ORIGINAL STUDY

Pyruvate Kinase, Glucose 6-phosphate Dehydrogenase and Glutathione Reductase Deficiencies and Neonatal Jaundice in Basrah, Iraq

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Abstract:

Hyperbilirubinemia is a common problem of term and preterm neonates. Two hundred four jaundiced neonates admitted to Basrah Maternity and Childrens Hospital over a 6-month period were studied to determine the frequency of pyruvate kinase (PK) deficiency, glucose-6-phosphate dehydrogenase (G6PD) deficiency and glutathione reductase (GSSG-R) deficiency. Forty two neonates (20.5%) had PK deficiency, 68 (33.3%) G6PD deficiency and 40 (19.6%) GSSG-R deficiency. Interaction of more than one enzymopathy was found in 36 neonates (17.6%). Other hemolytic causes of jaundice were ABO incompatibility in 50 (24.5%) of neonates and Rh. incompatibility in 18 (8.8%). In 38 neonates no cause of jaundice was identified. There was a statistically significant increase in the frequency of G6PD deficiency and more than one enzymopathy with increasing severity of jaundice. The highest frequency of kenricterus was found in those with more than one enzymopathy.

Red cell enzymopathies are an important cause of jaundice in Iraqi neonates and the presence of more than one enzymopathy carries a greater risk of developing severe jaundice.

Introduction:

Enzyme deficiencies, especially those protecting the red blood cells from oxidation, may lead to hemolysis and hyperbilirubinemia. Pyruvate kinase (PK), glucose-6-phosphate dehydrogenase (G6PD) and glutathione reductase (GSSG-R) are involved in the metabolism of erythrocytes and in the protection of red cells from oxidant stress[1-4].

Pyruvate kinase (PK) deficiency, a congenital hemolytic anemia, occurs in persons homozygous for an autosomal recessive gene[1]. Distribution of PK deficiency is world wide, but its prevalence in many parts of the world is still unknown[3]. Population surveys suggest a gene frequency of 1%[4]; ranging from 0.24% in the Spanish population[5], 1.4% in the American population[6], 2.4% in Saudi Arabia[7] and 3.4% in Hong Kong[8]. Clinical manifestations vary from a severe neonatal hemolytic anemia to mild, well-compensated hemolysis noted first in adulthood. Severe jaundice and anemia requiring exchange transfusion may occur in the neonatal period[1-4,9].

PK and G6PD deficiencies together constitute most of the cases of chronic hemolytic anemia due to erythrocyte enzymopathies[3]. Cases of PK deficiency with associated G6PD deficiency have been reported[3,4]. Glutathione reductase (GSSG-R) deficiency has been reported in several populations. This may be due to a mutation in the GSSG-R gene resulting in GR variants with low activity or to a nutritional deficiency of riboflavin leading to an acquired deficiency of GR[10].

The association of these enzyme deficiencies with neonatal jaundice and the high frequency of G6PD deficiency in Basrah (where it ranges from 13.1-14.06%[11,12]; while that of PK is 1.4%[11] made it necessary to determine the extent of PK, G6PD and GSSG-R deficiencies as contributing factors for neonatal jaundice.

Patients and Methods:

This study was made from 1st July 2000 until 31st December 2000 on neonates who were admitted to the neonatal nurseries of Basrah Maternity and Childrens Hospital either for the management of jaundice or who developed jaundice during the course of their hospitalization. A complete medical history was taken and a thorough physical examination was made for each neonate. The history included age of the neonate, sex, age of onset of jaundice, parental consanguinity, history of pallor, poor feeding, lethargy or abnormal body temperature. Physical examination included general and systemic examinations in addition to the assessment of gestational age using Dubowitz criteria.

A blood sample from each neonate was drawn into EDTA anti-coagulated tubes for the following investigations which were completed with 48 hours : Complete blood picture, reticu-

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locyte count and blood film for morphology, blood group and Rh, total serum bilirubin, Coomb's test, PK, G6PD and GSSG-R estimations, in addition to blood grouping and Rh. of the mother.

The activity of G6PD was determined by the fluorescent spot test described by Beutler[13]. Moderate enzyme activity was considered if the spot showed weak fluorescence after 15 minutes, while severe deficiency was detected by spots showing no fluorescence after 30 minutes[14]. G6PD activity was measured according to the WHO method[15]. Pyruvate kinase activity was determined by the fluorescent spot test[16] and also by a procedure recommended by the International Committee for Standardization in Hematology[17]. PK enzyme deficiency was considered partial or severe if the fluorescence disappeared after 25 minutes and 45 minutes respectively. GSSG-R activity was estimated only qualitatively by the fluorescent spot test[16]. Enzyme deficiency was considered moderate or severe if the fluorescence disappeared after 25 minutes and 45 minutes respectively

The treatment given each neonate and its outcome were recorded on discharge from hospital.

Statistical analysis:

The Chi-square (X^2) test was used to determine the relative importance of various variables. The comparison between mean groups was performed with one-way analysis of variance (ANOVA). A p-value less than 0.05 was considered to be statistically significant; $p< 0.01$ as highly significant and $p< 0.001$ as extremely significant.

Results:

Two hundred and four neonates (129 males and 75 females) with jaundice were included in the study; ages ranged from 2-22 days, with duration of jaundice ranging from 1-15 days. Forty two (20.5%) were found to have PK deficiency; 68 (33.3%) G6PD deficiency and 40 (19.6%) GSSG-R deficiency (***Table 1***). Forty nine (72%) of those with G6PD deficiency were male and 19 (28%) were female ($p = 0.08$). No significant difference was found in the frequency of PK deficiency and GSSG-R deficiency amongst the sexes.

Interaction of more than one red cell enzymopathy was detected in 36 neonates; six (2.9%) had PK and G6PD deficiencies, eight (3.9%) PK and GSSG-R deficiency, 16 (7.8%) G6PD and GSSG-R deficiency, while in six (2.9%) neonates all the enzymes were low in activity. Other hemolytic causes of jaundice were ABO incompatibility in 50 neonates (24.5%) and Rh incompatibility in 18 cases (8.8%). In 38 neonates (18.6%) no detectable cause of the jaundice was identified. ***Table 2*** illustrates the distribution of cases in relation to bilirubin level and causes of jaundice. There was an increase in the frequency

Table 1: Frequency of enzyme deficiencies in neonates with jaundice.

Sex	No. examined		PK def[1]		G6PD def.[2]		GSSG-R def.[3]	
	No.	%	No.	%	No.	%	No.	%
Male	129	63.2	23 Partial 21 Severe 2	54.8	49 Moderate 6 Severe 43	72	23 Moderate 17 Severe 6	57.5
Female	75	36.8	19 Partial 16 Severe 3	42.2	19 Moderate 10 Severe 9	28	17 Moderate 12 Severe 5	42.5
Total[4]	**204**	**100**	**42**	**20.6**	**68**	**33.3**	**40**	**19.6**

Enzyme activity (IU/gm Hb) in hemolysates of jaundiced neonates.

*(1) **PK:** Mean normal activity is 14.1 + 2.7 IU/gm Hb. Deficiency is defined with activity ranges: Partial = 3.1–8.3; Severe = 1.0–3.0 IU/gm Hb.*

*(2) **G6PD:** Mean normal activity is 9.5 + 2.3 IU/gm Hb. Deficiency is defined with activity ranges: Moderate = 2.2–5.2; Severe = 0.0-2.1 IU/gm Hb.*

*(3) **GSSG-R:** Activity was estimated only qualitatively by fluorescent spot test.*

(4) Thirty six neonates had more than one enzymopathy.

Table 2: Distribution of cases in relation to bilirubin level and hemolytic causes.

Bilirubin level	≤ 14 mg/dl		5-20 mg/dl		> 20 mg/dl		Total
Causes of jaundice	No.	%	No.	%	No.	%	
PK deficiency	6	27.3	5	22.7	11	50	**22**
G6PD deficiency	12	30	10	25	18**	45	**40**
GSSG-R deficiency	2	20	4	40	4	40	**10**
> one enzymopathy	3	8.3	14	38.9	19*	52.8	**36**
ABO incompatibility	19	24.5	14	28	17	34	**50**
Rh. incompatibility	4	8.8	5	27.8	9	50	**18**
Others	11	29	14	36.8	13	34.8	**38**

4 neonates had ABO incompatibility and Rh. incompatibility

** P = 0.05 ** P = 0.04*

of red cell enzymopathies and Rh incompatibility with increasing severity of jaundice (as indicated by bilirubin level) but this increase was statistically significant only in neonates with G6PD deficiency ($p = 0.04$) and if there was more than one red cell enzymopathy in the same neonate ($p = 0.05$). The percentage of neonates with G6PD deficiency alone increased from 30% of neonates with total serum bilirubin (TSB) < 14 mg/dl to 45%

of neonates with TSB >20 mg/dl, while the percentage of neonates with more than one red cell enzymopathy increased from 8% of neonates with TSB < 14 mg/dl to 53% of neonates with TSB > 20 mg/dl). Exchange transfusion was done for 93 (45.6%) neonates as indicated by their age, TSB level and general condition, while phototherapy was required for 70 neonates (34.3%).

The mean total serum bilirubin level, mean hemoglobin level and mean reticulocyte counts for neonates with and without enzymopathies are presented in ***Table 3***. There was no statistical difference in mean total serum bilirubin level and mean hemoglobin level amongt the different groups. However, there was a statistically significant difference in the mean reticulocyte count; neonates with Rh. compatibility had a significantly higher reticulocyte count when compared with neonates with jaundice due to other causes ($p < 0.05$ to 0.001).

Table 3: Mean hemoglobin, reticulocyte count and bilirubin levels in jaundiced neonates with and without enzymopathy.

Condition	No.	Hemoglobin gm/dl	Reticulocyte %	T S B mg/dl
PK deficiency	22	14.2 ± 2.6	3.8 ± 2.4*	20.6 ± 7
G6PD deficiency	40	14.4 ± 2.2	3.7 ± 2.3*	20.8 ± 6.9
GSSG-R deficiency	10	14.7 ± 2.14	3.9 ± 2.5*	20.7 ± 7
ABO incompatibility	50	15.1 ± 2.1	4.5 ± 2.6*	17.5 ± 6.8
Rh. incompatibility	18	14 ± 3.3	5.8 ± 4.5	21.4 ± 8.3
> one enzympoathy	36	14 ± 1.83	3.86 ± 3.1*	22 ± 6.27
Others	38	15.1 ± 2.5	3.8 ± 2.8*	178.6 ± 7.2

Values were expressed as mean ± SD.
** p < 0.05 Rh. incompatibility versus all other groups*
TSB = Total Serum Bilirubin

One hundred and seventy four of the neonates (85.3%) were full term, 30 (14.7%) were preterm. Poor feeding was present in a significant number of jaundiced neonates especially those with more than one enzymopathy (44.4%) and those with no detectable cause of jaundice (31.6%), as shown in ***Table 4***. Pallor was detected in more than 22% of neonates with PK deficiency, neonates with more than one enzymopathy and those with no obvious or detected cause of the jaundice. There were no statistically significant differences in the frequencies of pallor and poor feeding among neonates with PK deficiency, GSSG-R deficiency, those with more than one enzymopathy, Rh. incompatibility, ABO incompatibility and those with no obvious or detectable cause ($p > 0.05$). However, the frequency of pallor and poor feeding were significantly lower in neonates with G6PD deficiency when compared with those without obvious cause ($p<0.05$ and 0.002 respectively). Clinical signs of kernicterus

Table 4: Clinical characteristics of jaundiced neonates in relation to the cause of jaundice.

Cause	Total	Pallor		Poor Feeding		Kernicterus	
		No.	%	No.	%	No.	%
PK deficiency	22	5	22.7	5	22.7	1	4.5
G6PD deficiency	40	4*	10	2**	5	2	5
GSSG-R deficiency	10	1	10	1	10	-	-
> one enzymopathy	36	8	22.2	16	44.4	7@	19.4
ABO incompatibility	50	8	16	9	18	2	4
Rh. incompatibility	18	2	11.1	4	22.2	2	11.1
Others	38	10	26.3	12	31.6	2	5.3

** P < 0.05 ** P < 0.002 @ P < 0.06*

were present in 5-19.4% of neonates with different enzymopathies. The highest frequency of kernicterus was present in neonates with more than one enzymopathy (19.4%), where it was marginally statistically significant ($p > 0.06$) when compared to other groups.

Discussion:

Hyperbilrubinemia continues to be a common problem of term and preterm neonates. Although the need to diagnose and treat hyperbilirubinemia in healthy neonates has been controversial, recent reports of detrimental neurological effects from elevated bilirubin in healthy neonates makes scrutiny prudent [18]. In this study, analysis of 204 neonates with jaundice revealed G6PD deficiency in 68 (33.3%); PK deficiency in 42(20.5%) and GSSG-R deficiency in 40 (19.6%). Other studies in different countries amongst various ethnic groups have also established that G6PD deficiency may be the cause of neonatal hyperblirubinemia[19-22]. Some researchers have even suggested the need for a neonatal screening program for G6PD deficiency to prevent severe neonatal jaundice[23]. In comparison with a 1987 study in Basrah with a frequency of 51% G6PD deficiency in jaundiced neonates[24], our study found a lower percentage, possibly due to different methods for estimating G6PD.

It has been suggested by Chen et al[25] that the diminished ability of NADPH generation in G6PD-deficient erythrocytes might contribute directly to the more extensive peroxidation of the cells. Thus the defective capability of NADPH production, which resulted in the weakened facility of antiperoxidation and finally the lysis of erythrocytes, was one of the important mechanisms in the development of jaundice in G6PD-deficient neonates.

GSSG-R deficiency is a common disorder in several populations, the reported frequencies ranging from 0.04% in the Span-

ish population[5], through 3.6% in hospitalized patients in the USA[26] to 31.9% in the Saudi population[10]. The exact frequency of GSSG-R deficiency in the Iraqi population is not known. However the frequency of PK deficiency in the Basrah population was found to be 0.82%[11]. This report is the first reporting of GSSG-R and PK deficiency in neonatal jaundice. The study has revealed high frequencies of both PK deficiency and GSSG-R deficiency in jaundiced neonates in comparison with other studies; PK deficiency and GSSG-R deficiency were found in 2.5% and 0.5% of jaundiced neonates in Spain[18]. GSSG-R deficiency may be the cause of jaundice and at the same time it may be an adverse effect of phototherapy which destroys the riboflavin needed for full activity of GSSG-R. Some studies have suggested using vitamin B_2 to prevent that side effect[27]. Interaction of more than one enzymopathy was found in a significant number of jaundiced neonates (36/204); these were prone to severe jaundice, as indicated by their clinical and biochemical parameters.

In addition, interaction between ABO incompatibility or Rh incompatibility and enzymopathies are reported from this study; 29/50 neonates with ABO incompatibility, also had enzymopathies. A previous study in Thailand[20] showed that ABO incompatibility was associated with G6PD deficiency in a higher frequency than those without G6PD deficiency. However, ABO incompatibility and G6PD deficient neonates compared to these with either condition alone are not at increased risk of hemolysis or hyperbilirubinemia[20].

We conclude that red cell enzymopathies constitute an important cause of jaundice in Iraqi neonates. The presence of more than one enzymopathy carries a greater risk of developing severe jaundice in these neonates compared to those with a single red cell enzymopathy. We hope that our study will add to the limited information available on the prevalence of red cell enzymopathies in neonates with jaundice. However, further studies are required concerning the prevalence and role of different red cell enzymopathies in apparently healthy subjects as well as subjects with different diseases and to determine the molecular basis of these defects.

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