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Original Article

Neonatology Section

Relation between Neonatal Icter and Gilbert Syndrome in Gloucose-6-Phosphate Dehydrogenase Deficient Subjects

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ABSTRACT

Background and Aim: The pathogenesis of neonatal hyperbilirubinemia hasn't been completely defined in Gloucose-6-Phosphate Dehydrogenase (G6PD) deficient newborns. The aim of this study was to detect the relationship between Gilbert's syndrome and hyperbilirubinemia in Gloucose-6-Phosphate Dehydrogenase (G6PD) deficient neonates.

Materials and Methods: This case-control study was conducted in Amirkola pediatrics teaching hospital, Babol, Iran. A total number of one hundred four infants were included in the study (51 infants with neonatal jaundice and Gloucose-6-Phosphate Dehydrogenase (G6PD) deficiency admitted to phototherapy or transfusion were selected as the case group and 53 infants with Gloucose-6-Phosphate Dehydrogenase (G6PD) deficiency admitted for other reasons than jaundice were selected as the control group). Exclusion criteria were ABO or Rh incompatibility or other reasons that made Coombs test positive, sepsis, hepatosplenomegaly, metabolic diseases, medical treatment and phototherapy. The promoter and coding regions of Uridine diphosphate Glucuronosyl Transferase 1A1 (UGT1A1) of genomic DNA were amplified by polymerase chain reaction (PCR) isolated from leukocytes. We used chi-square test and t-test to compare cases and controls.

Results: Distribution of Gilbert genome was not significantly different between the two groups; among cases, 33.3% were homozygote, 35.3% heterozygote, and 31.4% normal. Among controls, 22.6% were homozygote, 34% heterozygote, and 43.4% normal (p-value=xxx). Hyperbilirubinemia family history didn't differ significantly between these two groups.

Conclusions: We showed that in Gloucose-6-Phosphate Dehydrogenase (G6PD) deficient neonates, there was no significant association between Gilbert's syndrome (promoter polymorphism) and hyperbilirubinemia.

Keywords: G6PD deficiency, Neonatal jaundice, Uridine diphosphate, Glucuronosyl-Transferase 1A1

INTRODUCTION

Neonatal jaundice or hyperbilirubinemia, defined as a total serum bilirubin level exceeding 5 mg/dL, is a frequent problem that affects about 60% of full term and 80% of preterm neonates in their first week of life. It is responsible for 75% of hospital readmissions in the first week after birth [1-3]. Neonatal jaundice can be a life threatening disease and could lead to neonatal morbidity and mortality especially in developing countries [4].

There are different mechanisms responsible for neonatal hyperbilirubinemia, such as increased bilirubin load on the liver cells via bilirubin production or enterohepatic circulation, decreased clearance of bilirubin from plasma through impaired uptake, conjugation, or excretion [5,6].

The conjugation process is catalyzed by the Uridine diphosphate Glucuronosyl Transferase (UGT) enzymes. UDP-GlucuronosylTransferase 1A1 (UGT1A1) is one of nine transferases encoded on the UGT1 locus. The UGT family contains several isoforms but only the A1 isoform (UGT1A1) participates in conjugation of bilirubin. This gene determines the structure of GlucuronosylTransferase enzyme. The UGT promoter consists of a TATA box which is a DNA sequence of thymine (T) and adenine (A). UGT1A1 promoter and coding sequence gene variants decreases hepatic bilirubin conjugation Mutations in the 1A1 exon or its promoter will affect bilirubin conjugation and results in Gilbert syndrome and the Crigler-Najjar syndrome [6-8].

Gilbert syndrome is the most common inheritable condition resulting in transient unconjugated hyperbilirubinemia. Nucleotide TA insertion into the TATA box-like sequence of the promoter region of the UGT1A1 gene is thought to be involved in this condition. This insertion generates A (TA) 7TAA (UGT1A1 * 28 allele) instead of AA (TA) 6TAA (UGT1A1*allele).

Gilbert syndrome is a benign chronic or recurrent unconjugated hyperbilirubinemia affects approximately 6% to 9% of the general population. Many studies have shown that Gilbert syndrome can play a role in the pathogenesis of neonatal hyperbillirubinemia. Some studies have revealed that the combination of the Gilbert genotype with other icterogenic factors such as breast feeding, G6PD deficiency, ABO incompatibility and pyloric stenosis dramatically increases a newborn risk of hyperbilirubinemia and also complications such as bilirubin encephalopathy [9].

This study was designed to assess the probable relationship between icter in neonates with G6PD deficiency and Gilbert syndrome.

MATERIALS AND METHODS

This was a case-control study conducted in Amirkola Children's Hospital of Babol University of medical sciences, North of Iran. Fiftyone infants with neonatal jaundice and G6PD deficiency admitted for phototherapy or transfusion enrolled through a simple sampling method, through 2005-2007 were compared with 53 matched infants with G6PD deficiency admitted for other reasons than jaundice during the same period.

Exclusion criteria were as followings: ABO and Rh incompatibility and other reasons that make coomb's test positive, sepsis, hepatosplenomegaly, metabolic diseases and other conditions resulted in hospitalization, medical treatment or phototherapy.

For assessment of Gilbert's syndrome, 3 cc of peripheral blood sample was collected in tubes where EDTA and DNA extraction was done by salting out. The promoter and coding regions of UGT1A1 were amplified by polymerase chain reaction (PCR) from genomic DNA isolated from leukocytes specific primer for each mutation, cutting the product of PCR with restricting enzyme for

each mutation and observing the length of them on Acryl amide gel. Normal allele included 6 repeat (AT) and the length of the proliferated segment was 90 couples base. Variants of alleles included 7 repeat (AT) and the proliferated segment was 92 couples.

PCR product had been put on 10% Acryl amide gel and stained with Ethium bromide. Numbers of repeating (AT) in gene promoter were determined with measurement or comparison of the length of the segments. Primers' sequences were as followings:

F: 5' ATTAACTTGGTGTCGATTGG3'

R: 5' AGCCATGGCGGCCTTTGCTC3'

STATISTICAL ANALYSIS

Statistical analyzes was done with SPSS-15 software. We used chi-square and t-test to analyze the differences in icter between those with or without Gilbert's syndrome.

RESULTS

The characteristics of cases and controls are provided in [Table/Fig-1]. There was not a significant relationship between the sex of neonates and having icter due to the G6PD. Male to female ratio was 5.37 in icteric patients while it was 3.07 in non-icteric one (p-value=0.331).

Icteric neonates had significantly higher weight than non-icteric ones (3.2 ± 0.4 versus 2.9 ± 0.7, p= 0.016). On the other hand, the family history of hyperbilirubinemia was not significantly different between two groups (p-value=0.623).

There was no significant difference between two groups with regard to the presence of the Gilbert syndrome (68% versus 56.6%, p-value=0.353). The difference remained non-significant when both homozygote and heterozygote forms of Gilbert syndrome were classified into one group (p-value=0.353) [Table/Fig-2].

Variable	Mean ± SD
Bilirubin	18.5 ±3
Icter onset	2.7±1.3
Hemoglobin	18.8±2.8

[Table/Fig-1]: Mean bilirubin, hemoglobin and age of onset of jaundice in icteric and G6PD deficient

Group	Icteric (cases) No. (%)	Non-icteric (controls) No. (%)
G6PD Homozygote	17 (33.3)	12 (22.6)
G6PD Heterozygote	18 (35.3)	18 (34)
Normal	16 (31.4)	23 (43.4)
p-value	0.353	

[Table/Fig-2]: Comparing G6PD deficient neonates and normal ones regards to the icter

DISCUSSION

Our study showed that while Gilbert syndrome was not distributed differently in icteric and non-icteric neonates with G6PD deficiency. Although homozygote form of Gilbert syndrome was more seen in icteric neonates, the difference was not statistically significant.

It has been showed that in Caucasians, the most prevalent gene polymorphism associated with Gilbert syndrome is an additional TA in the UGT1A1 promoter region and the normal gene in TATA Box included 6(TA) nucleotides in each allele [5]. Being homozygote for 7(TA) is associated with Gilbert syndrome, although not all homozygotes for 7(TA) have clinical manifestations and other factors like male gender may play a role in phenotype [5]. In Asians UGT1A128 is seen less and Gilbert syndrome could be induced by mutation in G71R coding area [5].

There are some reports about the relationship between Gilbert syndrome and G6PD deficiency [10]. Homozygote carriers of 211G to A in gene location of UGT1A1 have an additional risk factor of icter in male neonates with G6PD deficiency [3]. Also UGT1A1 analysis in a case-control study showed that 56% of cases had a mutation in nucleotide 211 exon 1 [11]. Akaba et al., in Japan reported a 19% frequency of G71R mutation in 159 neonates [12].

In a case-control study in Iran, Saki et al., reported a 14.8% prevalence of G6PD deficiency in parents of 115 neonates with severe unexplained indirect hyperbilirubinemia by using rifampin test in order to diagnose Gilbert syndrome. The prevalence of Gilbert syndrome was not significantly different between parents of hyperbilirubinemia group (22.2%) and the control group (19.13%). This study revealed that severe indirect hyperbilirubinemia could be plausible only when Gilbert syndrome combines with other factors like G6PD deficiency [9]. Similarly, Huang et al., have shown that variation 211 in G71R of UGT1A1 is a risk factor of progressing hyperbilirubinemia in neonates [13].

But some other studies reported no relationship between Gilbert syndrome and G6PD deficiency [5,9,14,15]; their findings were similar to the present study and no significant differences were observed between icteric and non-icteric neonates with regards to the Gilbert homozygoticity or hetrozygoticity.

CONCLUSION

Our study suggested that there is no significant relationship between Gilbert syndrome (promoter polymorphism) and hyperbilirubinemia in G6PD deficient neonates. Given the limited sample size of our study, and its being restricted to one geographic area, additional studies are needed to clarify this relationship. As the previous studies showed promoter mutations are seen more in Caucasians and in Asians, it could be more prevalent in coding area; more investigations are needed on polymorphism of coding area.

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