

Pyruvate kinase deficiency mimicking congenital dyserythropoietic anemia type I

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ABSTRACT

Background. Pyruvate kinase (PK) deficiency is the most common enzyme abnormality in the glycolytic pathway. Here, we describe two siblings with PK deficiency that mimicked congenital dyserythropoietic anemia (CDA) type I.

Case. The siblings were referred to our hospital for evaluation of anemia when they were newborns. Their PK enzyme activities were normal. Their bone marrow aspirations and electron microscopies showed CDA-like findings. A CDA panel with next-generation sequencing showed no mutation. Though their PK enzyme levels were normal, a molecular study of the *PKLR* gene showed a homozygous variant c.1623G>C (p.Lys541Asn) in exon 12 of our patients.

Conclusions. Although the diagnosis of pyruvate kinase deficiency is difficult, it can be confused with many other diagnoses. Bone marrow findings of these cases are similar to congenital dyserythropoietic anemia. In patients with normal pyruvate kinase enzyme levels, the diagnosis cannot be excluded and genetic analysis is required.

Key words: pyruvate kinase deficiency, congenital dyserythropoietic anemia, *PKLR* gene.

Pyruvate kinase (PK) deficiency is the most common enzyme abnormality in the glycolytic pathway, which leads to an anemia secondary to decreased ATP synthesis.¹ The disease exhibits autosomal recessive inheritance and is caused by mutations in the *PKLR* gene.² The protein encoded by this gene is a pyruvate kinase that catalyzes the phosphorylation of phosphoenolpyruvate into pyruvate and ATP, therefore a mutation in the *PKLR* gene leads to ATP deficiency in erythrocytes. This ATP deficiency presumably results in a reduced capacity to maintain the red cell membrane and diminished erythrocyte deformability, resulting

in a shortened lifespan and destruction in the spleen. Defects in this enzyme, due to gene mutations or genetic variations, are the common cause of chronic hereditary nonspherocytic hemolytic anemia.³

The diagnosis of PK deficiency is based on the presence of clinical signs and symptoms of hemolytic anemia, evidence of extravascular hemolysis in laboratory findings, measurement of PK activity or antigen levels and detection of mutations in the *PKLR* gene.⁴ The clinical severity of PK deficiency varies widely, ranging from mild anemia and jaundice to severe transfusion dependent hemolytic anemia. Even within the same family, individuals can have different symptoms and severity.^{5,6}

In some patients with PK deficiency, surprisingly, PK levels are normal. PK levels depend on the age of red blood cells and younger cells have higher PK levels. Reticulocytosis and

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large quantities of nucleated red blood cells in the peripheral blood due to hemolysis produce higher levels of PK.⁷ Multiple and frequent transfusions can obscure enzymatic defects and finally, the PK isozyme in white blood cells cannot be totally eliminated from the sample, which can influence detection of PK levels.⁸

Here, we describe two siblings with PK deficiency that was misdiagnosed as congenital dyserythropoietic anemia (CDA) type I.

Case Report

Patient 1 was referred to our hospital for the evaluation of anemia when she was 32 days old. She was born as a term baby of a first pregnancy and her birth weight was 3550 g. She was hospitalized in another hospital due to anemia and jaundice on the first postnatal day and received phototherapy and erythrocyte suspension. The initial family history was unremarkable except that parents were first-degree cousins. On physical examination, she had an icteric appearance, but did not display hepatosplenomegaly. Complete blood count revealed a hemoglobin of 4.8 g/dl (normal range 12-18.5 g/dl), mean corpuscular volume 92 fl (normal range 86-118 fl), mean corpuscular hemoglobin 24.9 pg/cell (normal range 29-36 pg/cell), mean corpuscular hemoglobin concentration 33 g/dl (normal range 28-38 g/dl), white blood cell count of $13.5 \times 10^9/L$ (normal range $5-20 \times 10^9/L$) and platelets $387 \times 10^9/L$ (normal range $150-450 \times 10^9/L$). The corrected reticulocyte count was 8.6% (normal range 0.5-1.5%). Indirect bilirubin level was 2.12 mg/dl and LDH 432 U/L. Her peripheral blood smear revealed mild hypochromia, polychromasia and rare schistocytes. Direct and indirect antiglobulin tests were negative. Serum folic acid, ferritin, vitamin B12 and haptoglobin levels were normal. The patient was transfused for the second time because of anemia at 32 days of age. On follow-up, pyruvate kinase, glucose-6-phosphate dehydrogenase and 5'-nucleotidase enzyme activities, hemoglobin electrophoresis and osmotic fragility tests were

normal 3 months after the first transfusion when the patient was 4 months old. In further investigations, a diagnosis of paroxysmal nocturnal hemoglobinuria was excluded since CD59 and CD55 were normally expressed on erythrocytes. Her parent's complete blood counts and erythrocyte morphologies were normal. She underwent bone marrow aspiration at 5 months of age. Erythroid hyperplasia with many bi-nucleated erythroblasts with nuclei of different maturities and internuclear chromatin bridges, which raised the concern of CDA, was seen in bone marrow aspiration. Spongy appearance (Swiss cheese appearance) of heterochromatin in all normoblasts and expansion of the perinuclear areas and the extension of the cytoplasm towards the nucleus in some, were observed with electron microscopy. These findings were assumed to be compatible with CDA type I. In her follow-up, since the hemoglobin values were between 5-6 g / dl, she was enrolled in a transfusion program.

Patient 2, the four year younger brother of patient 1, was admitted to our clinic with pallor and icterus at the age of one month. He did not have hepatosplenomegaly on physical examination. On peripheral blood smear, mild hypochromia and polychromasia were seen but red blood cell morphology showed no acanthocytes or nucleated erythrocyte. His corrected reticulocyte count was 6.5%. Serum folic acid, ferritin, vitamin B12 and haptoglobin levels were normal similar to patient 1. Activities of pyruvate kinase, glucose-6-phosphate dehydrogenase and 5'-nucleotidase enzyme activities and osmotic fragility test were normal. Bone marrow examination showed erythroid hyperplasia with significant dyserythropoiesis similar to his sister and electron microscopy was not performed (Fig. 1). He was enrolled in an erythrocyte transfusion program due to consistent anemia (Hb < 7 g/dl).

A congenital dyserythropoietic anemia panel with next generation sequencing (NGS) including *CDAN1*, *C15orf41*, *SEC23B*, *KLF1* and *GATA1* genes showed no mutations in the patients. While they were being followed-up, a

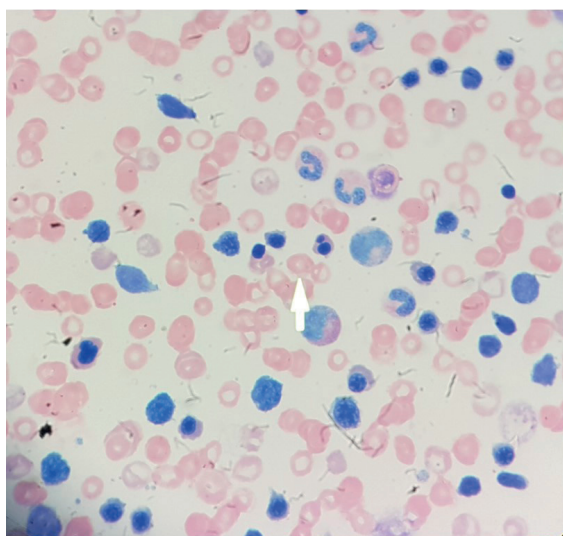


Fig. 1. Bone marrow smear showing erythroid hyperplasia with bilobulated erythroblasts and internuclear chromatin bridges.

newborn cousin with similar complaints was examined and diagnosed with PK deficiency due to a homozygous c.1623G>C (p.Lys541Asn) mutation in the *PKLR* gene in another center. Though PK enzyme levels of patients 1 and 2 were normal, a genetic test for PK deficiency was performed. In the molecular study of the *PKLR* gene, a homozygous c.1623G>C (p.Lys541Asn) in exon 12 was found in our patients. Subsequently, the heterozygous c.1623G>C mutation was identified in DNA samples of their parents by sequence analysis. Informed consent was obtained from the patients' families.

Discussion

Pyruvate kinase deficiency is a rare cause of hemolytic anemia and the differential diagnosis includes a heterogeneous group of both congenital and acquired hemolytic disorders. Though jaundice, severe indirect hyperbilirubinemia and significant anemia requiring transfusions are frequent in the neonatal period, as with our patient, some newborns have no evidence of jaundice or severe anemia.⁵ In children with PK deficiency, the most frequent symptoms are those related

to anemia, splenomegaly, jaundice, gallstones and secondary hemochromatosis.^{1,9} Due to the increased red cell 2,3-DPG content, which is responsible for a rightward shift in the oxygen dissociation curve of hemoglobin, the anemia may also be well tolerated. However, many patients with PK deficiency have fatigue related to their anemia.¹⁰

Our patients had neonatal onset severe hemolytic anemia with significant dyserythropoiesis. Dyserythropoiesis is considered to be present when erythroblasts show dysplastic features such as bi/multinuclearity, nuclear karyorrhexis, ring sideroblasts, cytoplasmic vacuolation, cytoplasmic PAS positivity, maturation asynchrony, megaloblastic changes, nuclear budding, internuclear chromatin bridges or duplication of the nuclear membrane. This can be seen in several congenital and acquired disorders such as CDA, some thalassemia syndromes, iron deficiency, vitamin B12 or folate deficiency, aplastic anemia, malaria and kala azar.¹¹ Onset of CDA also generally occurs in childhood, even clinical signs can occasionally be observed in the neonatal period.

Congenital dyserythropoietic anemia type I is characterized by erythroid hyperplasia and binucleated erythroblasts of different size and shape and thin chromatin bridges between nuclei of erythroblasts in the bone marrow.¹² The electron microscopy reveals unusual and significant morphological aberrations selectively within the erythroid series with progressing maturation. The pores of the nuclear envelope become more numerous and wider than normal and the nucleoli lack the filamentous component and have a purely granular appearance. This Swiss cheese appearance of heterochromatin, which is characteristic for CDA was also seen in our patient.¹³ Because the electron microscopy findings alone were not sufficient to diagnose CDA, we analyzed the mutation of our patients. However, no mutation was detected in the CDA type I related genes, *CDAN* and *C15orf41*. A 3-month-old child with evidence of dyserythropoiesis in bone marrow electron microscopic findings

suggesting CDA type I and coexistent unilateral multicystic dysplastic kidney and persistent PK deficiency was reported.¹⁴ Pereira et al.¹⁵ described another patient with clinically significant anemia who had dyserythropoiesis in the bone marrow suggestive of CDA and low PK activity. Of interest, coinheritance of *PKLR* and *GATA1* mutations were detected in that patient. In another study, Roy et al.¹⁶ analyzed 57 patients with congenital anemia with a novel 33-Gene targeted resequencing panel. In their study, an 18-month-old transfusion dependent girl was diagnosed with CDA because her bone marrow analysis showed erythroid hyperplasia with significant dyserythropoiesis and electron microscopy showed 'Swiss cheese heterochromatin'. Molecular analysis revealed compound heterozygosity for *PKLR* mutations, low PK levels were found and the bone marrow morphology was felt to be consistent with non-specific dyserythropoiesis rather than CDA.

In our patients; the c.1623G>C (p.Lys541Asn) mutation was found in exon 12 of the *PKLR* gene, which was reported previously as a novel mutation from Turkey.¹⁷ Unal et al.¹⁸ also described two patients with homozygous c.1151C>T and a novel homozygous c.880G>A mutation in the *PKLR* gene with erythroid hyperplasia along with double and multinucleated erythroid precursors, which suggested CDA. Recently, Hamada et al.¹⁹ performed whole-exome sequencing (WES) for 10 CDA patients who did not carry *CDAN1*, *SEC23B*, and *KLF1* mutations and reported that WES unexpectedly identified *G6PD* and *SPTA1* gene mutations known to cause congenital hemolytic anemia in two patients.

The causative mechanism for such hemolytic anemia exhibiting dyserythropoiesis similar to that exhibited in CDA remains unknown. Some authors thought dyserythropoiesis was secondary to ineffective erythropoiesis due to ATP depletion.¹⁴ However, others hypothesized that impaired erythrocyte membrane proteins cause incomplete chromosome segregation and cytokinesis in erythroblasts, resulting in

dyserythropoietic morphology.¹⁹ Coexistence of abnormalities of transcription factors or epigenetic modifiers of erythropoiesis might have a role in the development of dyserythropoiesis.

Given the rarity and the clinical heterogeneity, the diagnosis of these diseases can be difficult, mostly in atypical forms. Patients with PK deficiency are typically diagnosed in the neonatal period or early childhood. Patients have mild-to-moderate anemia that can be misdiagnosed as hemoglobinopathies such as thalassemia, more common hemolytic anemias such as hereditary spherocytosis, or inflammatory anemia. It is important to evaluate patients in terms of thalassemia mutation and other congenital membrane defects. Complications such as hyperbilirubinemia and gallstones can often be mistaken as Gilbert syndrome or hereditary hemochromatosis.

PK deficiency should be considered in the differential diagnosis of CDA. In addition to the enzyme activity, comprehensive genetic analysis is warranted for more effective diagnosis of patients with suspected CDA and congenital hemolytic anemia.

Ethical approval

Informed consent was obtained from the patients' families.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AKY, NYÖ, NY; data collection: AKY, AYE, DK; analysis and interpretation of results: AKY; draft manuscript preparation: AKY, NY. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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