An Autopsy Case of Pyruvate Kinase Deficiency Anemia Associated with Severe Hemochromatosis

Hideo NAGAI, Eisuke TAKAZAKURA, Hiroyuki ODA, Hiroshi TSUJI,
Yasuhiro TERADA, Hiroshi MAKINO, Hiromasa YAMAUCHI and Katsuhiko SAITOH*

We report an autopsy case of pyruvate kinase deficiency anemia with severe hemochromatosis. This anemia is rarely associated with hemochromatosis. In this case, the autopsy findings showed hemochromatosis of the heart, pancreas, liver, kidneys, thyroid gland, adrenal glands, testes and skin. Microscopic examination showed iron depositions in these organs, but not in the bone marrow. A family study showed negative data for iron overload and no known HLA type suggestive of idiopathic hemochromatosis. To explain this rare association, we suggest that this patient's iron overload was an acquired type, which might have mainly been caused by increased iron absorption due to the severe hemolytic anemia.

(Internal Medicine 33: 56–59, 1994)

Key words: congenital hemolytic anemia, iron overload, iron absorption, erythropoiesis, HLA type

Introduction

Pyruvate kinase (PK) deficiency anemia was initially described in 1961 (1). Since then more than 300 cases have been reported. This anemia is rarely associated with iron overload (2–4). We report an autopsy case of PK deficiency anemia, who died of heart failure, associated with severe hemochromatosis, and discuss the possible reasons as to why this patient developed this rare association.

Case Report

The patient was a 44-year-old man with severe anemia and insulin-dependent diabetes mellitus. There was no consanguineous marriage and no family history suggestive of anemia or jaundice. He had no medical history of long-term iron intake or of administration of large doses of ascorbic acid. His alcohol intake was minimal. His anemia was identified in childhood. At the age of about 20 he underwent cholecystectomy and splenectomy. At the age of 30 he had cardiomegaly and glycosuria. At age 32 he consulted Kurobe City Hospital and his hepatomegaly was pointed out. At the same time he was diagnosed as having diabetes mellitus and insulin injections were begun. In 1983, at age 39 while admitted to our hospital for anemia work up, he was diagnosed as having PK deficiency anemia. On physical examination his skin was brownish. His conjunctivae were anemic and icteric. An enlarged liver was

palpable 5 finger breadths on the right-midclavicular line. Laboratory data on admission are shown in Table 1, which were obtained before blood transfusion. His anemia was of a normochromic macrocellular pattern. Reticulocytes were increased in the peripheral blood, but echinocytes were not seen. The total bilirubin was 2.1 mg/dl and the indirect type was dominant. The serum iron level and the ferritin level were highly increased. The transferrin saturation was 89.8%. The ⁵¹Cr RBC life span was 11.8 days. Coombs' test, Ham test, osmotic fragility test, and sugar-water test were negative. The bone marrow picture showed hypercellular marrow with marked erythroid hyperplasia. Erythrocyte enzyme activity was measured (Table 2). PK activity was 1.6 U/gHb, which was decreased compared with the normal control, whereas 2, 3-diphosphoglycerate, which is intermediate in the glycolytic pathway, was increased. Other enzymological parameters were not determined. The patient's HLA type was not examined. Since that time he received blood transfusion of 3,800 ml. On October 5, 1988, he was admitted to our hospital because of congestive heart failure and hyperglycemia. On the 55th hospital day he died of heart failure. During the last admission, he required a blood transfusion of 3,600 ml because of progression of his anemia.

An autopsy showed severe hemochromatosis of the heart, pancreas, liver, kidneys, thyroid gland, adrenal glands, testes and skin. Microscopic examination also showed iron depositions in these organs, but not in the bone marrow. The liver was

From the Department of Internal Medicine, Kurobe City Hospital, Kurobe and *the Second Department of Pathology, Kanazawa University, Kanazawa
Received for publication March 15, 1993; Accepted for publication December 1, 1993

Reprint requests should be addressed to Dr. Hideo Nagai, the First Department of Internal Medicine, Kanazawa University, 13-1, Takara-machi, Kanazawa, 920

Table 1. Laboratory Data before Blood Transfusions

RBC	257×10 ⁴ /mm ³	Na	141 mEq/l
Hb	9.4 g/dl	K	4.3 mEq/l
Ht	29%	Cl	97 mEq/l
MCV	112.8 μm ³	BUN	26.7 mg/dl
MCH	36.6 pg	Cr	0.8 mg/dl
MCHC	32.4%	TTT	7.5 MU
WBC	6,400/mm ³	ZTT	20.2 KU
Seg.	39%	T. Bil.	2.1 mg/dl
Band.	4%	D. Bil.	1.6 mg/dl
Eo.	0%	AST	34 IU/l
Baso.	0%	ALT	42 IU/l
Lymph.	55%	γ-GTP	36 IU/l
Mono.	2%	LDH	330 IU/l
Plts	31.1×10 ⁴ /mm ³	ALP	8.7 KAU
Reticulocytes	60‰	TP	8.2 g/dl
ESR (1 hr)	40 mm	alb.	61.8%
CRP	(-)	γ-glob.	22.7%
PT	11.4 sec	T. Chol.	152 mg/dl
Fibrinogen	260 mg/dl	TG	97 mg/dl
Serum iron	266 μg/dl	BS	354 mg/dl
TIBC	239 μg/dl	HbA1	11.4%
UIBC	27 μg/dl	HbA1c	9.7%
Ferritin	1,200 μg/l		
Haptoglobin	64 mg/dl		

MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, PT: prothrombin time, TIBC: total iron binding capacity, UIBC: unsaturated iron binding capacity, BUN: blood urea nitrogen, Cr: serum creatinine, TTT: thymol turbidity test, ZTT: zinc sulfate turbidity test, T. Bil.: total bilirubin, D. Bil.: direct bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, γ-GTP: γ-glutamyltranspeptidase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase, TP: total protein, T. Chol.: total cholesterol, TG: triglyceride, BS: blood sugar.

Table 2. Assays of Erythrocyte Enzymes and Intermediates in the Glycolytic Pathway

	Control	Patient
Enzymes (U/gHb)		
Glucose 6-phosphate dehydrogenase	6.1	9.0
Hexokinase	0.1	0.3
Phosphoglycerate kinase	175.0	185.0
Phosphofructokinase	5.0	5.5
Pyruvate kinase	2.9	1.6
Lactate dehydrogenase	80.1	94.7
Glucosephosphate isomerase	42.0	45.7
Aldolase	0.38	0.39
Intermediates (μmols/ml.cells)		
2,3-Diphosphoglycerate	2.87	4.06
Adenosine triphosphate	1.39	1.81
Pyruvate	0.035	0.046
Phosphoenolpyruvate	0.040	0.040
2-Phosphoglycerate	0.003	0.002
Fructose-1,6-diphosphate	0.045	0.070

Fig. 1. An autopsy specimen of the liver. a) Iron-positive granules were seen in the hepatic cells and stromal histiocytes (Berlin blue stain, ×40). b) Fine nodular changes in the parenchyma and fibrotic changes in the stroma were seen (Azan stain, ×40).

enlarged and cirrhotic with fine nodules. Microscopic examination showed iron depositions in the cytoplasm of the hepatic cells (Fig. 1a), and fine nodular changes in the parenchyma and fibrotic changes in the stroma (Fig. 1b). The bone marrow was mildly hyperplastic in erythroid series, but contained few iron granules (Fig. 2a, b). Family study of three brothers of the patient showed negative data for iron overload and no known HLA type suggestive of idiopathic hemochromatosis (Table 3).

Discussion

PK deficiency associated with hereditary non-spherocytic anemia was initially described by Valentine et al in 1961 (1). PK deficiency anemia is the most common hemolytic anemia due to the enzyme abnormality in the Embden-Meyerhof pathway. In this anemia, clinical symptoms are observed in the homozygous or doubly heterozygous state. Although the PK activity of the present patient was 55% that of normal control, he showed clinical symptoms. Therefore this case was in the doubly heterozygous state, that means one of PK variants. The

Fig. 2. An autopsy specimen of the bone marrow. a) Erythroid series were in the hyperplastic state (HE stain, $\times 80$). b) Few iron granules were found (Berlin blue stain, $\times 40$).

fact that 2, 3-diphosphoglycerate was increased, was compatible for PK deficiency anemia. But the reason why phosphoenolpyruvate was not increased, remained uncertain. This anemia is an autosomal recessive inherited disorder, but there were no remarkable family histories.

Iron overload is a common complication of anemias marked by ineffective erythropoiesis. A particularly serious form of hemochromatosis develops in patients with thalassemia major or with certain varieties of sideroblastic anemia. These patients hyperabsorb iron inappropriately (5). However, hemolytic

anemias, such as hereditary spherocytosis and PK deficiency anemia, are not accompanied by ineffective erythropoiesis. PK deficiency anemia is uncommonly associated with non-transfusional hemochromatosis (2–4). When the present patient was transfused the first time, he had already developed diabetes mellitus, hepatomegaly, skin pigmentation and cardiomegaly due to hemochromatosis. So this case is non-transfusional.

In this rare association, two mechanisms are suggested. One possibility is that the patient's iron overload was caused by increased iron absorption due to the severe hemolytic anemia, which means a secondary phenomenon. The other possible mechanism is an association with idiopathic hemochromatosis. Salem et al reported two cases of PK deficiency anemia with non-transfusional hemochromatosis, and cited increased absorption of oral iron due to increased erythropoiesis secondary to anemia as the mechanism of this rare association (3). The same explanation was suggested by Barry et al for hemochromatosis with hereditary spherocytosis (6). These authors suggested that the iron loading is a secondary phenomenon. However, the HLA type was not noted in these reports. Rowbotham and Roeser reported that a patient with PK deficiency anemia had the HLA genotype (A3, B14), and that the prevalence of this HLA type in a population of patients with idiopathic hemochromatosis was forty times higher than in the normal population, suggesting the coexistence of two independent genetically determined diseases (4). Blacklock and Meerklin (7) and Edwards et al (8) reported hereditary spherocytosis associated with HLA-linked hemochromatosis, and suggested that the iron loading in hereditary spherocytosis is not a secondary phenomenon, but rather is due to the coincident occurrence of idiopathic hemochromatosis.

Several investigators reported that HLA A3, B7, and B14, and haplotypes A3 B7, A3 B5, A3 B14, A11 B5, and A11 B35 are significantly more frequent in idiopathic hemochromatosis patients than in controls in various countries (9–13). The present family study showed negative data for iron overload and no known HLA types suggestive of idiopathic hemochromatosis. We believe that the possibility of an association between PK deficiency anemia and idiopathic hemochromatosis is unlikely, and that our patient's iron overload might not have been HLA-related.

There are many factors that influence the development of iron overload, for example large doses of ascorbic acid, massive blood transfusion, an excess of dietary iron, alcohol intake, liver cirrhosis and malnutrition. The present patient had no evidence

Table 3. Data of Family Study

Age and Gender	Serum iron ($\mu\text{g/dl}$)	TIBC ($\mu\text{g/dl}$)	Transferrin saturation (%)	Serum ferritin ($\mu\text{g/l}$)	HLA type				
					A	B	C	DR	DQ
57 M*	56	319	17.6	167.1	A24(9)	BW52(5)	—	DR2	DQW1
53 M*	67	280	23.9	171.9	A24(9)	BW52(5) BW54(W22)	CW2	DR2 DR4	DQW1 DQW4
43 M**	152	339	44.8	195.4	A24(9)	BW52(5) BW54(W22)	CW1	DR2 DR4	DQW1 DQW4

M: male, TIBC: total iron binding capacity, *patient's elder brothers, **patient's younger brother.

of these factors. The clinical manifestations of PK deficiency are highly variable, ranging from pronounced neonatal jaundice requiring multiple exchange transfusions to a fully compensated hemolytic anemia. Splenectomy is effective for PK deficiency patients with severe anemia (14). In the present case, his anemia was identified in childhood, and it was still severe 20 years after splenectomy. Because of this severity, the erythropoiesis in our case might be more increased than in PK deficiency patients with mild or compensated anemia. In patients with PK deficiency anemia, reticulocytes are highly increased in the peripheral blood after splenectomy, but in our case reticulocytes was 60%. The fact that the autopsy specimen of the bone marrow showed little iron deposition, might indicate that the iron reserve of the bone marrow had been depleted because of increased erythropoiesis, that is, the consumption of iron exceeded the storage in the bone marrow. Therefore our patient's erythropoiesis was not highly increased because of relative iron deficiency in the bone marrow, and reticulocytes in the peripheral blood were not highly increased.

In conclusion, the present case was non-transfusional and might not have been HLA-related. Therefore, we believe that our case is one of acquired iron overload mainly caused by increased iron absorption due to the severe hemolytic anemia, although any additional factors which might have influenced the development of iron overload can not be completely ruled out.

Acknowledgements: The assays of erythrocytic enzyme activity were performed by Assistant Professor Tomoda and colleagues in the First Department of Biochemistry of the Medical School of Kanazawa University. We gratefully acknowledge their cooperation.

References

- 1) Valentine WN, Tanaka KR, Miwa S. A specific erythrocyte glycolytic enzyme defect (pyruvate kinase) in three subjects with congenital non-spherocytic hemolytic anemia. *Trans Assoc Am Physicians* **74**: 100, 1961.
- 2) Noel D, Duron F, Thomas M. Hemochromatose et deficit en pyruvate kinase. *Ann Med Interne* **130**: 679, 1979.
- 3) Salem HH, Van Der Weyden MB, Firkin BG. Iron overload in congenital erythrocyte pyruvate kinase deficiency. *Med J Aust* **1**: 531, 1980.
- 4) Rowbotham B, Roeser HP. Iron overload associated with congenital pyruvate kinase deficiency and high dose ascorbic acid injection. *Aust N Z J Med* **14**: 667, 1984.
- 5) Jandl JH. Erythropoietic hemochromatosis. in: *Blood, Textbook of Hematology*, Little, Brown and Company, Boston, 1987, p. 204.
- 6) Barry M, Scheuer PJ, Sherlock S, Ross CF, Williams R. Hereditary spherocytosis with secondary hemochromatosis. *Lancet* **2**: 481, 1968.
- 7) Blacklock HA, Meerkin M. Serum ferritin in patients with hereditary spherocytosis. *Br J Haematol* **49**: 117, 1981.
- 8) Edwards CQ, Skolnick MH, Dadone MM, Kushner JP. Iron overload in hereditary spherocytosis; association with HLA linked hemochromatosis. *Am J Hematol* **13**: 101, 1982.
- 9) Ritter B, Safwenberg J, Olsson KS. HLA as a marker of the hemochromatosis gene in Sweden. *Hum Genet* **68**: 62, 1984.
- 10) Simon M, Le Mignon L, Fauchet R, et al. A study of 609 HLA haplotypes marking for the hemochromatosis gene: (1) mapping of the gene near the HLA-A locus and characters required to define a heterozygous population and (2) hypothesis concerning the underlying cause of hemochromatosis-HLA association. *Am J Hum Genet* **41**: 89, 1987.
- 11) Milman N, Graudal N, Nielsen LS, Fenger K. HLA determinants in 70 Danish patients with idiopathic haemochromatosis. *Clin Genet* **33**: 286, 1988.
- 12) Panajotopoulos N, Piperno A, Conte D, et al. HLA typing in 67 Italian patients with idiopathic hemochromatosis and their relatives. *Tissue Antigens* **33**: 431, 1989.
- 13) Czink E, Gyodi EK, Nemet K, Hollan S. HLA antigens in Hungarian patients with idiopathic haemochromatosis. *J Clin Pathol* **44**: 79, 1991.
- 14) Miwa S. Pyruvate kinase deficiency. *Nippon Ketsueki Gakkai Zasshi* **50**: 1445, 1987.