Case Report

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Pyruvate Kinase Deficiency: A Near Miss

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Abstract

Hemolytic anemias are a myriad of conditions with similar signs and symptoms, but having numerous possible treatment options and outcomes. Hence, an accurate diagnosis is very important for treatment, counseling and prognostication of the disease. We present a male child with transfusion dependent anemia, jaundice and splenohepatomegaly. The standard tests for etiological work-up of anemia were normal/negative. He then underwent next generation sequencing, which revealed a mutation in the PKLR gene (confirming the pyruvate kinase deficiency). The child (at present) is being treated with regular packed red cell transfusions and monitoring of the ferritin level (for iron overload). It is emphasized that physicians treating anemic patients must maintain high level of suspicion for pyruvate kinase deficiency (PKD) in equivocal cases. Also, they need to be aware of the newer diagnostic methods available, like next generation sequencing including clinical exome sequencing.

Keywords: Hemolytic anemia; Hyperbilirubinemia, Next generation sequencing, PKLR gene, Pyruvate kinase, Transfusion.

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Introduction

Hemolytic anemias represent a group of disorders where there is excessive destruction of red blood cells. As our knowledge regarding the pathophysiology, etiology and genetics of these disorders expands, so does the plethora of the diagnostic tools. Every little detail added to the diagnosis can have a major impact on the management decisions and hence, an accurate diagnosis is important. Pyruvate Kinase is the most common glycolytic enzyme defect causing hereditary hemolytic anemia [3,7]. It has varied presentations and is difficult to diagnose based on standard tests due to frequent false negative reports [7]. It should therefore, be considered in the differential diagnosis of anemia where the standard etiological workup is unyielding [4]. Although there is no data on the prevalence of PK deficiency in the Indian population, majority of these cases are reported from Maharashtra, Gujarat and Uttar Pradesh [2]. We present a case of pyruvate kinase deficiency, who presented to us as an undiagnosed transfusion-dependent hemolytic anemia. He was extensively investigated for the etiology of anemia at our center and was finally diagnosed as pyruvate kinase deficiency on the basis of exome sequencing.

Case Report

Our patient, first born male (third degree consanguineous marriage) following up with us (since 3.5 months of age) with transfusion-dependent anemia used to require packed red cell transfusions every 3 to 4 months. There was no history suggestive of other blood cell line (white blood cells and platelets) affection. He had history of having undergone exchange transfusions on day 4 and day 7 of life for neonatal hyperbilirubinemia. It was, at that time, attributed to Rh-incompatibility, as the mother's blood group was B-Negative and child's blood group was B-Positive. There was absence of similar family history. On clinical examination at 5.5 years of age, the child had pallor, icterus, stunted growth and splenohepatomegaly (spleen 5 cm & liver 2 cm palpable). All the tests performed to ascertain the etiology of anemia had turned out to be normal/ negative. These included vitamin-B12 level, iron studies, Coomb's test (direct and indirect), hemoglobin electrophoresis, G6PD level and RBC membrane studies. Results of these investigations are presented in Table 1. A peripheral smear showed macrocytic non-spherocytic hypochromic anemia, reticulocytosis (count of 18%) and normal WBC and platelet counts. The bone-marrow examination (done at the age of 8 months) showed erythroid hyperplasia and other (normal) cell lines, suggestive of peripheral destruction of red cells. Pyruvate kinase (PK) and other red cell enzyme levels (done at 8 months of age) were also within normal limits. He finally underwent clinical exome sequencing for hemolytic anemia (at the age of 5 years), which showed a mutation in the PKLR (Pyruvate Kinase LR) gene (c.880G>A p.val 294> met), which is known to cause pyruvate kinase (PK) deficiency. The child is now being treated with regular packed red cell transfusions (every 3 to 4 monthly) with monitoring of ferritin levels.

Discussion

Pyruvate kinase deficiency (PKD) is inherited as an autosomal recessive condition and hence clinical features are seen only in homozyogous and compound heterozygous individuals [7]. The clinical features of PKD often vary over a spectrum from severe deficiency manifesting as anemia with hydrops fetalis in-utero to mild anemia that can evade detection until adulthood [1]. Jaundice can be the sole presenting feature if the hemolysis is well compensated for [3]. Neonates, with PKD, can develop hyperbilirubinemia requiring prolonged

Table 1: Details of investigations for anemia in our patient (done at age of 8 months).

Sr. No.	Investigation	Result	Normal values
1.	Hemoglobin (g/dL)	5.1	12.5 (11.5)*
2.	Reticulocyte count (%)	18	0.5- 1
3.	Mean corpuscular volume (fL)	89.5	81 (75)*
4.	Mean corpuscular hemoglobin concentration (%)	25.9	34 (31)*
5.	Total leukocyte count (cells/cumm)	13,800	4500-13500
6.	Platelet count (Lakhs/cumm)	2.54	1.5-3.5
7.	Peripheral smear	Anisopoikilocytosis + Crenated RBC + Few tear drop cell	
8.	Sr. Vitamin B12 (pg/mL)	266	200-800
9.	Hemoglobin electrophoresis:		
	HbA2 (%)	2.7	<3.5
	HbF (%)	1.1	<2
	Abnormal Hb	None	None
10.	Red-cell enzyme levels (IU/gm Hb):		
	Pyruvate kinase	9.5	7.5 to 11.0
	G6PD	7.9	6.5 to 13.0
	Phosphoglycerate Kinase	159.7	150 to 250
	Glucose phosphate isomerase	53.1	45 to 75
11.	Coomb's test (direct and indirect)	Negative	
12.	Red cell membrane studies (for hereditary spherocytosis): Flow cytometry of red cell Eosin-5-maleimide in mean channel fluorescence units (MCF)	1145.96	900 to 1300

* Values are Mean (-2SD)

phototherapy or exchange transfusions (as was necessary in our case in the neonatal period) [6]. Transient exacerbations of anemia and jaundice are also known with intercurrent illnesses, stress and pregnancy [3]. The gold standard for diagnosis of PKD is measurement of enzyme activity within erythrocytes [1]. However, this enzyme-activity is red-cell age dependent (being higher in younger cells and decreasing as the RBC ages) and may be masked by reticulocytosis [3]. It should be noted that even though pyruvate kinase levels are normal, rest of the red cell age-dependent enzyme activity is elevated [1]. This however was not seen in our case. The accurate diagnosis in such cases (where the standard tests are inconclusive) requires advanced diagnostic tools such as next generation sequencing [8].

PK enzyme, in humans, exists as a tetramer of tissue specific sub-units. Four of these subunits are known and include R sub-unit found in red cells, L in the liver, M1 in muscle, heart and brain and M2 in early fetal tissues [7]. Neonates developing severe hyperbilirubinemia in the absence of significant hemolysis is attributed to deficiency of the liver isoenzyme [6]. A single gene PKLR, present on chromosome 1q21 codes for the R and L sub-units [9]. More than 200 mutations are reported in this gene, including missense, splice site mutations and insertion-deletions [7]. The mutations most commonly seen in Indian population include c.1436G>A (18.33%), c.992A>G (11.66%) and c.1456C>T (11.66%) as reported in a study (in the year 2013) [8]. The mutation seen in our case, is a novel mutation and has not yet been reported [8,10].

The treatment of PKD is symptomatic with regular red cell transfusions (as curative therapy is not available) and supportive with chelation for the iron overload [1]. The frequency of the red cell transfusions slowly decreases, as the child gets older [3]. Adults and adolescents, thus, require fewer transfusions. Newborns often develop hyperbilirubinemia and may require phototherapy and/or exchange transfusions [6].

In 1966, splenectomy was first described as a treatment option for PKD [5]. Since then, several studies have evaluated the effectiveness of this intervention in PKD and have found it useful especially in older children and adults with high transfusion requirements [4]. Partial splenectomy where 80-90% of the spleen is removed has also been tried [1]. Due to rarity of the disease and varied presentation, there are no standards/ guidelines available for the optimal hemoglobin levels, frequency of transfusions or ideal candidates for splenectomy [1]. All patients planned for splenectomy should receive vaccination against capsulated organisms including pneumococcal and meningococcal vaccines and penicillin prophylaxis after the splenectomy [5]. Curative hematopoietic stem cell transplant has also been attempted, however, it remains an experimental treatment [1]. The major complications of PKD include iron overload in both, transfusion dependent and independent patients [3]. The onset and severity of iron overload is determined by the frequency of transfusions required and thus the severity of enzyme deficiency [7]. Other complications include post-splenectomy infections with capsulated organisms and post-splenectomy thrombosis [1]. Gallstones are more common in patients with PKD than in other hemolytic anemias (and may be seen even after splenectomy) [1]. Successful pregnancies have been reported in these patients [1]. However, it should be noted that the rate of hemolysis may increase during pregnancy [3]. Therefore, pregnant PKD patients should receive regular transfusions and should not receive iron, as there is increased risk of iron overload [3].

Conclusion

Pyruvate kinase deficiency should be suspected as the cause for unexplained hemolysis and neonatal hyperbilirubinemia when the standard tests for etiological diagnosis of the hemolytic anemia do not reveal any abnormality or are equivocal. It is essential to employ the newer diagnostic modalities such as next generation sequencing to establish the diagnosis in such cases.

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