

UDP-Glucuronosyltransferase Promoter Polymorphism in Iranian Neonates with Idiopathic Hyperbilirubinemia

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Abstract- To determine the association between polymorphism of UGT1A1 gene and idiopathic hyperbilirubinemia in Iranian neonates. Fifty neonates with idiopathic hyperbilirubinemia and Serum total bilirubin (STB) more than 15mg/dl and 50 neonates with idiopathic hyperbilirubinemia and Serum total bilirubin (STB) less than 15mg/dl enrolled in this study. Thymine-adenine (TA) repeats in the promoter region of UGT1A1 gene investigated by means of polymerase-chain reaction (PCR) DNA sequencing. Demographic characteristics did not differ significantly between groups while STB was higher in case group (17.5±1.9 vs. 10.4±1.8, p value <0.001). Among one hundred neonates evaluated in this study, TA6/6, TA6/7 and TA7/7 genotypes found in 52%, 42% and 6%, totally. TA6/7 and TA7/7 genotypes observed in case group more than the control group (P<0.001). STB levels were significantly higher in cases with TA6/7 and TA7/7 genotype pattern (P<0.001). Heterozygous and variant homozygous genotypes of the promoter region of UGT1A1 gene in healthy Iranian neonates with idiopathic hyperbilirubinemia should be considered.

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Introduction

Neonatal jaundice is a challenging issue for many pediatricians. Near 60% of all term neonates, show clinical icter during 7 days after birth, although fewer have pathological basis (1). Physiologic jaundice characterized with total serum bilirubin up to 6 mg per dL which decreases over the first week while pathologic reasons can be identified by developing jaundice within 24 hours after birth, total serum bilirubin level more than 17 mg per dl, serum conjugated bilirubin level higher than 2 mg per dL or more than 20 percent of the total serum bilirubin concentration (2). Previous studies showed that neonatal hyperbilirubinemia is higher in Asian neonates and in near 50%, the exact cause is not identifiable (3).

As glucuronidation of unconjugated bilirubin forms the first step of bilirubin excretory pathway, reduction in UDP-glucuronosyltransferase

1A1 (UGT1A1) enzyme activity results in bilirubin

glucuronidation impairment which is the most cause of hyperbilirubinemia in the white population (4). 30-70% reduction in enzyme activity will take place by mutation in the promoter region and exon of this enzyme gene. Insertion of additional thymine-adenine (TA) nucleotides in the normal sequence A (TA)₆ TAA of the TATAA box promoter of the gene is a normal polymorphism in Caucasian people (5).

Prevalence of different mutations of UGT1A1 gene differs widely among different ethnical population.

The goal of this study was to determine the association between polymorphism of UGT1A1 gene and idiopathic hyperbilirubinemia in Iranian neonates.

Materials and Methods

This prospective study conducted in Imam Khomeini Hospital and Mirzakuchakhan Hospital between March 2010 and March 2011.

Fifty neonates with idiopathic hyperbilirubinemia

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and Serum total bilirubin (STB) more than 15mg/dl and 50 neonates with idiopathic hyperbilirubinemia and Serum total bilirubin (STB) less than 15mg/dl enrolled in this study. The highest bilirubin level at the first week of life considered as STB.

Inclusion criteria were:

Gestational age more than 37 weeks, birth weight \geq 2500 g and icter development during 24 hours of birth.

Exclusion criteria were: presence of ABO incompatibility or hemolysis (decreased HB, increased reticulocyte count and positive peripheral smear for spherocyte, nucleated RBC, anisopoikilocytoma), direct hyperbilirubinemia and systemic disease (such as sepsis, urinary tract infection, polycythemia, G6PDD (glucose-6-phosphate dehydrogenase deficiency), hypothyroidism, cephalohematoma) and history of exchange. All parents asked to fill informed consent before study. Study had been approved by local ethics committee of Tehran University of medical sciences.

2 ml venous blood sample drawn from the antecubital vein and sent to laboratory by means of EDTA vacutainers. Phenol-chloroform procedure applied for genomic DNA isolation. Oligonucleotide primers were used to magnify and sequence thymine-adenine (TA) repeats. The primer designed in size of 495 bp (6).

1 μ l DNA in 4 deoxynucleotide triphosphates (5 μ l from each, 10 pmol/ μ l) 3 μ l from each primer and 5 μ l M_gCL_2 (1 u/ μ L) – 5 μ L of Tag Polymerase and 1x buffer used as amplification reaction mixture. The PCR reaction performed with a DNA thermal cycle as 30 cycles at 94°C for 30 s, 55°C for 15 s, 72°C for 1 min, and 72 V for 3 min. Electrophoresis with 2 % agarose

gel USED FOR PCR confirmation. EZ-10-Spin Column PCR Purification Kit (BIO BASIC Inc., Ontario, Canada) as DNA sequencing templates used for PCR products treatments.

Due to manufacturer's instructions, Dye Terminator Cycle Sequencing with Beckman CEQ 8000 Generic Analysis System (Beckman Coulter Inc., Fullerton, CA, USA) applied for DNA sequencing.

According to the promoter sequencing, bearing the sequence (TA)₆ TAA in the TATAA element of both alleles considered as normal homozygote, variant homozygote with the sequence (TA)₇TAA in both alleles (TA₇/7) and heterozygote with one of each in the individual allele (TA₆/7).

STB measured by spectrophotometric method (B-105 Digital bilirubinometer, Erma Inc, Japan). G6PD enzyme activities measured by spectrophotometric method, too (Trinity Biotech Procedure No: 345-UV, Ireland). ABO incompatibility considered whether neonate blood group was A or B and mother's blood group was O.

Data analyzed using SPSS version 18 and presented as Mean \pm SD. The Student's *t*-test, One-way ANOVA applied for continuous as well as the Pearson X² test with Fisher's exact test were used for categorical variables assessment, respectively.

P-value<0.05 was considered statistically significant.

Results

Demographic characteristics did not differ significantly between groups while STB was higher in case group (Table 1).

Table 1. Demographic characteristics and baseline laboratory findings of participants.

| | Case group | Control group | <i>P</i> value |
|------------------------------|------------------|--------------------|----------------|
| Sex (M/F) | 26/24 | 25/25 | 0.5 |
| Birth weight | 3293 \pm 406.8 | 3199.6 \pm 460.7 | 0.2 |
| Hyperbilirubinemia age onset | 2.9 \pm 0.8 | 4.3 \pm 1 | <0.001 |
| Gestational age | 38.4 \pm 0.6 | 38.3 \pm 0.8 | 0.09 |
| STB | 17.5 \pm 1.9 | 10.4 \pm 1.8 | <0.001 |

Table 2. Frequency of different genotypes in two groups.

| | Case | Control | <i>P</i> value |
|--------------------|---------|---------|----------------|
| TA ₆ /6 | 15(30%) | 37(74%) | <0.001 |
| TA ₆ /7 | 29(58%) | 13(26%) | |
| TA ₇ /7 | 6(12%) | 0 | |

Table 3. STB levels in different genotype pattern.

| | TA6/6 | TA6/7 | TA7/7 | P value |
|---------------|----------|----------|----------|---------|
| Case group | 15.8±0.3 | 17.8±1.6 | 20.3±1.4 | <0.001 |
| Control group | 9.7±1.4 | 12.3±1.2 | | <0.001 |

History of hyperbilirubinemia was present in 24 and 12 neonates in the case and control group, respectively ($P=0.01$). History of familial marriage of parents did not differ significantly between two groups (15 vs. 12, $P=0.3$). Among one hundred neonates evaluated in this study, TA6/6, TA6/7 and TA7/7 genotypes found in 52%, 42% and 6%, totally. TA6/7 and TA7/7 genotypes observed in case group more than the control group.

STB differ significantly due to genotypes in each group (case and control ones).

Discussion

In this study, genotype TA6/7 (polymorphism in the promoter region of UGT1A1 gene) found to be related with higher STB level in both groups. We found TA7/7 genotype in 12% of neonates with STB more than 15 mg/dl. This rate is higher than the rates reported in previous studies. Ergin *et al.*, compared 50 neonates with idiopathic hyperbilirubinemia with 54 healthy controls and found genotype TA6/6 more common in healthy ones (88.8% vs. 20%) while genotypes TA6/7 and TA7/7 found further in case group (TA6/7:68% vs. 11.2%, TA7/7 12% vs 0) (6). Further studies reported prevalence of TA7/7 genotype as 0.6%, 8.5%, 9% and 10% in neonates with hyperbilirubinemia (7-10).

Sunil *et al* evaluated 77 hyperbilirubinemia and 50 healthy neonates in India and reported a lower rate of UGT1A1 gene polymorphism in healthy ones than hyperbilirubinemia cases (50% vs. 89.6%, p value<0.05) (11). In another study, Laforgia *et al.*, observed that UGT1A1 gene polymorphism (TA7/7 genotype) is significantly higher in Italian neonates with STB level more than 13 than cases with lower STB concentration (12). By measuring STB in second and fourth days after birth, Bancroft *et al* and Chowdhury *et al* found that in neonates with TA7/7 genotype in the promoter region of UGT1A1 gene, STB concentration is upper than healthy ones (13,14).

On the other hand, other previous studies showed that UGT1A1 gene polymorphism is not related with indirect hyperbilirubinemia in different regions of Turkey (7-10). These differences can be due to ethnical and regional differences or different study subjects and methods.

Not only TA7/7 homozygote polymorphism but also TA6/7 heterozygosity will result in UGT1A1 enzyme activity reduction (4). Like Ergin *et al* findings (6), in this study we found a higher level of STB in TA6/7 carriers than TA6/6 carriers.

TA7/7 only found to be associated with higher serum bilirubin while TA7/7 polymorphism along with ABO incompatibility, G6PDD, or thalassemia could cause severe hyperbilirubinemia (15,16).

Breast milk feeding is the most causative factor of hyperbilirubinemia in neonates, however, in cases with no evident risk factor, UGT1A1 gene variation should be considered as the gene product is one of the key enzymes of the bilirubin conjugation pathway. Promoter region polymorphism can cause a reduction in enzyme production while enzyme structure abnormalities and dysfunction can be results of coding region variation (17,18). In conclusion, heterozygous and variant homozygous genotypes of the promoter region of UGT1A1 gene in healthy Iranian neonates with idiopathic hyperbilirubinemia should be considered.

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