Multicenter Study of Pyruvate Kinase Deficiency in Argentina

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Abstract: The red blood cell (RBC) *pyruvate kinase deficiency* (PKD) is the most common recessive congenital defect of glycolytic enzymes associated with non-spherocytic hemolytic anemia. It is a rare hereditary disorder caused by >300 variants in the *PKLR* gene. This is a retrospective study of 19 patients from different centers from Argentina with confirmed molecular diagnosis of PKD. Clinical follow-up was carried out from birth in most cases. Five consanguineous patients from "gypsy" community, were homozygous for the "PK-Gypsy deletion" (PK-Gd). During the neonatal period they developed anemia with icterus. Transfusion exchange was required in 60%, light therapy in 80%, and RBC transfusion in 80%. During the follow-up iron overload was detected in the 100%, cholecystectomy was indicated in 40%, and splenectomy in 60%. Thirteen cases had 2 missense variants (MS), being the Mediterranean variant (p.Arg486Trp) the more frequent detected (26%).Only 1 patient had a missense-splicing mutation combination. During the neonatal period, 86% had anemia and icterus. Light therapy was required in 78%, transfusion exchange in 21% and RBC transfusion in 64**%.** During the follow-up iron overload was detected in 57% and splenectomy was indicated in 43%. Transfusions (pre-splenectomy and post-splenectomy) were more required in PK-Gd cases as compared with patients with point mutations (100%/60% vs 71%/29% respectively). Our data indicates a high clinical-therapeutic-molecular heterogeneity in PKD patients with the PK-Gd group presenting the most severe cases.

Keywords: Pyruvate Kinase Deficiency, Anemia, RBC transfusion, Iron overload.

INTRODUCTION

Pyruvate kinase deficiency (PKD) is a rare congenital disorder characterized by reduced activity of the Pyruvate kinase (PK) enzyme in red blood cells (RBCs), leading to lifelong chronic hemolysis and associated with common complications such as anemia, jaundice, gallstones, iron overload, endocrinopathies, and bone disease [1]. It was suggested that PKD frequency is likely between 3.2 and 8.5 cases per million in Western populations [2], and the variability in the reported prevalence, could be a consequence of under-diagnosed PKD patients due to a mild or no clinical expression [3].

PKD is a recessive disorder caused by more than 300 variants in the *PKLR* gene. These large number of *PKLR* variants and its different combinations, may have variables functional impacts on enzyme activity and probably contributes to its remarkable clinical

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heterogeneity [4]. The clinical symptoms can range from the most severe forms, such as hydrops fetalis, intrauterine death, or severe neonatal jaundice to fully compensated hemolytic anemia.

PK is the glycolytic enzyme that catalyzes the irreversible conversion of phosphoenolpyruvate to pyruvate, generating the synthesis of an ATP molecule to maintain the integrity and function of RBCs. Therefore, PKD is related to inadequate ATP production that can affect red blood cells homeostasis and induced, their premature elimination from the circulation by the spleen [5]. Hemolytic crisis and RBC transfusions are directly related with body iron overload with increased iron stores in liver and heart.

Iron overload in PKD can also develop in absence of RBC transfusions, mimicking a dyserythropoietic phenotype. Consequently, severe forms of PKD can be misdiagnosed as congenital dyserythropoietic anemias (CDA), as well as other congenital hemolytic anemias. Patients with PKD and low hepcidin levels have been recently reported, similar to cases with CDA, suggesting that an increased intestinal iron absorption can also contribute to iron overload [6].

Hepatic iron deposition can be quantified by magnetic resonance T2-star (T2*) technique for the measurement of tissue iron, with validation to chemical estimation of iron in patients undergoing liver biopsy. Myocardial iron deposition can be reproducibly quantified by magnetic resonance T2-star (T2*) technique, T2* (in mseconds) has an inverse correlation with the cardiac level tissue iron load [7].

Here we describe the clinical, therapeutic and molecular features of 19 cases of PKD registered by the Erythropaties Commission of Argentine Society of Hematology (SAH) in Argentina.

PATIENTS

A retrospective and descriptive study of 19 patients with confirmed molecular diagnosis of PKD is presented. The age at clinical diagnosis ranged between 12.6 to 240 months (median 107.1 months). The follow-up time ranged between 3.5 moths to 1365 months. The more relevant clinical features as anemia, jaundice, gallstones, iron overload, were analyzed from the neonatal period and subsequent follow-up.

HEMATOLOGICAL AND BIOCHEMICAL ANALYSIS

The routine hematologic studies were performed to rule out other causes of chronic hemolytic anemia. The corresponding laboratory values as complete blood count, hemoglobin, serum ferritin, serum bilirubin, reticulocyte count (%), were evaluated before and after different therapeutics as RBC transfusion, light therapy, splenectomy. Only in two patients PK enzymatic activity could be evaluated.

MOLECULAR ANALYSIS OF THE *PKLR* GENE

Point variants and small deletions/insertions in the *PKLR* gene were analyzed by PCR-Sanger Sequencing [8] and the PK-GYPSY deletion was evaluated by GAP-PCR as previously described [9].

IRON OVERLOAD EVALUATION BY SERUM FERRITIN AND LIVER/HEART MRI STUDIES

The iron overload was evaluated by serum ferritin (SF) and magnetic resonance imaging estimation of somatic iron overload at liver and heart level (7; 10).

TREATMENTS

Treatments included exchange transfusion, light therapy and RBC transfusion. All cases with indication of splenectomy received immunizations, with the pneumococcal, meningococcal, and haemophilus influenza vaccines, and no post-splenectomy complications were observed.

Iron chelation therapy with Deferasirox (DFx) was administered in patients with iron overload [11].

RESULTS

Five patients belonging to Gypsy community were consanguineous and presented the PK Gypsy deletion in homozygous state. This group corresponds to the 26% of all patients analyzed. During the neonatal period all these patients presented anemia and jaundice; four patients required RBC transfusion, 4 light therapy and 3 transfusion exchange. During the follow up time all required regular RBC transfusion (>6/year). Three patients had splenectomies and reduced TGR requirement to 1-5 per year post splenectomy. Two patients required cholecystectomy and the five patients of this group presented iron overload and received iron chelation therapy (Figure **1** and **2**).

The remaining 14 patients (74%) were compound heterozygous or homozygous for point variants in the *PKLR* gene (Table **1**): 13 presented 2 missense variants (MS) and 1 had the combination of a missense with a null variant affecting splicing. Four of these variants (*) were novel and have been recently reported by members of our group [8]. Of the 27 alleles

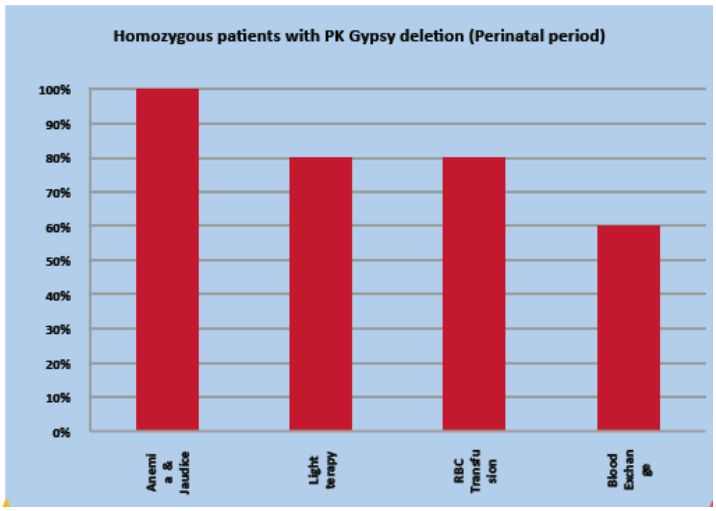

Figure 1: Main clinical features of patients with PK-Gypsy deletion during perinatal period.

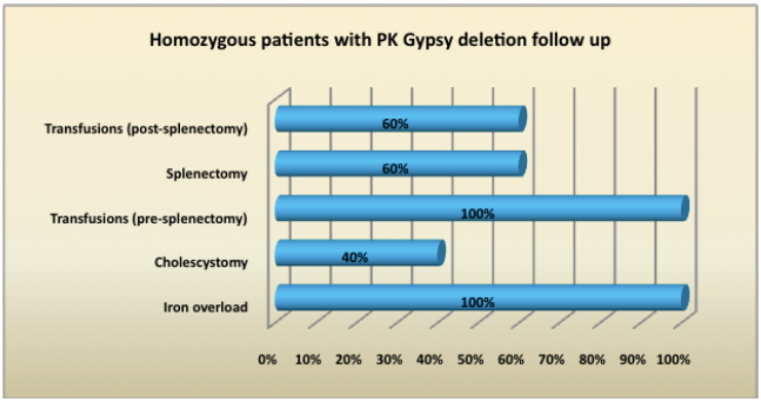

Figure 2: Requirement for blood exchange, light therapy and RBC transfusion in patients with PK-Gypsy deletion during the follow-up.

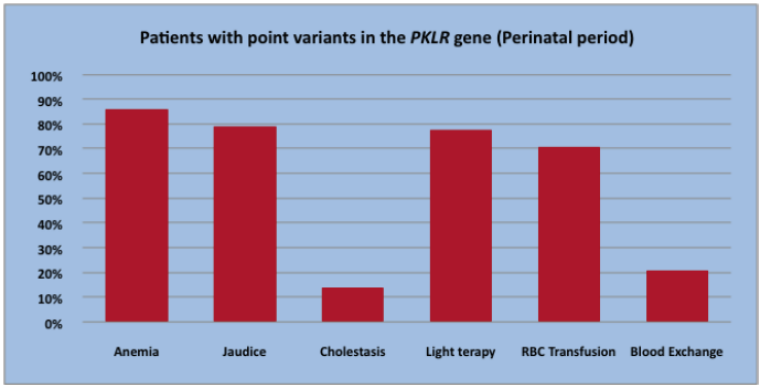

Figure 3: Main clinical features of patients with point variants in the PKLR gene.

with MS variants, 7 (26%) carried the frequent variant in the Mediterranean population p.Arg486Trp [4]. During the neonatal period 12 patients presented anemia, 11 jaundice and 2 cholestasis. Ten patients required RBC transfusion, 3 transfusion exchange and 11 light therapy. During the follow up time 10 patients continued with RBC transfusion requirement; regular in 7 and sporadic in 3. Six patients had splenectomy and reduced RBC transfusion requirement: 4 required sporadic RBC transfusion while two stopped having a transfusion requirement. In this group only 8 patients (57%) presented iron overload and received iron chelation therapy (Figure **3** and **4**).

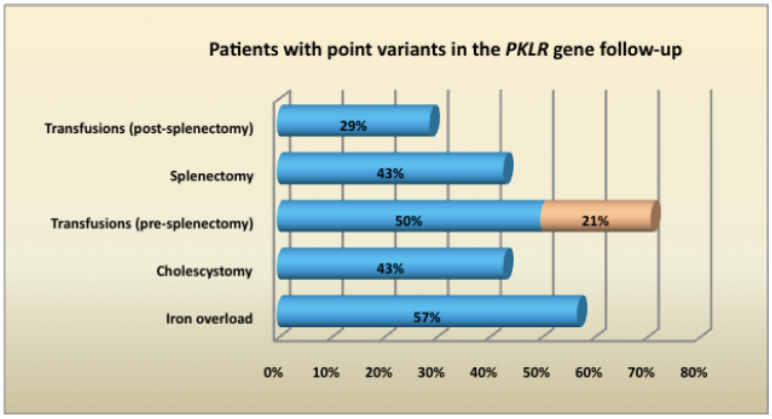

Figure 4: Requirement for blood exchange, light therapy and RBC transfusion in patients with point variants in the PKLR gene during the follow-up.

Table 1: Genotype of the 19 Pyruvate Kinase Deficient Patient Included in this Study

Patient #	Genotype allele 1	Genotype allele 2
#1	NM_000298.4:c.1178 A>G (p.Asn393Ser)	NM_000298.4:c.1670 G>C (p.Gly557Ala)
#2	NM_000298.4: c.1436 G>A (p.Arg479His)	NM_000298.4: c.1436 G>A (p.Arg479His)
#3	NM_000298.4: c.1269 G>A (p.Ala423Ala)	NM_000298.4: c.1456 C>T (p.Arg486Trp)
#4	NM_000298.4: c.1021G>A (p.Gly341Ser) *	NM_000298.4: c.1456 C>T (p.Arg486Trp)
#5	NM_000298.4: c.1021G>A (p.Gly341Ser) *	NM_000298.4: c.1706 G>A (p.Arg569Gln)
#6	NM_000298.4: c.347 G>A (p.Arg116Gln) *	NM_000298.4: c.1232 G>T (p.Gly411Val) *
#7	NG_011677.1: g.13980_15119delPK Gypsy	NG_011677.1: g.13980_15119delPK Gypsy
#8	NG_011677.1: g.13980_15119delPK Gypsy	NG_011677.1: g.13980_15119delPK Gypsy
#9	NM_000298.4: c.1456 C>T (p.Arg486Trp)	NM_000298.4: c.1456 C>T (p.Arg486Trp)
#10	NM_000298.4: c.1456 C>T (p.Arg486Trp)	NM_000298.4: c.1456 C>T (p.Arg486Trp)
#11	NG_011677.1: g.13980_15119delPK Gypsy	NG_011677.1: g.13980_15119delPK Gypsy
#12	NM_000298.4: c.1529 G>A (p.Arg510Gln)	NM_000298.4: c.1595 G>C (p.Arg532Pro) *
#13	NM_000298.4: c.1223 C>T (p.Thr408Ile)	NM_000298.4: c.1223 C>T (p.Thr408Ile)
#14	NM_000298.4: c.1483 G>A (p.Ala495Thr)	NM_000298.4: c.1516 G>A (Val506Ile)
#15	NM_000298.4: c.1021G>A (p.Gly341Ser) *	NM_000298.4: c.1456 C>T (p.Arg486Trp)
#16	NM_000298.4: c.695-2 A>T	NM_000298.4: c.1511 G>T (p.Arg504Leu)
#17	NG_011677.1: g.13980_15119delPK Gypsy	NG_011677.1: g.13980_15119delPK Gypsy
#18	NG_011677.1: g.13980_15119delPK Gypsy	NG_011677.1: g.13980_15119delPK Gypsy
#19	NM_000298.4:c.118C>T (Arg40Trp)	NM_000298.4:c.347 G>A (p.Arg116Gln) *

DISCUSSION

Although the number of patients presented is limited, they are the total number of cases that could be registered by the members of the erythropathies commission of the SAH, therefore, they are a representative sample of the PKD in Argentina.

The possibility of PKD should be considered in patients with active hemolysis with no findings suggestive of an acquired autoimmune process, red cell membrane defects, or hemoglobinopathies. Pyruvate Kinase deficiency (PKD) is a rare cause of hereditary hemolytic anemia (AHH), usually moderate to severe. PKD is difficult to diagnose, because it

presents characteristics that overlap with other hereditary hemolytic anemias, and requires the performance of different types of studies for a differential diagnosis and molecular tests for its confirmation. Patients can have a variable phenotype and normal or near-normal red cell morphology. During long time, enzyme testing has been the gold standard for diagnosis. More recently, *PKLR* gene testing has become clinically available and is now used as a confirmative test. This allowed the molecular characterization of the *PKLR* gene in this group of Argentinian patients, and documented in the SAH records.

PK-Gypsy deletion homozygous patients presented a clinical picture of chronic hemolytic anemia with higher red blood cell transfusion (RBC-t) requirement and with a significant iron overload. In these patients, splenectomy was more required as compared with other mutations. In all cases splenectomy decreased the RBC-requirement, demonstrating the importance of its indication. Splenectomy can be associated with a life-long risk of sepsis with encapsulated organisms; and additionally, it has been associated with risk of thromboembolic disease [12]. Our pediatric patients who had splenectomies did not present with complications related to this procedure.

The patients with heterogeneous and non-deletional molecular alterations presented variable clinical phenotype since compensated/chronic hemolytic anemia to chronic hemolytic anemia with regular RBC-t requirement. As has been previously reported, missense variants are the more prevalent in *PKLR* gene and phenotype- genotype correlation for this type of mutations is difficult since two different variants are present in the two alleles in most patients. These prevalent genotypes explain in turn the clinical heterogeneity that this group of patients presents (4;13).

In our study, care of the newborn infant with PKD was critical because severe hyperbilirubinemia was a common finding, even in patients who later presented mild phenotypes, and these newborns often required phototherapy and/or exchange transfusions.

In patients with PK-GYPSY deletion iron overload was the most frequent complication, highlighting the need for chelation treatment and periodic controls to improve morbidity. The early application of iron chelation therapy has made it possible to avoid complications due to cardiac iron overload and reduce its morbidity.

The importance of including PKD as a differential diagnosis in all pediatric patient with hemolytic anemia is highlighted, since diagnostic delay can lead to inappropriate therapeutic behaviors. Enzymatic detection of PK is not very accessible in our setting, but molecular studies allowed diagnostic confirmation of all the cases presented here.

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