Unstable Hemoglobinemia, Hb Buenos Aires, Bryn Mawr, Followed Up for Eighteen Years

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https://doi.org/10.15017/19249
Case Report

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Abstract  A 20-year-old man has been under observation for 18 years because of unstable hemoglobinemia, Hb Buenos Aires, Bryn Mawr (β-globin, Phe85Ser). At the age of 19 years, he was hospitalized because of fever and hemolytic crisis, and the symptoms resolved after infusion of antibiotics. Nucleotide sequencing of the β-globin gene confirmed that the patient was heterozygous for the mutation. The patient's erythrocytes showed an increased affinity for oxygen and a prolonged glycerol lysis time. We review a previously reported single family and 5 other cases, and discuss the clinical significance of splenectomy and plasma-derived haptoglobin.

Introduction

Unstable hemoglobinemia, Hb Buenos Aires, Bryn Mawr is a rare hemoglobinopathy. Although one family and 5 cases have been reported, the clinical phenotype, and the oxygen affinity and membrane properties of the affected erythrocytes have not been fully described1-6. Here we report a 20-year-old man who was diagnosed as having the disease at the age of 2 years. We found that the oxygen affinity and glycerol lysis time of the patient's erythrocytes were altered.

Case Report

A Japanese boy was first investigated at the age of 2 years at Nagasaki University Hospital in 1985, because of jaundice, splenomegaly and pigmenturia, and was diagnosed as having unstable hemoglobinemia, Hb Buenos Aires, Bryn Mawr. Activities of the RBC enzymes glucose-6-phosphate dehydrogenase and pyruvate kinase were normal. He remained apparently healthy during boyhood, and his height and weight were within the normal ranges. At the age of 17 years, he developed a self-resolving upper respiratory infection and hemolytic crisis; there had been no documented hemolytic or aplastic crisis up to that time.

At the age of 19 years, he was referred to Fukuoka Dental College Hospital because
of general fatigue, fever, sore throat and lower abdominal pain. His height was 1.73 m, weight 53 kg, body temperature 39.2 °C, and pulse 100/min. The pharynx was injected, and the tonsils mildly swollen. There was no swelling of the cervical lymph nodes. Jaundice and splenomegaly extending over the midline of the abdomen were evident. The peripheral blood WBC count was 10.0 × 10⁹ /L, RBC 2.76 × 10¹² /L, Hb 9.0 g/dl, Ht 27.8%, platelets 112×10⁹ /L, reticulocytes 9.2%. A peripheral blood smear showed anisocytosis, but no notable spherocytes or poikilocytes. No Heinz bodies were found in smears by neutral red staining. Coombs, HAM and sugar water tests all gave negative results. AST was 130 IU/L, ALT 137 IU/L, LDH 932 IU/L, total bilirubin 16.26 mg/dl and indirect bilirubin 10.08 mg/dl. Serum haptoglobin was <7.9 mg/dl, transferrin 179.0 mg/dl, ferritin 417.5 ng/ml, and C-reactive protein 10.0 mg/dl. The patient was negative for HBsAg and HCV-Ab, rheumatoid factor and the TPHA test. Ultrasonography showed severe splenomegaly (20 × 30 cm), but no gallstones. The patient was diagnosed as having hemolytic crisis, upper respiratory tract infection and suspected abdominal infection including appendicitis.

After hospitalization, he received intravenous cefazolin 1 g twice a day for 3 days. He became afebrile in a day, and his general fatigue and appetite loss improved. During these three days, 3,000 ml of saline was infused daily in order to prevent renal insult due to free heme. He was discharged on the 10th day with mild jaundice and moderate splenomegaly, but without any other sequelae.

Laboratory findings one month after discharge were: WBC 5.3 × 10⁹ /L, Hb 13.7 g/dl, platelets 124×10⁹ /L, reticulocytes 5.2%. Blood chemistry gave the following values: AST 25 U/L, ALT 12 IU/L, LDH 254 IU/L, total bilirubin 2.85 mg/dl, CRP 0.1 mg/dl.

Diagnosis of Hb Buenos Aires, Bryn Mawr

Screening Tests for Hemoglobinopathies

To see if the patient had unstable hemoglobinemia, the isopropanol test was performed⁷, and this gave a positive result (Fig. 1A). Both cellulose-acetate membrane electrophoresis of hemoglobins and isoelectric focusing yielded abnormal bands (Fig. 1B, C). The amounts of HbF and HbA₂ were within the normal ranges (Fig. 1C).

Biochemical Diagnosis of Hb Buenos Aires, Bryn Mawr

To determine which globin was affected, the globins extracted from the hemolysate were subjected to reverse-phase HPLC.

The optical density of the eluting solution showed an abnormal peak between heme and the normal β-globin peaks. The ratio of the abnormal peak to that of normal HbA was 0.61 : 1 (Fig. 2A). The estimated ratios of the hemoglobins were HbA 59.7 %, abnormal Hb 36.5 %, HbF 1.0 % and HbA₂ 2.8 %. Therefore, it was concluded that the patient had an abnormal β-globin.

To determine the altered amino acid residue (s), the abnormal β-globin was purified by extraction as a brick-red precipitate, followed by urea CM-52 ion-exchange column chromatography (Fig. 2B). The purified abnormal β-globin chain was digested with trypsin and run in a C18 reverse-phase column. The HPLC elution profile demonstrated absence of the normal peptide βT10 and presence of an abnormal peak eluting just after βT3 (Fig. 2C). The abnormal peak fraction was collected and
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Fig. 1 Screening tests for hemoglobinopathy. A, Isopropanol test. The isopropanol stability test was carried out by the method of Carrell and Key. The patient's sample is turbid, suggesting that he has unstable hemoglobins. B, Cellulose-acetate membrane electrophoresis of hemoglobin. The hemolysate was dissolved in buffer containing of 6 M urea, 45 mM Tris-borate and 1 mM EDTA. After electrophoresis, the membrane was stained with Ponsau-R. The bar indicates an abnormal band. C, Isoelectric focusing of hemoglobin in an ampholine-acrylamide gel, pH 6 - 9. Lane pairs on the left and right are those before and after staining with Coomasie brilliant blue.

then analyzed by an amino acid analyzer. The amino acid composition of the abnormal peptide was identical to that of the normal βT10 fragment, except for lack of Phe and a double amount of Ser (Table 1). Therefore, it was concluded that the patient's abnormal β-globin had a single amino acid substitution from Phe to Ser at position 85 (Phe85Ser).

**Nucleotide sequencing of β-globin gene**

To confirm the Phe85Ser mutation, the patient's β-globin gene was amplified by polymerase chain reaction and the nucleotide sequence was determined. Direct sequencing of exon 2 showed coexistence of T and C at the second letter of codon 85 (Fig. 3). Therefore, the patient was heterozygous for the β-globin gene with a normal (TTT for Phe) and a mutated (TCT for Ser) codon 85.

**Glycerol Lysis Time and Oxygen Affinity of Erythrocytes**

To know the membrane property of the erythrocytes, the median glycerol lysis time (GLT50) was determined. Twenty microliters of whole blood was mixed with 5 ml of isotonic phosphate buffer, pH 7.4. One milliliter of the mixture was mixed with 2.0 ml 0.3 M glycerol, and the optical absorbance at 625 nm was measured in a 10-mm-path cuvette. As results, GLT50 of the patient was 57 s, whereas those of the reference samples were 22 - 55 s.

To see the function of the abnormal Hb, oxygen affinity of erythrocytes, i.e. P50 that indicates the oxygen pressure required to saturate 50% of the erythrocytes, was measured. As shown in the oxygen dissociation curve (Fig. 4), the patient's P50 was 21 mmHg, whereas the control value was 26 mmHg.
Fig. 2 Detection of abnormal $\beta$-globin. A, Reverse-phase HPLC of whole blood showing an abnormal peak between heme and the normal $\beta$-globin peaks. Cells were lysed in distilled water, centrifuged 12,000 x g for 15 min, and the supernatant was run in a TSK gel ODS120T column. The ratio of the abnormal Hb to normal HbA is 0.61:1. B, Large preparation of abnormal $\beta$-globin by CM-52 ion-exchange column chromatography of urea-lysed brick-red precipitate of hemolysate, in 8 M urea-phosphate buffer, pH 6.8 with a 5 - 35 mM NaCl gradient. C, C18 reverse-phase column chromatography (μ Bondpack) of the trypsin-digested abnormal $\beta$-globin with a 0.1 - 50 % linear gradient of acetonitrile, showing absence of normal $\beta$T10 peptide fragments and emergence of an abnormal peak.

Discussion

We have described the clinical course of the third episode of hemolytic crisis in a patient with Hb Buenos Aires, who has been followed for 18 years. Unlike the abnormal hemoglobinemia Hirosaki ($\alpha$-globin Phe43Leu), which shows various clinical phenotypes, no phenotypic variations have been evident among reported cases of Hb Buenos Aires (Table 2). Jaundice and splenomegaly were present in all the cases reported. The levels of Hb and percentages of reticulocytes at a steady state without hemolytic or aplastic crisis were 11.5 - 14 g/dl and 3.5% - 18%.

Table 1 Amino acid composition of the abnormal $\beta$T10 peptide

<table>
<thead>
<tr>
<th></th>
<th>Pt</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>0.97 (1)</td>
<td>1.02 (1)</td>
</tr>
<tr>
<td>Thr</td>
<td>1.89 (2)</td>
<td>1.90 (2)</td>
</tr>
<tr>
<td>Ser</td>
<td>1.69 (2)</td>
<td>0.88 (1)</td>
</tr>
<tr>
<td>Glu</td>
<td>1.03 (1)</td>
<td>1.10 (1)</td>
</tr>
<tr>
<td>Gly</td>
<td>1.02 (1)</td>
<td>1.02 (1)</td>
</tr>
<tr>
<td>Ala</td>
<td>1.09 (1)</td>
<td>1.16 (1)</td>
</tr>
<tr>
<td>Cys</td>
<td>0.98 (1)</td>
<td>0.96 (1)</td>
</tr>
<tr>
<td>Leu</td>
<td>2.17 (2)</td>
<td>1.97 (2)</td>
</tr>
<tr>
<td>Phe</td>
<td>- (0)</td>
<td>0.92 (1)</td>
</tr>
<tr>
<td>Lys</td>
<td>1.09 (1)</td>
<td>1.02 (1)</td>
</tr>
<tr>
<td>His</td>
<td>1.05 (1)</td>
<td>0.99 (1)</td>
</tr>
</tbody>
</table>

The abnormal $\beta$T10 peptide was analyzed by a peptide analyzer. Phenylalanine was not detected in the abnormal peptide, whereas the amount of serine in abnormal peptide was twice that of normal peptide.
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Fig. 4 Oxygen-dissociation curve. The patient's P50, the oxygen pressure required to saturate 50% of the erythrocytes, was 21 mmHg, whereas the control value was 26 mmHg. The curves were determined by the mixing technique of Edwards and Martin9. P50 values were corrected to pH 7.4 using the Bohr correction curves published by Rorth10.

In the present case, absence of Heinz bodies in fresh blood at both the time of hemolytic crisis and non-crisis steady state suggested that the spleen was efficiently removing Heinz bodies from erythrocytes and dealing with the abnormal erythrocytes. However, a blood sample left at 4°C for 48 h showed no Heinz body formation. Moreover, neither nibbled erythrocytes nor poikilocytosis were observed in the patient's blood smears. These findings suggested that no Heinz bodies were formed in our patient, although the amount of degenerated Hb was sufficient for precipitation with isopropanol. Shortened erythrocyte survival is mediated by two processes: Heinz body formation and oxidant damage to membrane lipids and proteins12. The latter process appears to have been dominant in our patient.

The P50 of the two reported cases and the present case ranged from 19.4 to 22.2 mmHg, suggesting that the abnormal Hb, Buenos Aires, has increased affinity for

respectively. The amounts of abnormal Hb were 21.3% and 36.5% in two of the cases described. Therefore, in cases of Hb Buenos Aires, hemolysis is continuous but is compensated by increased erythropoiesis. Incompatibilities among reported cases have been presence/absence of gallstones and Heinz bodies.

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Fig. 3 Nucleotide sequence of exon 2 of the β-globin gene, showing coexistence of T and C at the second letter of codon 85. Exon 2 of the patient’s β-globin gene was amplified by polymerase chain reaction using the primers β51-2: 5’taggcactgactctctctgctcatt and β8-2: 5’ccttcctcctctgctcatt. The nucleotide sequence of the amplified product was determined by ABI PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Tokyo) and autosequencer ABI 373A (Applied Biosystems, Tokyo).
Table 2  Reported cases of hemoglobin Buenos Aires

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Gender</th>
<th>Country</th>
<th>Hb*** (g/dl)</th>
<th>Abnormal Hb (%)</th>
<th>Reticulocyte*** (%)</th>
<th>Heinz body</th>
<th>Splenomegaly</th>
<th>Gallstone</th>
<th>C50 Patient / Ref (mmHg)</th>
<th>GLT50</th>
<th>Other findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-M</td>
<td>M**</td>
<td>Argentina</td>
<td>12.6</td>
<td>9.0</td>
<td>pos</td>
<td>yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>anisocytosis, basophilic stippling [1]</td>
</tr>
<tr>
<td></td>
<td>M**</td>
<td></td>
<td>13.5</td>
<td>4.0</td>
<td>yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>splenectomy at 30 years of age</td>
</tr>
<tr>
<td></td>
<td>M**</td>
<td></td>
<td>14.0</td>
<td>3.5</td>
<td>yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>aplastic crisis with Parvovirus B19 infection [3]</td>
</tr>
<tr>
<td>7-F</td>
<td>USA</td>
<td>12-15</td>
<td>11.5</td>
<td>10</td>
<td>yes</td>
<td>no</td>
<td></td>
<td></td>
<td>22.2 / 27.0</td>
<td></td>
<td>anisocytosis [6]</td>
</tr>
<tr>
<td>9-F</td>
<td>Japan</td>
<td>11.5</td>
<td>12.6</td>
<td>9.0</td>
<td>yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[This case]</td>
</tr>
</tbody>
</table>

*: not described, **: at diagnosis, ***: a family study, age not provided, ****: steady state without hemolytic or aplastic crisis

Oxygen. According to the Perutz model[21],[3], the amino acid substitution from Phe to the smaller molecule Ser at position 85 (the first position in the F helix) would allow the F helix to be closer to the heme. This structural alteration tends to adopt the oxy - (R-) configuration. In addition, the amino acid substitution of a hydrophilic amino acid for a hydrophobic one decreases the heme pocket's hydrophobicity, allowing oxygen molecules to enter the space more easily.

Such decreased hydrophobicity also allows a water molecule (H₂O) to enter the heme pocket. This would further decrease the space's hydrophobicity, and the pocket would become incapable of retaining the heme. The loss of heme would in turn cause the unstable hemoglobin to become denatured. In addition, the Phe85Ser substitution might also affect the globin-to-globin contact regions and destabilize the tetramer structure.

Splenectomy is a matter for consideration in patients with hemolytic anemias. It is well known that splenectomy can dramatically resolve the continuous and episodic hemolysis in cases of hemolytic anemia such as hereditary spherocytosis, caused by abnormal membrane proteins. However, little is known about its significance in hemoglobinopathies. Only one reported patient with Hb Buenos Aires underwent splenectomy, but its effect was not documented[1] (Table 2). The potential demerits of splenectomy in patients with hemoglobinopathies include thromboembolic complications such as deep venous thrombosis or priapism[14]. In our patient, the potential merit of total or partial splenectomy was considered small because (1) the abnormal hemoglobin had higher affinity for oxygen, and thus the net ability of peripheral blood to supply oxygen to peripheral tissues would not have been improved by increasing the amount of abnormal Hb, and (2) the anemia was well compensated and the patient did not require transfusion, even at hemolytic crisis. Therefore, we have continued to observe the patient without recommending splenectomy.

Human plasma-derived haptoglobin for intravenous infusion is available in Japan, and is an option for the treatment of
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hemo-lytic uremic syndrome, unmatched transfusion and severe burns\textsuperscript{15}. It is believed that, by binding to free heme and bringing it to hepatocytes, the infused haptoglobin prevents free heme from directly damaging the endothelial cells of the nephron and collecting tubules. However, as there are few clinical examples of its benefit, and no criteria or guidelines for its application, we did not perform haptoglobin infusion.

**Acknowledgments**

We express our regret at the passing of Prof. Takaoki Miyaji, who initially analyzed the hemoglobin and diagnosed the present case in 1986. We wish to thank Prof. Shiro Miwa for examining the erythrocyte enzymes. Some of the results reported in this article were previously deposited on the Globin Gene Server (http://globin.cse.psu.edu/) in 1997.

**References**


(Received for publication October 6, 2004)
不安定ヘマログビン症 Hb Buenos Aires, Bryn Mawr:
18年の経過観察

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