml (1,616 $\pm$ 307 ml)
Total blood volume	5,507 ml (3,725 $\pm$ 596 ml)
Total plasma volume	2,126 ml (2,104 $\pm$ 400 ml)
Erythropoietin	21.7 mIU/ml (9–40 mIU/ml)
2,3-DPG	4.18 $\mu\text{mol/ml}$ (1.8–2.2 $\mu\text{mol/ml}$ RBC)
Vitamin B ₁₂	551 $\mu\text{g/dl}$ (210–840 $\mu\text{g/dl}$)
Serum iron	183 $\mu\text{g/dl}$ (60–180 $\mu\text{g/dl}$)
Bone marrow biopsy	
normocellular	

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Received for publication September 13, 1993; Accepted for publication February 17, 1994

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Methods and Results

Cation exchange high pressure liquid chromatography (HPLC) of hemoglobin fraction

HPLC (Fig. 1) revealed an abnormal variant peak (abn.Hb), which emerged after HbA and HbA₂ using cation exchange HPLC (Micropeal BPG-17, 7.5×150 mm column). Absorbance (415 nm) was measured to determine the elutes of the individual hemoglobin bands (4).

Hemoglobin fractionation by isoelectric electrophoresis

Using a polyacrylamide gel plate (1), isoelectric electrophoresis resolved two major components, HbA and abn.Hb (Fig. 2), which were demonstrated as abnormal slow-moving bands.

Separation of hemoglobin α -chain and β -chain by carboxyl-methyl (CM) cellulose column

Hemoglobin was biosynthesized with incorporation of tritium-labeled leucine (5), and then subjected to uread CM-cellulose column chromatography. The abnormal β peak was eluted after the peak of the normal β chain (Fig. 3).

Isolation of the abnormal hemoglobin peptide

The β chain was aminoethylated, digested with trypsin, and the obtained peptide fragments were separated by reversed phase on HPLC (SG120 column, 4.6×250 mm) (6) (Fig. 4). The peak for the β T-15 peptide was absent, but no abnormal peak was observed. Amino acid analysis of each peak disclosed that β T-15 had emerged with β T-6,7,8 peptide (Table 2), and that histidine had been substituted for tyrosine in the β T-15 (Table 2). This substitution, β 145 Tyr → His, has already been reported in Hb Bethesda.

Analysis of the DNA sequence in β -chain globulin gene

To further clarify the results of the amino acid analysis, DNA sequencing was done. The nucleotide sequence in the β -chain globulin gene was analyzed using DNA obtained from the patient's peripheral blood (7). Two amplification primer sets were used for the PCR method (8). The amplification of DNA was carried out by the dideoxy method for sequencing. β Bethesda and β normal clone were obtained. Then the reaction samples were analyzed by electrophoresis on a polyacrylamide gel.

Both T and C were observed for the first base of the β 145 codon, indicating that the patient globulin was a heterozygote

Fig. 1. Cation exchange HPLC of hemolysate. An abnormal Hb (abn.Hb) was eluted after HbA and HbA₂.

Fig. 2. Isoelectrofocusing of hemolysates on a polyacrylamide gel plate.

Fig. 3. Urea CM-cellulose column chromatography of the ³H-leucine-labeled globin. —: absorbance, ----: radioactivity.

Fig. 4. Separation of the tryptic digest of the aminoacetylated β -Bethesda chain by HPLC on a Capcell Pak 5C₁₈ SG120 column (4.6 mm I.D. × 250 mm) with a linear gradient of acetonitrile (from 0 to 50%) in 9 mM trimethylamine-acetic acid buffer (pH 6.0) at a flow rate of 0.7 ml/min for 100 minutes.

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Table 2. Amino Acid Composition of a Peptides Mixture Containing the Abnormal β T-15

Amino acid	Found (nmol)	Theoretical molecular ratio			
		T-6	T-7	T-8	T-15
Glycine	7.33		1		
Alanine	7.93		1		
Valine	6.18	1			
Tyrosine	0.00				1
Lysine	16.85	1	1	1	
Histidine	19.91		1		1

of Hb Bethesda. Furthermore, clones of the β globin genes were separated, and the results assigned either TAT (tyrosine) or CAT (histidine) for codon 145 (Fig. 5).

The oxygen equilibrium curve

The oxygen equilibrium curves were measured on whole blood that had been diluted 150 times with isotonic phosphate buffer solution (0.15M K_2HPO_4 +0.15M NaH_2PO_4), pH of 6.9 or 7.45, at 37°C. The final concentration of hemoglobin was adjusted to 60 μ M on hemo basis (9).

The oxygen equilibrium curves of the patient's red cell suspension did not show the typical sigmoid shape especially at low O_2 saturation (Fig. 6).

Comparison of the P_{50} values indicated that the Hb Bethesda red blood cells had an oxygen affinity 2.7 to 2.9 times greater than the normal red blood cells. The low saturation region of the oxygen equilibrium curve for this patient's red blood cells showed the loss of cooperative intersubunit interaction.

Family study

The data on this family are presented in Table 3. His father and his younger sister carried the same abnormal hemoglobin and had similar polycythemia.

Discussion

There have been six previously reported cases of Hb Bethesda [β 145(HC2)Tyr $\rightarrow$ His], including North European Caucasians (5, 10, 11), a French Canadian (12), and a Chinese living in America (13). This is the first report of Hb Bethesda in Japan. All Hb Bethesda patients had secondary polycythemia because of inefficient O_2 dissociation from the abnormal hemoglobin.

Fig. 5. DNA sequence of β -Bethesda gene. β 145 codon was changed from TAT (tyrosine) to CAT (histidine).

Fig. 6. Oxygen equilibrium curves of erythrocytes from a heterozygote of Hb Bethesda (Beth./A) and a normal control (A/A). The curves were obtained using the automated method of Imai et al (9). —●—: Beth./A (pH 7.45), —○—: Beth./A (pH 6.9), —×—: A/A (pH 7.45), —□—: A/A (pH 6.9).

Table 3. Hematologic Findings of the Family

	Proband	Father	Sister
RBC $\times 10^4/\text{mm}^3$	607	653	675
Hb g/dl	19.1	19.1	14.1
Hct %	53.6	53.6	45.6
HbF (0.1–1.2%)	0.32	0.22	0.15
HbA ₂ (2.2–3.2%)	2.86	2.28	2.07
Abnormal Hb (%)	49.5	48.6	48.3

Oxygen equilibrium curve of the carriers is shifted to the left and the normal sigmoid curve was replaced by a straight line at the low saturation region.

An elevated value for 2,3-DPG was observed in our patient in contrast to previous reports of a normal level (10, 12, 13). 2,3-DPG facilitates dissociation of oxygen from hemoglobin which results in improved oxygenation of the tissues (14).

The neutrophil alkaline phosphatase score, serum vitamin B₁₂ and erythropoietin levels were consistent with previously reported cases of Hb Bethesda by Schmidt (12) and Bunn (13).

The only clinical manifestation in Hb Bethesda is persistent cutaneous flush due to the polycythemia. Cardiac and cerebrovascular complications have not been reported, and fetal oxygenation seemed undisturbed in a mother with Hb Bethesda (15). In this case, only the proband was found to have cutaneous flush.

Acknowledgments: Authors appreciate Dr. Kiyohiro Imai, Department of Physiology, Osaka University, School of Medicine, for his technical assistance of oxygen saturation curve.

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