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Glucose-6-Phosphate Dehydrogenase Deficiency Among Newborns with Indirect Hyperbilirubinaemia in Bani Sueif Governate

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ABSTRACT

Background: Jaundice is the most common condition requiring attention in newborn. G6PD deficiency is among the causes of severe neonatal hyper-bilirubinemia with the potential of kernicturs.

Objectives: The aim of this work is to detect cases of G6PD deficiency among newborns with indirect Hyperbilirubinaemia in our country before recommending it as an obligatory screening test for all newborns using the quantitative method and for early diagnosis of G6PD deficiency.

Patients and Methods: This study was conducted on 200 full term neonates with neonatal jaundice including 158 males and 42 females. They were selected from the NICU in Beni-Suef University teaching hospital. Cases were recruited during the study period from May 2010 to January 2011.

Results: Out of the 200 neonates, 8 patients (4%) were G6PD deficient using the screening test.

Conclusion: Despite low incidence of G6PD deficiency in our study, we recommend screening for G6PD deficiency in any neonate presenting with jaundice or any neonate with positive family history for G6PD deficiency not only to detect the etiology of jaundice, but also to prevent kernicterus and future hemolytic episodes.

Key Words: *G6PD deficiency – Neonatal jaundice – Neonatal screening – Quantitative enzyme assay.*

INTRODUCTION

Hyperbilirubinemia is one of the most common problems in the neonatal period, and is a benign condition in most cases **[1]**.

Nonetheless, untreated severe indirect hyperbilirubinemia is potentially neurotoxic, and conjugated direct hyperbilirubinemia often signifies a serious illness **[2]**.

Kernicterus is a neurologic syndrome resulting from the deposition of unconjugated bilirubin in the brain cells. The risk in infants with erythroblstosis is directly related to serum bilirubin levels. The relationship between serum bilirubin level and kernicterus among healthy term infants is uncertain **[3]**.

The mechanisms of neonatal hyperbilirubinaemia are variable including: Bilirubin overproduction which occurs in hemolytic diseases with either positive Coombs test (ABO incompatibility, Rhesus incompatibility, and minor blood group antigens) or negative Coombs test (red blood cell membrane defects, e.g., spherocytosis, elliptocytosis, and/or red blood cell enzyme defects, such as glucose-6-phosphate dehydrogenase (G6PD) and pyruvate kinase deficiencies) Nonhemolytic causes as cephalohematoma, bruising, polycythemia or decreased Bilirubin conjugation as Crigler-Najjar syndrome types 1 and 2, Gilbert syndrome, Hypothyroidism, Breast milk jaundice and Impaired bilirubin excretion as infection, metabolic disorder, chromosomal abnormality, drugs **[4]**.

The prevalence of G-6-PD deficiency among Caucasian populations ranges from less than 1 in 1000 among northern European populations to 50 percent of the males among Kurdish Jews. G-6-PD deficiency is also found among certain Chinese populations and in Southeast Asia but it is rare in Japan. G-6-PD deficiency of the A-type is very common in West Africa, and the prevalence among African American males is approximately 11 percent. Some 16 percent of African American males carry the non-deficient G-6-PD A+ gene **[5]**.

Icterus neonatorum in G-6-PD deficiency probably is due principally to inadequate processing of bilirubin by the immature liver of G-6-PD-deficient infants, although shortening of red cell life span may play a role. Severe jaundice due to G-6-PD deficiency seems to be limited to infants who have also inherited a mutation of the (UDPGT-1) gene promoter **[6]**.

Evaluation of G6PD deficiency should be considered, especially for infants who are older than four days, have a positive family history, or are of East Asian, Greek, Mediterranean, or African descent.

SUBJECTS AND METHODS

This study was conducted on 200 full term neonates with neonatal jaundice including 158 males and 42 females. They were selected from the NICU in Beni-Suef University teaching hospital. Cases were recruited during the study period from May 2010 to January 2011. The protocol was approved by the IRB of the Faculty of Medicine, Beni-Suef University and informed consent was obtained from children's guardians.

Inclusion criteria:

All full term neonates with indirect hyperbilirubinaemia.

Exclusion criteria:

Cholestasis and Preterm infants.

Methods:

All cases were subjected to: Careful history taking focusing on factors that might lead to neonatal jaundice as gestational age, mode of delivery and instrument use (forceps, or ventouse); factors that might lead to sepsis and urinary tract infections as premature rupture of membranes, maternal fever, vaginal discharge; pattern of breast feeding adequacy; passage of meconium; family history of jaundice in a previous sibling, RH or ABO incompatibility, family history of chronic haemolyticanaemia presenting in the neonatal period (spherocytosis & elliptocytosis); history of G6PD in a family member.

Thorough clinical examination: Vital data: Heart rate, respiratory rate, temperature. Complexion: Jaundice or pallor. Anthropometric measurements: Weight, length, head circumference and abdominal circumference; Modified Ballard Scoring to include full term only (above 37 weeks); decreased perfusion & lethargy; organomegaly and palpable flank masses. Complete systemic examination: GIT, chest, cardiac and neurological examination.

Investigations:

1- Serum bilirubin (total and direct) using automated (Cobas C 111) (Roche) **[7]**.

2- Complete blood picture using Electronic counter Sysmexkx– 21N.

3- Reticulocytic count by Brilliant Cresyl blue stain (supravital stain).

4- Coomb's test (direct) **[8]**.

5- Maternal & neonatal blood group & RH.

6- Serum C- reactive protein (CRP) using Latex serology test.

7- G6PD enzyme assay: Quantitative method using spectrophotometer Bayer RA-50 chemistry analyzer Biosystem according to manufacturer instructions.

Statistical analysis:

Analysis of data was done by using SPSS (statistical program for social science version 15) as follows Description of quantitative variables as mean, SD and range Description of qualitative variables as number and percentage Chi-square test was used to compare qualitative variables between groups Unpaired *t*-test was used to compare two groups as regard quantitative variable in parametric data (SD <50% mean). Mann Whitney test used instead of unpaired *t*-test in non parametric data (SD >50% mean) Spearman correlation test was used to rank different variables against each other either positively or inversely.

- *p*-value >0.05 insignificant.
- $p<0.05$ significant.
- $p<0.01$ highly significant **[9]**.

RESULTS

The present study included 200 full term neonates admitted for neonatal jaundice. (Table 1) presents the characteristics of the study group. The majority of the cases were males (79%) and the majority was in 4-7 days old at the time of testing.

As regards the maternal history of the studied neonates, the majority (60%) had irrelevant history, urinary tract infection, Premature rupture

of membrane were each encountered in 15%, 2% had placenta previa while 8% had other conditions as maternal diabetes, instrumental delivery and maternal medication.

The laboratory findings of the studied neonates are presented in (Table 2).

The causes of hyperbilirubinemia in the studied cohort are presented in (Table 3). In the majority of cases (69%) no cause could be detected. Sepsis was the most common cause followed by ABO incompatibility. G6PD deficiency was detected in 8 cases (4%).

No statistically significant difference was encountered in any of the laboratory parameters between neonates with normal and those with deficient G6PD. As regards maternal history, 4/8 cases had irrelevant history while UTI and PROM were detected in 2/8 cases each.

No statistically significant correlation could be detected between G6PD level on one side and either age, birth weight, bilirubin level, Hb, TLC, Platelets or reticulocyte count on the other side.

Table (1): Characteristics of 200 neonates with hyperbilirubinemia.

Variable	No.	%
Age (days):		
≤3	78	39
4-7	84	42
≥8	38	19
Mean±SD	4.5±2	(1-15)
Gestational age:		
37 weeks	60	30
>37	140	70
Gender:		
Male	158	79
Female	42	21

Table (2): Description of laboratory data of 200 neonates with hyperbilirubinemia.

Parameter	Mean	±SD	Range
HB g/dl	13.1	1.9	11-16
HCT %	38.8±5	5	35-42
MCV fl	97	9	95-99
MCH pg	33.5	2	29-35
WBCs/cmm	9.9	2	4-11
Lymphocytes %	28	11	0-30
Neutrophils %	48	20	4-58
RDW %	14	2	11-16
Reticlocytes %	2.7	2	0.9-3
Platelets/cmm	280	100	150-350
Total bilirubin (mg/dl)	16	3	8-18
Direct bilirubin (mg/dl)	0.78	0.07	0.04-1.01

Table (3): Causes of indirect hyperbilirubinemia in 200 neonates.

Cause	Number	%
ABO incompatibility	18	9
Rheusus incompatibility	4	2
Cephalhematoma	2	1
Sepsis	30	15
G6PD deficiency	8	4
Undetermined (exaggerated physiological jaundice, breast feeding or breast milk jaundice, unknown)	138	69
Total	200	100

DISCUSSION

Glucose-6-phosphate dehydrogenase deficiency, the most common enzyme deficiency worldwide, causes a spectrum of disease manifestations including neonatal hyperbilirubinemia, acute hemolysis, and chronic hemolysis. Persons with this condition also may be asymptomatic. This X-linked inherited disorder most commonly affects persons of African, Asian, Mediterranean, or Middle-Eastern descent. Approximately 400 million people are affected worldwide **[10]**.

The relationship between G6PD deficiency and hyperbilirubinemia in the newborn period is well recognized. Severe neonatal hyperbilirubinemia resulting in kernicterus is the most serious complication of this enzyme deficiency in the newborn period. Thus early neonatal screening programmes should be instituted especially in countries where the prevalance of enzyme deficiency is high **[11]**.

Hyperbilirubinemia in G6PD-deficient neonates is thought to be secondary to reduced hepatic conjugation and excretion of bilirubin, rather than increased bilirubin production resulting from hemolysis. Thus no difference was encountered in reticulocyte count or hematocrit level between G6PD-deficient and normal groups **[12]**.

In the present study, the G6PD deficiency was found in 4% of the studied population. Other studies reported slightly lower incidence of 1.57% in Spain **[11]** 2.1% in Zanjan province of Iran, 2.5% in Singapore **[13]** and 3.5% in Turkey **[11]**. On the contrary a much higher

incidence of 30%, 40% and 14% were reported in Al-Houfuf area in Saudi Arabia **[14]** in Nigerean neonates **[15]** and in Black Americans **[16]** respectively.

Our results showed that the G6PD deficient newborns are all males thus there is no G6PD deficient female neonates, this result comes in agreement with those obtained by Huang et al. **[17]** and Yu et al. **[18]**; they reported that G6PD deficient females are not at increased risk for the development of neonatal hyperbilirubinemia in Taiwan. Similarly another study in Taiwan reported that the prevalence of G6PD deficiency was 3.54% in males and 1.57% in females **[19]**. Thus the prevalence of G6PD deficiency in males was significantly higher than females in this study. The percentage of boys was higher than girls in other studies as well, such as the study by Koosha & Rafizadeh **[20]** which reported that 3.6% of males and 0.6% of females were G6PD deficient. Similarly the ratio between male: Female G6PD deficient neonate was 3: 1 in the study by Atay et al. **[11]**.

However, such results were not matched with the reports obtained by Tan **[21]** in Singapore and by Kaplan and Abramov **[22]** in Israel that showed higher incidence of neonatal hyperbilirubinemia in G6PD-deficient females. Another study by Omran et al. **[15]** in Saudi Arabia showed higher incidence of G6PD deficiency in females may be in part due to the high rate of consanguinity among the Saudi population, leading to increased numbers of female homozygotes.

In the present study, no significant relationship was noted between the severity of jaundice and morphological changes in the neonates' RBCs, their reticulocyte count or their hemoglobin concentration. Such findings were also reported by Kaplan et al. **[23]** who suggested that jaundice is thought to be secondary to reduced hepatic conjugation and excretion of bilirubin, rather than increased bilirubin production resulting from hemolysis. This is in agreement with Abolghasemi et al. **[24]** who reported that jaundice may not necessarily be related to hemolysis, but probably to transferase activity in liver cells. This is supported by the fact that in jaundiced G6PD deficient neonates there are lower levels of bilirubin diglucuronide, normal packed cell volumes (PCV), normal reticulocyte counts and insignificant rise of carboxyhemoglobin **[25]**.

Statistical analysis of our results showed that there were no differences in the highest total bilirubin concentration, reticulocyte count, or the lowest haemoglobin level between normal G6PD and G6PD-deficient newborns. This comes in agreement with the results obtained by Koosha and Rafizadeh **[20]** and Al-Omran et al. **[15]**. Also Atay et al. **[11]** in Turkey reported that no statistical difference was detected between G6PD deficient and normal groups in relation to reticulocyte count. These findings do not suggest significant hemolysis as a cause of jaundice in these infants, which is a common observation in G6PD deficient neonates. On the contrary, a Nigerian study done by Kaplan et al. **[23]** documented that the difference in the mean total Hb value of the G6PD deficient group compared with controls was extremely significant. These findings suggest accompanying hemolysis as a cause of this difference.

In the current study, we demonstrated that G6PD deficiency by itself is a risk factor for the development of neonatal hyperbilirubinemia even without exposure to chemicals that might cause hemolysis. As the Apgar scores of these neonates in our study showed that the stress from birth process was not likely the major cause to induce neonatal hyperbilirubinemia in G6PD-deficient neonates in the nursery, our findings implied that the possible cause of neonatal hyperbilirubinemia was not directly related to hemolysis, but was secondary to reduced hepatic conjugation and excretion of bilirubin. Our results came in concordance with another study by Weng et al. **[20]** in Taiwan.

However, the mechanism of the relationship between G6PD activity and neonatal hyperbilirubinemia is not clear. The presence of other genetic factors has been postulated in the pathogenesis of neonatal hyperbilirubinemia in G6PD deficiency. Kaplan et al. **[23]** reported that UGT1A1 gene mutation, diminishing activity of the conjugating enzyme UGT, was associated with neonatal hyperbilirubinemia in G6PD deficiency. However, this was not confirmed by Galanello et al. **[26]**. Thus the interaction between UGT1A1 and G6PD genes remains to be verified.

In conclusion, G6PD deficiency is encountered in 4% of neonatal hyperbilirubinema in our study. Despite its low incidence, we recommend screening for G6PD deficiency in any neonate presenting with jaundice or any neonate with positive family history for G6PD deficiency not only to detect the etiology of jaundice, but also to prevent kernicterus and future haemolytic episodes.

Genetic counseling is recommended; parents should know that the condition is an X linked disease, transmitted by the asymptomatic mother to affect 50% of her sons. So, the recurrence rate in subsequent pregnancies is 50% in male offsprings. In male siblings (brothers) of patient, the enzymatic activity of G6PD should be studied for early diagnosis of G6PD deficiency. In addition, we recommend that measurement of the enzyme UGT be made available for the clinical use in the evaluation of neonatal hyperbilirubinaemia.

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