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Study of RBCs Pyruvate Kinase Deficiency in Neonatal Pathological Indirect Hyperbilirubinemia

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ABSTRACT

Background: Pyruvate kinase (PK) enzyme deficiency is an autosomal recessive disorder with both male and female equally affected. It is the most common enzyme abnormality in the glycolytic pathway of red blood cell metabolism. Clinical presentation of PK deficiency ranges from hydrops fetalis to mild compensated hemolytic jaundice.

Objective: The aim of this work was to determine prevalence of RBCs pyruvate kinase enzyme deficiency among neonates with pathological indirect jaundice in Sharkia Governorate.

Patients and Methods: This Cross-sectional study was done in Neonatal Intensive Care Unit, Pediatric Department at Zagazig University Hospitals during the period between June 2018 and December 2018. This study included 95 patients with pathological indirect hyperbilirubinemia.

Results: There was statistically significant positive correlation between total bilirubin and all of reticulocytes' count, and platelet count. There was statistically significant negative correlation between total bilirubin and all of hemoglobin, red blood cells (RBCs), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). There was non-significant correlation between it and other parameters. Pyruvate kinase deficiency was diagnosed in 4.2% of 95 patients with indirect hyperbilirubinemia. Glucose-6-phosphate dehydrogenase (G6PD) deficiency was diagnosed in 16.8%.

Conclusion: Red blood cells (RBCs) pyruvate kinase enzyme deficiency among neonates with pathological indirect jaundice is the second enzyme deficiency cause indirect jaundice after G6PD and is a risk factor related to pathological jaundice in neonates.

Keywords: Indirect hyperbilirubinemia, Pyruvate kinase enzyme, non-spherocytic, hemolytic anemia, Neonatal jaundice.

INTRODUCTION

Mature erythrocytes completely depend on glucose as a source of energy. Glucose is catabolized to pyruvate and lactate in the Embden-Meyerhof pathway, which is the major anaerobic glycolytic pathway [(1)]. In glycolytic pathway the enzyme pyruvate kinase acts on phosphoenolpyruvate and convert it to pyruvate, so generate adenosine triphosphate [(2)].

Four known PK isoenzymes (M1, M2, L and R), encoded by two separate genes (PK-M and PK- LR). PK-R isoenzyme is specific for RBCs [(3)]. Insufficient ATP formation due to pyruvate kinase deficiency affects RBCs metabolism and loss of RBCs membrane plasticity leading to its trapping and destruction in spleen resulting in hemolytic jaundice [(4)]. Unconjugated jaundice in neonates is one of the most frequent diseases in neonatal period. Many causes can be encountered in this condition, which may be physiological or pathological[(3)].

Pathological indirect hyperbilirubinemia in neonates may be immune or non-immune. Non-immune causes are RBCs enzymopathy, RBCs membrane abnormalities and haemoglobinopathies. Kernicterus is serious neurological sequelae of severe rise of indirect bilirubin[(5)]. Pyruvate kinase enzyme deficiency is an autosomal recessive disorder with both male and female equally affected. It is the most common enzyme abnormality in the glycolytic pathway of red blood cell metabolism[(4)].

Pyruvate kinase enzyme deficiency was described firstly 1961. Most of cases were found in north European area while few cases diagnosed in Middle East, Japan, China, Spain and Saudi Arabia. Clinical presentation of PK deficiency ranges from hydrops fetalis to mild compensated hemolytic jaundice. Severe enzyme deficiency presented early in life, while mild deficiency passes unnoticed till later age [(6)].

The second most common cause of neonatal non-spherocytic non-immune indirect hyperbilirubinemia in the United States is pyruvate kinase deficiency after G6PD deficiency [(7).] In India the prevalence of pyruvate kinase deficiency in neonates with indirect hyperbilirubinemia was 3.21%[(6)]. In Egypt, 2.8% was the prevalence of pyruvate Kinase deficiency in research study in Cairo University[(3)].

In our study we tried to know the prevalence of pyruvate kinase deficiency in Egyptian neonates with indirect hyperbilirubinemia in Sharkia Zagazig University.

PATIENTS AND METHODS

This cross-sectional study was done in Neonatal Intensive Care Unit (NICU), Pediatric Department at Zagazig University Hospitals during the period between June 2018 and December 2018. This study included 95 patients with pathological indirect hyperbilirubinemia.

Patient criteria:

Age group: less than 28 days of life (neonates).

Inclusion criteria: neonates with pathological indirect hyperbilirubinemia and need treatment with

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phototherapy with or without exchange transfusion in NICU.

Exclusion criteria: Neonates with indirect hyperbilirubinemia but not indicated for hospitalization. Immune indirect hyperbilirubinemia. G6PD enzymopathy cases. Spherocytosis cases, and parents refuse to participate research.

Study design: Cross sectional study.
Sample size: Assuming that number of cases which attending to Neonatal Intensive Care Unit at Zagazig University Hospitals with pathological non immune, non-spherocytic and non G6PD enzymopathy indirect hyperbilirubinemia are 100 at 6 months, prevalence of pyruvate kinase in neonates with pathological non immune indirect hyperbilirubinemia cases are 5%, confidence 95%, and power 80%.
Sample type: Simple random sample.

Operational design:
Methods: All patients enrolled in the study were subjected to the following:
1- Data collection regarding gestational age, age of NICU admission in days, sex and history of other siblings with neonatal jaundice, family members and consanguinity.
2- Detailed examination; general and systemic.
3- Laboratory investigation including:

- Reticulocyte count.
- Complete blood count.
- Blood grouping (ABO and RH).
- Total and direct serum bilirubin levels.
- Direct Coombs test.
- Osmotic fragility test.
- G6PD assay.
- Pyruvate kinase enzyme assay by ELISA technique.

Time schedule:
This study was done as: Writing review 6 months, collecting data 6 months, analysis of data 6 months, writing discussion 4 months and conclusion and recommendation 2 months.

Ethical consent:
An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Every caregiver of each patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis:
All data were collected, tabulated and statistically analyzed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA). Continuous quantitative variables were expressed as the mean ± SD and categorical qualitative variables were expressed as absolute frequencies (number) and relative frequencies (percentage). Continuous data were checked for normality by using Shapiro Wilk test. Independent t-test was used to compare normally distributed quantitative data. P value < 0.05 was considered significant.

RESULTS
Table (1) shows the baseline data of the studied patients.

Table (1): The baseline data of studied patients

	N=95	%
Gender:		
Female	46	48.4%
Male	49	51.6%
Consanguinity:		
Negative	74	77.9%
Positive	21	22.1%
Family history:		
Negative	82	86.3%
Positive	13	13.7%
Feeding:		
Formula	57	60%
Intravenous (IV)	10	10.5%
Mixed	23	24.2%
Parenteral nutrition (PN)	5	5.3%
Hardware security module (HSM)	2	2.1%
	Mean ± SD	**Range**
Weight (Kg)	2.91 ± 0.4	1.8 – 4
Age of admission (Days)	2.56 ± 0.56	1 – 5
Gestational age (week)	38.94 ± 2.46	33 – 42

Table (2) shows the level of the G6PD, pyruvate kinase, and bilirubin of the patients.

Table (2): Levels of the studied enzymes and levels of bilirubin of patients

	Mean ± SD	Median	Range
G6PD (U/g hemoglobin)	273.31 ± 62.32	166	1 – 807.6
Deficiency	16 (16.8%)		
Pyruvate kinase (U/g hemoglobin)	165.32 ± 41.32	167.6	11 – 353.6
Deficiency	4 (4.2%)		
Direct bilirubin (mg/dl)	0.695 ± 0.18	0.4	0.1 – 1.9
Total bilirubin (mg/dl)	162.92 ± 2.38	16	14 – 29

Table (3) shows that all patients underwent phototherapy while exchange transfusion was indicated in 6 patients only.

Table (3): Therapy received by the studied patients

	N=95	%
Phototherapy (days):		
Mean ± SD	4.07 ± 1.01	
Range	2 – 8	
Exchange transfusion		
No need	89	93.7%
Once	5	5.3%
Twice	1	1.1%

Table (4) shows that there was statistically significant positive correlation between total bilirubin and all of reticulosis count, and platelet count. There was statistically significant negative correlation between total bilirubin and all of hemoglobin, RBCs, MCV, and MCHC. There was non-significant correlation between it and other parameters**.**

Table (4): Correlation between serum bilirubin and the studied parameters

	Total bilirubin		Direct bilirubin	
	r	P	R	p
Gestational age (week)	-0.108	0.299	-0.126	0.222
Age at admission (Days)	0.106	0.308	-0.006	0.957
Weight (kg)	-0.053	0.611	-0.193	0.06
Hemoglobin (g/dL)	-0.514	<0.001**	-0.292	0.004*
RBCs (mcL)	-0.51	<0.001**	-0.32	0.002*
Reticulocytes' count (cells/mm^3)	0.466	<0.001**	0.227	0.027*
Mean corpuscular volume (MCV) (fL)	-0.14	0.177	-0.277	0.007*
Mean corpuscular hemoglobin concentration (MCHC) (g/dL)	0.13	0.208	-0.254	0.013*
WBCs (mcL)	-0.313	0.002*	-0.248	0.015*
Platelet count (mcL)	0.291	0.004*	0.264	0.01*
Osmotic fragility	0.012	0.906	0.016	0.881
G6PD (IU/g)	-0.233	0.023*	-0.11	0.288
Pyruvate kinase (mL)	0.038	0.716	0.018	0.861

*: Significant, **: Highly significant

Table (5) shows that there was statistically significant association between duration of phototherapy and all of G6PD deficiency, Coombs test and spherocytosis (all of them were significantly associated with longer duration of phototherapy).

Table (5): Relation between phototherapy and all of ABO incompatibility, G6PD, positive Coombs test and pyruvate kinase deficiency and spherocytosis

	Phototherapy Mean ± SD	P
ABO incompatibility:		
Present (n=23)	4.39 ± 1.01	0.24
Absent (n=72)	3.97 ± 0.90	
G6PD		
Deficiency (n=16)	5.13 ± 1.31	0.002*
Normal (n=79)	3.86 ± 0.84	
Coombs test:		
Negative (n=87)	3.93 ± 0.73	0.002*
Positive (n=8)	5.63 ± 1.21	
Pyruvate kinase deficiency	4 ± 0.81	0.92
Deficiency (n=4)	4.08 ± 0.90	
Normal (n=91)		
Spherocytosis		
High osmotic fragility (n=12)	3.93 ± 0.71	0.011*
Normal osmotic fragility (n=83)	5.08 ± 1.00	

*: Significant

Table (6) shows the distribution of anemic patients according to diagnosis. Out of 34 anemic patients, 5.9% had pyruvate kinase deficiency.

Table (6): Distribution of anemic patients according to diagnosis

	Anemia N= 34 (%)
ABO incompatibility:	
Present	11 (32.4%)
Absent	23 (67.6%)
G6PD	
Deficiency	10 (29.4%)
Normal	24 (70.6%)
Coombs test:	
Negative	30 (88.2%)
Positive	4 (11.8%)
Pyruvate kinase deficiency	
Positive	2 (5.9%)
Negative	32 (94.1%)
Spherocytosis	
High osmotic fragility	10 (29.4%)
Normal osmotic fragility	24 (70.6%)

DISCUSSION

This study included 95 patients with indirect hyperbilirubinemia, out of them 51.6% were males i.e., the percentage of males was greater than females. This is supported by the study of **Paul *et al.*** [7] where jaundiced males were significantly higher than females, and was in agreement with **Abdel Fattah *et al.*** [3] who studied two groups with severe neonatal jaundice and found that males were 58.5%.

In our study, pyruvate kinase deficiency was diagnosed in 4.2% of 95 patients. This was more than **Abdel Fattah *et al.*** [3] study, who reported that only 2 of the 69 patients included in their study (2.8%) had pyruvate kinase deficiency.

In our study, four cases were diagnosed with pyruvate kinase deficiency, 3 of them were presented with normocytic hemolytic anemia and the fourth was presented with macrocytic hemolytic anemia and the level of pyruvate kinase was severely low. This agrees with **Koralkova *et al.*** [8]**,** who found that in enzymopathies they present with normocytic normochromic anemia with no characteristic morphological abnormality as in red cell membrane defect or hemoglobinopathy**.** The true prevalence of PK deficiency in different parts of the world is unknown because it occurs relatively rare; diagnosis is generally done only in symptomatic cases in specialized centers, and some commercial and hospital-based laboratories[9]. In another study by **ElAlfy *et al.*** [10], at Ain Shams University, three cases out of 150 anemic patients were diagnosed with pyruvate kinase, 2 of them were presented with normocytic hemolytic anemia and the third was presented with macrocytic hemolytic anemia with no specific findings in blood film and the level of pyruvate kinase was severely low in 2 of them while borderline in the third for which genotyping was pending [10]**.**

The frequencies of enzyme deficiencies that cause hemolytic anemia among Iraqi people in southern Iraq have been studied. These hereditary red blood cell enzymes included glucose 6-phosphate dehydrogenase (G6PD), pyruvate kinase (PK) and hexokinase (HK). The most common enzyme deficiency was G6PD deficiency, an X-linked recessive trait. PK deficiency was associated with chronic hemolytic anemia[11]**.** In India, a study to screen newborns with jaundice for the presence of pyruvate kinase deficiency determined that 3.21% of all newborns with jaundice also had the deficiency, with a 30-40% reduction found in pyruvate kinase activity [12]**.** Screened children of survivors of the atomic bombs in Japan were studied and prevalence values of 1.4% (threshold < 66% of normal) were found among 3069 child [13].

Three studies [14-16] screened for patients with PK deficiency. Between Spanish populations appeared to be clinically free, PK deficiency prevalence values between 0.2% and 2.2% were observed. Given the extremely high prevalence values reported in these apparently healthy populations[14]. In Northeast India, with endemic malaria, population screened for PK deficiency, resulted in complete PK deficiency of 0.083%. Accuracy of enzyme assay and the poor generalizability of populations in malaria-endemic regions, may led to the risk of bias for this study to be high [15]**.** Study of PK deficiency in newborns of eastern Saudi Arabia in areas with high consanguinity reported populations described as clinically free. The prevalence was 3.1% [16].

In our results, G6PD deficiency was diagnosed in 16.8%, which was the most common enzyme deficiency followed by PK enzyme. This agrees with study in Iraq where G6PD deficiency was by far the most common of the three enzyme deficiencies that cause hemolytic anemia. G6PD deficiency is transmitted as a sex-linked trait with severe enzyme deficiency occurring only in hemizygote males and homozygote females [11]**.**

In our study all patients underwent phototherapy for period ranged from 2 to 8 days with mean 4.07 days. Exchange transfusion was indicated in 6 patients (5 of them received it once and only one patient received it twice). This is in contrary to **Abdel Fattah *et al.*** [3] study as 2 pyruvate kinase deficiency (PKD) cases needed exchange transfusion, but agrees with **ElAlfy *et al.*** [10], as no PKD cases needed exchange transfusion.

CONCLUSION

RBCs pyruvate kinase enzymopathy is the most common enzyme defect in the glycolytic pathway after G6PD enzyme deficiency and should be in mind as one of the causes of pathological neonatal jaundice.

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