FREQUENCY OF PYRUVATE KINASE (PK) DEFICIENCY IN NEONATES WITH HAEMOLYTIC ANAEMIA

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ABSTRACT

Objective: To determine the frequency of pyruvate kinase deficiency in neonates presenting with haemolytic anaemia.

Study Design: Cross sectional descriptive study.

Place and Duration of Study: Haematology department, Armed Forces Institute of Pathology, Rawalpindi (AFIP) from Jan 2011 to Jan 2012.

Material and Methods: Study was done in collaboration with neonatology department of Military Hospital. Informed consent from parents of neonates was obtained. Two hundred and twenty five neonates with haemolytic anaemia based on low haemoglobin (<14g/dl), raised reticulocyte counts (>5%) and indirect hyper bilirubinaemia (as per CDC nomogram for evaluation of hyper bilirubinaemia in term neonates) were selected. Qualitative pyruvate kinase enzyme assay was done using Bio vision PK assay kit. Estimation of enzyme was based on generation of pyruvate by addition of substrate with change in optical density (OD) of sample in presence of enzyme. Dilution of standard as recommended by manufacturer was made and standard graph was plotted. The OD was measured using wave length of 570 nm at two points (start and at 25 min). Cut off limit of less than 25% activity was considered positive for pyruvate kinase deficiency. Confirmation was done by running in parallel negative and positive controls (provided).

Results: Seven (3.1%Confidence Interval: ±3.33) out of 225 patients were found deficient. Among these 4 were male and 3 were female neonates. The age range was between 1 to 3 weeks. The mean age of presentation was 1.89 ± (0.832) weeks.

Conclusion: We conclude that pyruvate kinase deficiency is not uncommon in our setup and all patients with congenital non-spherocytic haemolytic anaemia where cause cannot be established should be screened for pyruvate kinase deficiency.

Keywords: Congenital Non Spherocytic Haemolytic Anaemia, Pyruvate Kinase Deficiency.

INTRODUCTION

Mature red blood cells (RBC) do not have mitochondria and their main source of energy is via anaerobic glycolysis (Embden Meyerhof Pathway). This glycolytic pathway converts glucose into pyruvate anaerobically yielding two ATP molecules. A number of enzymes are involved in the process. Pyruvate kinase (PK) converts phosphoenolpyruvate to pyruvate with gain of one ATP molecule[1]. Insufficient ATP generation due to deficiency of PK enzyme results in failure of normal metabolism in red cells leading to loss of membrane plasticity causing red cell to get trapped and destroyed in splenic vasculature. PK deficiency is a common enzymatic defect, inherited in autosomal recessive manner. Two genes PK-M, PK-LR, located on chromosome 15q and 1q code for the enzyme. Four isozymes are produced by post translational alternate splicing sites.

Deficiency or structural defect of PK results in congenital non-spherocytic haemolytic anaemia (CNSHA)[2]. Homozygous or compound heterozygous mutations results in clinical manifestations[3]. More than 180 different mutations have been identified with correlations established between amino acid substitutions and their structural and functional effects[4].

Clinical presentations vary from hydrops foetalis, in utero death to mild fully compensated haemolytic anaemia. Early presentations are associated with severe deficiencies while mild deficiency may go

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unnoticed till later age. Anaemia is well tolerated in PK deficient patients as a result of 2, 3 diphosphoglycerate (2, 3 DPG) accumulation causing right shift of O_2 dissociation curve[5].

PK deficiency is prevalent worldwide. In white population the incidence is 1 to 5%[5,6] and 3% in Hong Kong[6]. In UK it is 3.2 per million population based on enzymatic assays[8]. However no data is available for Pakistan. Therefore aim of this study was to estimate frequency of PK deficiency in our setup. The rationale of this study is to establish our own data and provide early diagnosis and treatment to the patients subsequently.

MATERIAL AND METHODS

This cross sectional descriptive study was carried out at AFIP in collaboration with neonatology department of MH from Jan 2011 to Jan 2012. Two hundred and twenty five neonates with HA were selected. Informed consent was taken from the parents. Blood samples were taken by aseptic technique in ethylene-diaminetetraacetic acid (EDTA) paediatric CP bottles. Inclusion criteria was set to demonstrate haemolysis by low haemoglobin HB (<14g/dl), raised reticulocyte counts (>5%) 1, raised lactate dehydrogenase and indirect hyper-bilirubinaemia (as per CDC nomogram for evaluation of hyperbilirubinaemia in term neonates).Consecutive non probability sampling was done. Patients not meeting inclusion criteria were excluded from study.

Colorimetric kinetic assay was done on washed red cells using Bio vision (US) PK assay kit. Two ml venous blood in EDTA was collected. RBCs were separated by centrifugation at 2000 revolutions per min (RPM) for five minutes. The supernatant containing platelets and white blood cells was discarded. RBCs were washed with normal saline. Haemolysate was prepared by using assay buffer (provided). Estimation of enzyme was based on generation of pyruvate by addition of substrate with change in optical density (OD) of sample in presence of enzyme. Generated pyruvate was oxidized by pyruvate oxidase to produce colour ($\lambda = 570$ nm). Dilution of standard as recommended by manufacturer was made and standard graph was plotted. The OD was measured using wave length of 570nm at two points (start and at 25 min). PK activity was measured against the standard (provided). Cutoff value of less than 25% activity was considered positive for PK deficiency. Data was analysed by using SPSS version 19. Data was summarized as means ± standard deviation (SD) number or percentage as appropriate. Fishers exact test and unpaired t test were used for comparison of variable and data as applicable. A *p* value of less than 0.05 was considered significant.

RESULTS

Two hundred and twenty five neonates with haemolytic anaemia were selected. Out of 225 neonates 130 (58 %) were male and 95 (42%) were female patients. Seven (3.1%CI: ±3.33) out of 225 patients were found deficient (PK activity < 25%). Among these 4 (57%) were male and 3 (43%) were female neonates. The age range was between 1 to 3 weeks. The mean age of presentation was 1.89 ± 0.832 weeks, with chief clinical presentation of neonatal jaundice. (Table).

DISCUSSION

The term CNSHA was first used by Dacie in 1952 to describe patients with congenital haemolytic anaemia, who presented with similar symptoms and clinical findings to those encountered in patients with hereditary spherocytosis (HS). However no spherocytes were present on peripheral blood film examination and

osmotic fragility was not corrected by addition of glucose[7].

There are number of causes of CNSHA in neonates. Hereditary haematological conditions such as haemoglobinopathies and enzymopathies are among common causes[8]. Neonatal RBCs are different from adult RBCs in metabolic requirements and have a shorter life span. PK activity reduces progressively in circulation[9] resulting in a greater susceptibility for destruction leading to haemolytic anaemia and neonatal jaundice[10]. PK deficiency was first noticed in northern European states however with advent of new technologies worldwide incidences have been reported. In India, screening of newborns with jaundice for PK deficiency revealed incidence of 3.21%[8]. Population survey demonstrated a heterozygote rate of 6% in Saudi Arabia, 1.4% in Germany, and 0.14% in Ann Arbour, Michigan. As with autosomal recessive disorder, the incidence increases with the consanguinity in society. Our results are closer to the Indian studies. One possible reason can be inter family marriages, being practiced in both societies since it is an autosomal recessive disorder. Other possibility includes endemic malarial infection in sub-continent region. Historically malarial parasite infection has been considered as a major driving force for many red cell abnormalities. Experiments have shown reduced malarial parasite proliferation in PK deficient cells[14] providing survival advantage to heterozygous carriers. Morbidity in the newborn with pyruvate kinase deficiency is usually the result of severe anaemia, hyperbilirubinaemia or both combined, with the adverse effects associated with the management of the condition. Clinically anaemia, jaundice, and splenomegaly were the major findings in the newborn with PK deficiency (100%).

Table: Characteristics of patients with and without pyruvate kinase deficiency.

	PK deficient	PK non deficient	*p*-value
Mean age (wks)	1.89 ± 0.832 (wks.)	2.42 ± 1.138	0.223
Gender Male Female	 4 (57%) 3 (43%)	 130 (58%) 95 (42%)	 1.000
Hemoglobin g/dl	7.8 ± 2.0l	10.4 ± 2.3l	0.0035
LDH IU/L	921 ± 621	583 ± 409l	0.035
Indirect Bilirubin mg/dl	4.7 ± 5.6	3.0 ± 1.7	0.02
Reticulocyte count (%)	12.9% ± 4.1	14.2% ± 3.9	0.386

Heterozygotes have intermediate enzyme levels and are asymptomatic; while homozygotes are clinically symptomatic. Severity of the condition widely varies, even among patients with the same level of deficiency. Therefore a linear correlation between clinical severity and PK activity is hard to establish. Only severe deficiency manifests in neonatal period, whereas mild deficiencies may remain unrecognised until adult life. Minimum age for presentation was 1 day old neonate reported in an Italian study[5]. The early onset of symptoms was, associated with a severe clinical course[15,11].

Differential diagnosis of CNSHA includes other enzymes involved in red blood cell metabolism. The PK deficiency should be considered in differential diagnosis of neonates with haemolytic anaemia and neonatal jaundice, where the cause of haemolysis is not established. The minimum workup should include CBC count, differential blood counts, reticulocyte counts, a serum bilirubin level

and peripheral blood film examination, to guide the clinician toward diagnosis of PK deficiency. Normochromic, normocytic, or macrocytic anaemia together with reticulocytosis in the absence of blood loss, is suggestive of haemolysis. A negative Coombs test excludes immune haemolysis.

Specific diagnosis requires Enzyme assay or DNA analysis involving polymerase chain reaction (PCR).

Large number of genetic mutations make PCR of limited use in population where mutations are unknown. If genetic defects in parents are known antenatal diagnosis can be done by using DNA analysis[13]. Screening index of families will help in evaluating true frequency in our population[16]. Genetic studies of such families would also facilitate establishing the more common mutations enabling health professionals to offer antenatal diagnosis to affected families[5]. We recommend that all neonates who present with a CNSHA, neonatal jaundice and do not show evidence of red cell allo antibodies, G6PD deficiency and haemoglobinopathies should be screened for detection of PK deficiency.

CONCLUSION

We conclude that PK deficiency is not uncommon in our setup and all patients with CNSHA where cause cannot be established should be screened for PK deficiency.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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