The Frequency of UDP-Glucuronosyltransferase 1A1 Promoter Region (TA)7 Polymorphism in Newborns and it's Relation with Jaundice

Summary

Increased bilirubin formation and decreased bilirubin conjugation play an important role in the pathogenesis of the newborn jaundice. Although physiologic jaundice is seen in most of the newborns, there are many risk factors that affect the severity and duration of hyperbilirubinemia. The latest studies showed that the frequency and severity of neonatal jaundice have been increased when mutations of the gene coding UDP-glucuronosyltransferase(UGT)1A1 coexist with other risk factors. Healthy term newborns weighing over 2500 g. were included in this study. The patient group consisted of 107 newborns either with total bilirubin level over 15 mg dl^{-1} within 7 days or 5 mg dl^{-1} after 15 days of age. The control group consisted of 55 newborns with bilirubin levels in physiological ranges. We investigated the frequency of promoter region [thymine–adenine(TA)]7 polymorphism in UGT1A1 gene. Factors which might cause pathologic and prolonged jaundice with coexisting polymorphism were also investigated. UGT1A1 6/7 genotype was found to be 11% in patient group and 13% in the control group. The difference between patient and control groups was not statistically significant. (TA)7 allele frequency was 0.069 and it is concluded that UGT1A1 promoter region polymorphism was not a risk factor for neonatal jaundice.

Key words: bilirubin, neonatal jaundice, UDP-glucuronosyltransferase 1A1, UGT1A1, promoter region, (TA)7 polymorphism

Introduction

Neonatal jaundice is the most common problem of the newborns. It can be seen in 60% of healthy term newborns and 80% of preterm newborns. Level of the plasma indirect bilirubin may increase due to the imbalance between bilirubin formation and conjugation rates. The increase in production rate and decrease in conjugation rate of bilirubin are both responsible for the occurrence of the neonatal jaundice, because neonates have red cells with high turnover and a shorter life span (60–90 days), and insufficient conjugation of bilirubin due to the deficient UDP-glucuronosyltransferase (UGT), which is the responsible enzyme in bilirubin conjugation [1–4].

Causes of increase in indirect bilirubin might be either physiologic or pathologic. This situation, which is called transient hyperbilirubinemia, is seen in the first week of life and when compared according to adult criteria is seen in 97% of the newborns biochemically. In two-thirds of these cases, bilirubin level exceeds 5 mg dl^{-1}, which is called visible jaundice [5, 6]. There are many factors that effect the duration and severity of the jaundice [7]. Among these are the ABO, Rh and subgroup incompatibilities, deficiency of glucose-6-phosphate dehydrogenase (G6PD) and pyruvate kinase, hereditary spherocytosis, defective hemoglobin synthesis, hypothyroidism, pylor stenosis, breast milk jaundice, Gilbert syndrome (GS), Crigler–Najjar syndrome (CNS) and cephalohematoma. However, these situations do not always cause pathologic jaundice in the same frequency and severity [8–11]. Molecular studies on GS in Caucasians showed that UGT1A1 gene had a normal coding region while an excess pair of nucleotide was found on the promotor region. Normally there is a pair of thymine–adenine (TA) repeating six times on the wild-type promotor region. The decreased transcription rate of UGT1A1 gene, the decreased activity of the enzyme and the relation between GS were shown in homozygous mutation for promotor region [5, 12–15]. The frequency of TA7 allele in Asian populations with GS was lower than Caucasians, while the frequency of the mutations in UGT1A1 exon line, especially, G71R mutation, were higher [16–18]. In recent years, it has been suggested that some newborns, who had pathologic jaundice showed features of GS and has been started to evaluate the relationship between newborn jaundice and UGT1A1 mutations [16, 18–23]. In this study, the frequency of the UGT1A1 gene promotor region TA7 polymorphism and its relationship between newborn pathologic jaundice has been evaluated.

Material and Methods

Term newborns with jaundice who were followed up in Mersin University and state hospitals and whose birth weights >2500 g were included in this prospective, randomized study. They had no known risk factors of maternal disease, blood group incompatibilities, hemolytic anemia, G6PD deficiency, infection, asphyxia, cephalohematoma, dehydration, hypothyroidism or liver disease. These conditions were evaluated by means of history, clinical and laboratory examinations such as full blood cell count, peripheral blood smear, reticulocyte count, hemoglobin electrophoresis, blood group and subgroups, G6PD activity, liver function tests, TSH, total T4 and total IgM. Newborns whose total bilirubin level exceeded 15 mg dl^{-1} within 7 days of

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TABLE 1
Clinical properties of the patient and control groups

	Patient ($n = 107$)	Control ($n = 55$)	p
Sex (male/female)	61/46	23/32	0.006
Birth weight (g)	3371.2 ± 489.4	3533 ± 483.1	0.046
Gestational age (week)	38.5 ± 1.0	38.6 ± 0.9	0.917
Number of births given by the mother	2.0 ± 1.3	2.0 ± 1.6	0.388
History of jaundice in brothers or sisters	12	0	0.009
Feeding (Formula/maternal milk)	3/104	0/55	0.552

life (pathologic jaundice, $n = 99$), and total bilirubin level still over $5\,mg\,dl^{-1}$ after the 15th day of birth (prolonged jaundice, $n = 8$) constituted the patient group ($n = 107$). Newborns with physiologic jaundice constituted the control group ($n = 55$). The parents of the infants gave their informed consent and the study approved by the institutional ethical committee.

DNA extraction and genotyping

Blood samples were collected in EDTA-containing tubes and stored at +4°C until DNA extraction. DNA was extracted from white blood cells by high pure polymerase chain reaction (PCR) template preparation kit (Cat no: 1 796 828, Roche Diagnostics, GmbH, Mannheim, Germany). UGT1A1 gene promotor region TA polymorphism were studied by using UGT1A1 (TA)6/7 Toolset for LightCycler (Order#: UGT1A1 (TA) 6/7–16, Genes-4U AG, Winterthur, Switzerland) and Fast Start DNA Master Hybridization Probe Kit (Cat no: 2 239 272, Roche Diagnostics, GmbH, Mannheim, Germany) by Light Cycler 2.0 Real-time PCR. Real-time PCR system performs rapid PCR and simultaneous polymorphism detection by melting curve analysis by monitoring the fluorescence. After amplification of the interested gene by using primers and hybridization probes, a melting curve was generated. As a result of melting curve analysis for UGT1A1 promotor region the melting temperature of polymorphic (TA)7 allele was found at 59.6 ± 2.5°C, and also the melting temperature of wild-type (TA)6 allele was found at 56.4 ± 2.5°C.

Statistical analysis

SPSS for Windows (version 10.0) statistical package software was used for the statistical analysis of the study. *Z*-test was used for ratio comparisons and chi-square test was used in order to test the differences between allele and genotype frequencies. To test the differences between the two independent groups, Student's *t*-test was used in order to compare the independent groups when the cases were in normal distribution and when not normally distributed, Mann–Whitney U-test was used.

Results

The clinical data of the patient and the control groups were summarized in Table 1. Male–female ratio and the rate of history of jaundice in siblings were significantly higher, while the mean birthweight was significantly lower in patient group compared with the control group ($p = 0.006$, 0.046 and 0.009, respectively). The gestational age, number of the gestations of the mother and feeding modalities in groups were not statistically significant.

The frequencies of (TA) 6/6, 6/7 and 7/7 genotypes were found as 89, 11 and 0% in patient group, and 85, 13 and 2% in control group, respectively (Table 2). The allelic frequencies of (TA)6 and (TA)7 in the patient and the control groups were found as 0.944 and 0.056, and 0.918 and 0.082, respectively (Table 3). The difference between groups for genotype and allele frequencies was not statistically significant ($p > 0.05$).

In the patient group, properties related to newborn jaundice of infants carrying a wild-type promotor region (6/6) and polymorphic allele (6/7 and 7/7) are given in Table 4. There were no correlation between (TA)7 presence of allele and the total bilirubin levels, onset of jaundice and need for blood exchange transfusion or phototheraphy (Table 4).

Discussion

Several studies on GS showed that the promoter region polymorphism of UGT1A1 gene has variable occurrence in different races. It was affecting up to 36% of Africans, but only 3% of Asians. However, some studies suggested that the TA repetitive polymorphism of promoter region of UGT1A1 gene might be related to the prolonged jaundice of newborn in European population, that it might be one of the risk factors of hyperbilirubinemia [18, 22, 24]. In our study, when we assessed the frequency of UGT1A1 promoter region polymorphism in neonates with hyperbilirubinemia, we found that no infant had 7/7 genotype and that 11% had 6/7 genotype. Similar to this genotype frequency the (TA)7 allele frequency also found as 0.056 in patient group, which was seen between 0.31 and 0.63 in Caucasians and between 0.02 and 0.17 in Asians [17, 18, 25, 26]. This finding is

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TABLE 2
(TA) 6/6, 6/7 and 7/7 genotype frequencies in patient and control groups

	6/6 (*n*)	6/7 (*n*)	7/7 (*n*)
Patient group	89% (95)	11% (12)	0
Control group	85% (47)	13% (7)	2% (1)

TABLE 3
(TA)6 and 7 allele frequencies in patient and control groups

	Patient group ($n=107$)	Control group ($n=55$)
(TA) 6 allele	0.944 (202)	0.918 (101)
(TA) 7 allele	0.056 (12)	0.082 (9)

TABLE 4
Effects of TA repetition number on jaundice in patient group

	Patient group ($n=107$)		
	TA 6/6	TA 6/7 + TA 7/7	*p*
Total bilirubin (mg/dl)	14.9 ± 9.0	12.9 ± 6.7	0.340
Onset of jaundice (day)	2.6 ± 1.1	2.2 ± 0.7	0.416
Phototherapy +	92/103	11/103	0.394
Blood exchange +	14/15	1/15	0.483

consistent with Asians rather than Caucasians. Also this finding might indicate that polymorphism is affected not only by the nations but also by the ethnic populations [20, 27]. Because other studies in Turkish population found higher prevalence of this polymorphism in neonatal hyperbilirubinemia [22, 23]. Different mutations, especially G71R mutation, are the leading genetic risk factor of the GS in Asian population [16, 17, 19]. Akaba *et al.* [28], Maruo, *et al.* [29] and Yamamoto *et al.* [30] also showed the relationship of this polymorphism with neonatal hyperbilirubinemia. In our regional population, studying G71R mutation which is never detected in Caucasians might come up with interesting results. In previous studies of Monaghan, *et al.* [16] and Babaoğlu, *et al.* [22] they were found that (TA)7 allele was seen more in prolonged newborn jaundice than in pathologic ones. Of the 107 infants enrolled in our patient group, eight had prolonged jaundice. The clinical difference of the patient group also might be the cause of lower frequency of this polymorphism in our regional population.

Maruo, *et al.* [31], Bihan, *et al.* [25], Bancroft, *et al.* [32] and Ülgenalp, *et al.* [23] found no relation between (TA)7 genotype with serum bilirubin levels. Similar to these studies no correlation was also found in our study between serum bilirubin levels and the polymorphism. Also, no correlation was found between TA(7) genotype and the onset of jaundice and treatment choice. It was suggested that the polymorphism of the promoter region of UGT1A1 gene alone would not be able to explain the causes of newborn jaundice in our study population.

Most factors which cause pathologic jaundice in newborns like blood group incompatibilities, hereditery spherocytosis, G6PD deficiency, usually do not show any signs or symptoms. Recent studies show that when these factors are alone, the disease frequently stays silent but when any of these come together, they increase the severity and the frequency of the newborn jaundice [33–42]. In such a situation, the association of above conditions with UGT1A1 gene mutation might also have significant effect on serum bilirubin levels.

Conclusion

Hyperbilirubinemia is the most common problem in neonates, and severe cases are treated by phototherapy or exchange transfusion. Nevertheless, the exact mechanism and the risk factors of neonatal hyperbilirubinemia have to be identified to determine a definitive treatment. Genetic mutations in the enzyme that is responsible for the bilirubin metabolism seem to be the most important risk factor in neonatal hyperbilirubinemia. But mutations found in UGT1A1 gene show differences between different races. In this study population, we found lower prevalance of the promoter region polymorphism of UGT1A1 gene. Our findings need additional studies on other mutations of this gene, especially G71A mutation. The leading mutations for a population have to be identified and the effect of any other contributing factors might also have to be taken into account.

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Switch from Antibiotic Eye Drops to Instillation of Mother's Milk Drops as a Treatment of Infant Epiphora

Summary

In a paediatric practice, the management of patients with signs and symptoms of congenital nasolacrimal duct obstruction (CNLO) was switched from topical antibiotic to topical mother's milk (MM) -based regimens. The conservative management of this condition includes frequent cleansing of the lids, digital lacrimal sac massage, and application of topical antibiotic drops when there is a mucopurulent discharge. The method for managing CNLO has evolved in our office of paediatrics during the past 7 years. This change was initiated by some mothers who have applied traditional therapy: MM eye drops. This evolution has been accompanied by a number of articles from the mid 1990s into the 2000s on safety of MM eye drops in case of neonatal conjunctivitis, and on the anti-inflammatory characteristics and antibacterial activity of topical MM.

Key words: breast feeding, human milk, lacrimal duct obstruction, infant, newborn, diseases

Professor John Davis noticed some 40 years ago that West Indian mothers at Hammersmith Hospital frequently treated neonatal sticky eye with breast milk [1]. In a paediatric practice, the management of patients with signs and symptoms of congenital nasolacrimal duct obstruction (CNLO) was switched from topical antibiotic to topical mother's milk (MM)-based regimens. The conservative management of this condition includes frequent cleansing of the lids, digital lacrimal sac massage, and application of topical antibiotic drops when there is a mucopurulent discharge [2]. The method for managing CNLO has evolved in our office of paediatrics during the past 7 years. This change was initiated by some mothers who have applied traditional therapy: MM eye drops. This evolution has been accompanied by a number of articles from the mid 1990s into the 2000s on safety of MM eye drops in case of neonatal conjunctivitis [3], and on anti-inflammatory characteristics [4] and on against micro-organisms activity [5] of topical MM. This is a retrospective analysis to compare results before and after management switching. Sixty-five patients who met the following selection criteria were evaluated: birth between 1 January 1999 and 1 June 2006, affiliation to this clinic within the first month of life, initially breastfed and follow-up until epiphora resolution. Twenty patients underwent a conventional treatment on antibiotics (A) and 45 patients underwent an alternative treatment on topical MM.

Study Population

There were no significant differences in birth gestation, type of delivery, birth weight, birth order or bilateral vs. unilateral CNLO between those who had received A and those who had received MM. There were significant differences in gender (unexplained): 15 males/5 females in case of A vs. 30 males/15 females in case of MM, $P = 0.014$; and in year of birth (the management was changed along several years): mean 2002.10 and SD 2.15 in case of A vs. mean 2003.42 and SD 0.32 in case of MM., $P = 0.023$. The Fisher's exact test was used to compare proportions and the *t*-test was used to compare different means. Table 1 gives the data for epiphora resolution by

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