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# Prevalence of Pyruvate Kinase Deficiency among the Newborns (Shiraz-Iran)

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## Abstract

**Background:** The frequency of pyruvate kinase (PK) deficiency, an autosomal recessive defect, is approximately 3 per 10,000 individuals in Shiraz and surrounding areas, and is increased due to high consanguinity marriage frequency. The purpose of this study is to obtain data on the frequency and spectrum of gene mutation of PK in newborns, from Shiraz and surrounding areas.

**Materials and Methods:** Two hundred eleven neonates with neonatal jaundice were studied for erythrocyte pyruvate kinase activity using the method recommended by International Committee for Standardization in Hematology (ICSH).

**Results:** In 22 the PK enzyme activity was below 60%, where the erythrocyte PK activity from 35 healthy cord bloods ranged from 3.9 – 9.8 IU/g Hb.

Genomic DNA analysis for PK-R gene mutation was examined in 12 out of 22 cases (heterozygote 3.8%, 95% CI=0.012-0.064; homozygous 1.8%, 95% CI=0.001-0.036). All mutations in four homozygote and 8 heterozygote neonates in this cohort have been reported from the region previously, except for mutation G1675C that has not been found before. Therefore, the rate of recurrence of PK-R gene defect is 4 times more frequent in those with neonatal jaundice in comparison with the regional frequency (0.038).

**Conclusion:** Erythrocyte enzymopathies, especially PK deficiency, should be considered in the differential diagnosis of non-immune hemolytic anemia as well as for genetic counseling in our area.

**Keywords:** Pyruvate Kinase, Mutation, Prevalence, Newborn, Iran.

## Introduction

Neonatal jaundice is the most common cause for hospital admission in the first 2 weeks of life. Hyperbilirubinemia and anemia at birth may be due to four major etiologies: immune incompatibility of ABO or Rh group, hereditary cell membrane defects, hemoglobinopathies, and red blood cell (RBC) enzymopathies.[1] Among the enzymopathies, glucose-6-phosphate-dehydrogenase (G6PD) and pyruvate kinase (PK) deficiencies are the most common defects in diverse ethnic groups.

PK converts phosphoenolpyruvate to pyruvate in anaerobic glycolysis and is critical for production of ATP in erythrocytes. In human, the enzyme is coded by PK-LR gene in erythrocytes. Homozygote or compound heterozygote state of abnormal allele causes non-spherocytic hemolytic anemia with variable clinical severity. Severely anemic patients may require regular blood transfusion and splenectomy. Some abnormal mutations may be associated with interauterine death.[2]

Pyruvate kinase deficiency which contributes in about 80-90% of cases of glycolytic and nucleotide enzymopathies is inherited as an autosomal recessive manner and until now, 180 PK mutations has been reported.[3,4]

The gene frequency of PK deficiency in the south provinces of Iran varies from 0.010 to 0.026,[5] and predicted by the Hardy-Weinberg equation, homozygote newborn in the region should be approximately 3 per 10,000 individuals. The major factor that can affect the frequency of

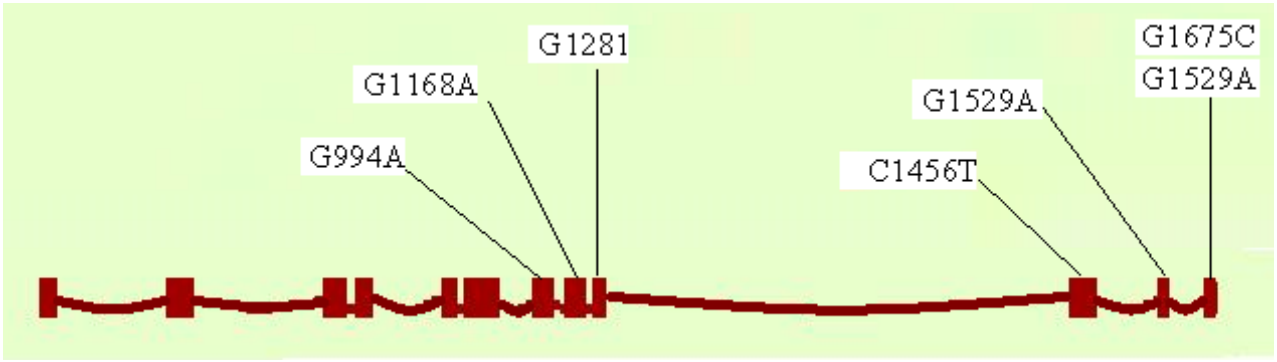


***Figure 1.*** *PK-R gene with twelve exons and position of detected mutations in the affected newborns.*

homozygosity is consanguinity. This study was performed to evaluate the prevalence of PK deficiency and spectrum of PK-R gene mutations in newborns with neonatal jaundice in referral university hospitals.

## Materials and Methods

In a period of three months, four hundred and eight hospitalized newborns, suspected to have neonatal jaundice, were candidates for PK evaluation. Two hundred eleven parents gave full consent for the study. None of them had any exchanged blood transfusion. The blood samples were collected in 1-2 mg/ml of EDTA and determination of enzyme activity was performed within 48 hours. The ICSH-recommended method was performed for the measurement of erythrocyte enzyme activities.[6] The enzyme activity of PK was measured at 37°C by lactate dehydrogenase coupled spectrophotometric assay. The results were calculated as U/g hemoglobin (Hb) and subsequently adapted to percentage of normal activity.[7]

### *Reference Subjects*

In order to define expected reference limit for the local newborns, 35 cord blood samples from normally delivered newborns that did not show any evidence of neonatal jaundice within 48 hours were selected, and the enzyme activity was determined.

### *Molecular Study*

Genomic DNA extraction and molecular analysis of 22 patients were carried out.[5] All exons of R-PK were amplified by polymerase chain reaction at 95°C for 60 seconds, 58°C for 45 seconds, and 72°C for 60 seconds in 35 cycles and final extension was 72°C for 2 minutes. Mutation analysis was screened by single-strand conformation polymorphism (SSCP) and restriction fragment length polymorphism (RFLP) for all exons product according to Zanella *et al* [8] and Baronciani *et al* [9], respectively.

## Results

Among 35 cord blood samples the measured range of RBC PK activity was between 4.5 and 15.9 IU/g Hb and 22 out of 211 term newborns with neonatal jaundice had enzyme activity below 60% of normal control.

### *Mutation in PK-R Gene*

To examine mutations of the PK-R type gene, we assumed that 22 cases have the total PK deficiency or partial deficiency.

Out of 24 chromatids examined, we detected 16 mutant alleles while the rest did not have any mutations in the 12 coding regions. The arrangement of mutant allele in 8 individuals was presented with the heterozygote patterns (3.8%; 95% CI=0.012-0.064), and in 4 cases with homozygous or double heterozygote status (1.8%; 95% CI=0.001-0.036).

The mutations that were revealed in this cohort study of newborns are summarized in table 1. All mutations except one (G1675C) have been identified in a previous regional population survey.[5] The distribution of each mutation is depicted on the gene (figure 1).

Total and indirect serum bilirubin levels in 4 cases with defect on both alleles were 13.7± 6.3 and 12.1±6.2 mg/dl, respectively and their hemoglobin level was 10.2±2.3 gm/dl.

***Table 1.*** *Allele Frequency of R-pyruvate kinase gene mutation found in 16 chromatids of neonates.*

| PK Mutations | No. of subjects (%) |
|---|---|
| G1168A/ G1168A | 2 ( 12.5) |
| C1456T /C1456T | 1 (6.25) |
| G1529A/C1492T | 1 (6.25) |
| G1529A/W | 3 (18.75) |
| G1492T/W | 2 (12.5) |
| G994A/W | 1 (6.25) |
| G1281/W | 1 (6.25) |
| G1675C*/W | 1 (6.25) |

* Formerly was not reported from Iran.

## Discussion

Except for G6PD and Phosphoglycerate kinase which are X-linked, the other 12 hereditary erythrocyte enzyme abnormalities that result in heridatery non-spherocytic hemolytic anemia (HNSHA) have autosomal recessive (AR) manner. It is believed that approximately 80% 0f HNSHAs are due to PK and G6PD,[10] and PK deficiency is the most common and heterogeneous AR defect. Up to now, more than 180 different mutations on PK-LR gene have been reported.[11] Each mutation has a different effect on enzyme activity and expresses a broad spectrum of clinical presentations from mild to severe transfusion-dependent hemolytic anemia resulting from either compound heterozygote or homozygote state. This variability of phenotype is associated with particular cruel mutations which may be frequent alleles, though their carrier state is not common in the pointed region or ethnic group.

Pyruvate kinase deficiency (PK; EC 2.7.1.40 and OMIM: 266200) is the most frequent enzyme abnormality of glycolytic pathway that affects generation of ATP in the cells.[12] A tissue specific expression of four isoenzymes (M1-, M2-, L-, and R-type) is mainly regulated by developmental, dietary, and hormonal control.[13] The four different PK isoforms are the products of two genes PK-M and PK-LR. The alternate tissue-specific promoters and splicing mRNA generate different isoenzymes.[14,15] The R-Type is restricted to the erythrocytes. Severe PK deficiency can cause neonatal anemia due to reduction in the ATP levels, since erythrocytes are completely dependent on their generation by glycolysis.[16] Mutation in PK-LR gene may result in abnormal enzyme synthesis in both erythrocytes and liver. Clinical symptoms are solely related to abnormal synthesis in erythrocytes, since the other isoenzyme in the hepatocyte compensates for defected L- or/and R-type PK.[17] The severity of hemolysis is variable and range from mild to severe, depending on the position of mutation and finding of homozygocity or double heterozygosity of mutated alleles. Hydrops fetalis and death in the neonatal period, [18,19] neonatal anemia, and chronic anemia and subsequent need for continuous transfusion support are possible adverse effects of the harsh abnormal enzyme.

The PK activity of four affected newborns was less than 2.7 IU/g Hb (range from 1.1 to 2.7) and hemoglobin levels were lower compared with normal controls. M-PK gene reactivation or conserved activity may be responsible for unexpected enzyme activity in mature erythrocytes.[20] Differential diagnosis of M-PK or R-PK at the isoenzyme levels were not performed in this study. In spite of lacking evidence for compensation in a PK deficient patient with null mutation,[21] it has been declared that quantitative decrease of PK enzyme is accompanied with persistent expression of M2 gene in the mature erythrocyte.[20] The controlling mechanism of compensation PK-M gene expression and potentially epigenetic factors is not clearly understood. The expected homozygosity for C1456T mutations is $1{:}4*10^6$ and usually associated with very severe hemolytic anemia.

Heterozygote carrier usually has no clinical manifestation and PK enzyme activity range from 40% to 60% of the normal mean value. In this study, eight heterozygote newborns had activity between 2.7 and 5.5 IU/g Hb and did not have anemia on admission. Among missense mutations, the C1456T and G994A alleles at homozygous state are usually associated with very severe hemolytic anemia.

PK deficiency has a world-wide distribution and the collective data show an increasing frequency from Europe to Asia population,[22-26] as well as from central area of Iran to the Arabian Peninsula.[26]

## Conclusion

Our study suggests that erythrocyte enzymopathies, especially PK deficiency, must be considered in the differential diagnosis of non-immune hemolytic anemia, also for genetic counseling. By screening of hyperbiliruinemic neonates which was done in this study, 4 homozygote cases of PK deficiency were revealed and had a moderate to severe clinical course. Hence, PK deficiency is not common in Iran but high rate of consanguinity increases the possibility of homozygote state.

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