Association between uridin diphosphate glucuronosylotransferase 1A1 (UGT1A1) gene polymorphism and neonatal hyperbilirubinemia

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Objective: To assess the prevalence of UGT1A1*28 and UGT1A1*60 polymorphisms of UGT1A1 gene and their association with hyperbilirubinemia. Study design: The study was performed at a single centre – at the Department of Obstetrics of the Medical University of Gdańsk in Poland. DNA was isolated from Guthrie cards of 171 infants. Only full term newborns (gestational age 38-42 weeks) were included in the study. Fluorescent molecular probes were used for UGT1A1 promoter variation analysis. The presence of UGT1A1*28 polymorphism was detected with a dual-probe system, and UGT1A1*60 with a SimpleProbe™. Result: Homozygous UGT1A1*28 and UGT1A1*60 genotypes were detected in 14.6% and 20.5% of the newborns, respectively. Homozygous (G/G) genotypes of UGT1A1*60 polymorphism were found in all of the UGT1A1*28 (i.e. (TA)6/(TA)6) homozygotes. More than 80% (55/66) of the children with “wild” type UGT1A1*28 genotype (where no polymorphism was detected) (i.e. (TA)6/(TA)6) carried the “wild” (T/T) genotype of UGT1A1*60 as well. The UGT1A1*28 polymorphism was detected more often among neonates with elevated bilirubin. Hyperbilirubinemia was diagnosed more frequently in boys. Conclusion: Polymorphisms of the UGT1A1 gene frequently co-exist in neonates. The presence of UGT1A1*28 polymorphism and male gender seem to predispose to neonatal hyperbilirubinemia.

Key words: UGT1A1 gene, polymorphism, hyperbilirubinemia, neonates

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INTRODUCTION

Hyperbilirubinemia is a relatively frequent condition of neonatal and early infantile period. Neonatal hyperbilirubinemia is defined as a total serum bilirubin level above 5 mg/dL (86 μmol/L) (Porter et al., 2002). Up to 50% of healthy, full term and 80% of preterm neonates demonstrate physiological jaundice (American Academy of Pediatrics Subcommittee on Hyperbilirubinemia, 2004; Scrafford et al., 2013). In some cases jaundice can be more severe and prolonged and it can be associated with a potential risk of neurotoxicity (Dore et al., 1999; Hammermann et al., 1998). Bilirubin monoglucuronide, degraded easier than the diglucuronide, is the only product of bilirubin conjugation in neonates. Moreover, the intestinal concentration of beta-glucuronidase, the enzyme responsible for elimination of glucuronic acid, is markedly higher in newborns than in adults, and only trace amounts of physiological bacterial flora reducing bilirubin to colorless tetrapyrole compounds are present. As a result, instead of being transformed into urobilinogen, most of unconjugated bilirubin is reabsorbed to the hepato-enteric circulation and can cross the blood-brain barrier causing neuronal injury (Hale et al., 2007). This phenomenon is referred to as bilirubin encephalopathy or kernicterus. The penetration of bilirubin into tissues starts with blood concentration exceeding 2.5 mg/dL (Murray et al., 2012).

The fact that the evident cause of neonatal or early infantile hyperbilirubinemia cannot be identified in more than half of the cases can suggest the presence of another unidentified risk factor of this condition. Racial predisposition suggests potential genetic background of neonatal hyperbilirubinemia (Maruo et al., 1999). Conjugation of bilirubin with glucuronic acid is catalyzed by a hepatic enzyme uridine diphosphate glucuronosylotransferase (UDP-glucuronosyltransferase), namely its 1A1 isoform (Clarke et al., 1997). The fact that the enzyme is not stable and its determination with classical methods is substantially biased, justifies its testing at a genetic level (Huang et al., 2002).

UDP-glucuronosyltransferase is encoded by the UGT1A1 gene. The A(TA)TA box polymorphism (UGT1A1*28), identified as the principal cause of the Gilbert syndrome, is relatively frequently detected in the Caucasian population (11–16%) (Bosma, 2003). The mutated allele is characterized by alterations in its promoter region, namely in the TATA box sequence. This sequence constitutes the binding site for transcription factor II D, which is vital for the initiation of the transcription process (Bosma et al., 1995). Decreased expression of the UGT1A1 gene results from the presence of additional TA repeat in the TATA box sequence, i.e. A(TA)TA instead of A(TA)TTA (Bosma, 2003). The presence of this variant is frequently associated with another alter-
ation in the UGT1A1 promoter, the polymorphism of Phenobarbital Responsive Enhancer Module (PBREM) (Sugatani et al., 2002). This polymorphism, resulting from a single nucleotide substitution (thymine to guanine, c.-3279T>G), is referred to as UGT1A1*60 (Costa et al., 2005; Maruo et al., 2004). Transcriptional activity of UGT1A1 in (TA)/(TA) homozygotes is decreased to 60-80%. Also, the presence of c.-3279T>G is associated with a decrease in the transcriptional activity to about 60% of its normal level. However, the co-existence of these two homozygous variants (UGT1A1*28 and UGT1A1*60) can be reflected by a decrease in the transcriptional activity of UGT1A1 down to 30% (Maruo et al., 2004).

Data on the influence of UGT1A1 gene polymorphism on the prevalence of hyperbilirubinemia in neonates from a Caucasian population are inconclusive (Kaplan et al., 2003). Bancroft et al. (1998), who analyzed the severity of jaundice by means of transcutaneous bilirubinometry, did not observe significant differences in peak bilirubin levels in children with UGT1A1*28 polymorphism when compared to controls. Nevertheless, they have shown an association between the presence of AT(A)/TA variant and the severity of jaundice during the initial two days of life (Monaghan et al., 1999). Another study, involving a group of Scottish newborns, revealed that nearly one-third of the children with prolonged jaundice were (TA)/(TA) homozygotes. Moreover, presence of the AT(A)/TA allele was suggested to predispose to jaundice in breastfed neonates (Monaghan et al., 1999). Also, results of the Italian and Greek studies suggest that newborns who carry the AT(A)/TA allele are characterized by a markedly higher prevalence and longer duration of hyperbilirubinemia, despite the lack of significant differences in peak concentrations of bilirubin between the carriers and controls (Laforgia et al., 2002; Roy-Chowdhury et al., 2002). Presence of the UGT1A1*28 polymorphism seems to predispose to hyperbilirubinemia in patients who are unexposed to other risk factors of this condition (Bancroft et al., 1998). However, opinions in this matter are inconclusive. According to Babaoglu et al. (2006), presence of the AT(A)/TA allele is not associated with the prevalence of hyperbilirubinemia among Turkish newborns. UGT1A1*28 was reported as the most common mutation causing the Gilbert’s syndrome in Caucasians; in contrast, a markedly lower prevalence of UGT1A1*28, corresponding to about 1-2%, was observed in the Asian population (Azlin et al., 2011; Tiwari et al., 2014). This suggests that the role of the UGT1A1 polymorphism in the pathogenesis of hyperbilirubinemia among newborns of Caucasian heritage is still not completely understood, justifying further research.

Therefore, the aim of this study was to assess the prevalence of UGT1A1*28 and UGT1A1*60 polymorphisms in a randomly selected group of Polish newborns from a single university hospital centre.

METHODS

Study group. The study included 171 newborns consecutively born at term (gestational age 38–42 weeks) at the Department of Obstetrics of the Medical University of Gdansk (University Clinical Center).

Only full term newborns were included in the study because prematurity is one of the risk factors of hyperbilirubinemia.

A detailed medical history, regarding the course of pregnancy (including maternal conditions and used medications), delivery (gestational age, Apgar score), familial history of systemic conditions and liver disorders, was obtained for every newborn. Also, anthropometric measurements at birth (birth weight, body length, head and chest circumferences) were recorded. Serum bilirubin level in all neonates was measured on the 2nd or 3rd day after birth. As the vast majority of subjects were breastfed and only few were subjected to mixed feeding, the effect of diet on bilirubin concentration was not analyzed. Detailed characteristics of the study participants are presented in Table 1.

ETHICS. The protocol of the study was approved by the Local Bioethical Committee of the Medical University of Gdansk (decision no. NKEBN/87/2008). Parents of all children gave their informed consent for their participation in the project. All of the samples were collected during a routine medical examination and the newborns participating in the study were not exposed to any additional invasive procedures.

Material. The examined material included 171 so-called Guthrie cards (Whatman, UK) with the dried samples of capillary blood obtained by pricking the heel. Moreover, the serum level of bilirubin was determined by means of the diazo method (Architect Systems™, USA) on the 2nd or 3rd day after birth.

The data was collected between November 2010 and January 2011.

Genetic tests. Genomic DNA was isolated from the Guthrie cards with an aid of Sherlock AX set (AbA Biotechnology, Poland). The methodology of detecting the studied UGT1A1 polymorphisms was described previously (Romanowski et al., 2009). UGT1A1 gene fragments of interest were amplified by means of asymmetric PCR. The presence of UGT1A1*28 polymorphism was detected with two molecular probes, a fluorescence-labeled anchor probe and a quencher-labeled (IowaBlackFQ; Integrated DNA Technology, USA) sensor probe, and the presence of UGT1A1*60 polymorphism was determined with a single fluorescent SimpleProbe™ designed with LightCycler Probe Design Software 2.0 (Roche Diagnostics, Germany). Genotyping was based on the differences in the binding stability (melting temperatures) of the probes and various allelic variants. Melting temperature profiles of the probes hybridized to PCR products were determined with the LightCycler 2.0 instrument (Roche Diagnostic, Germany) by increasing temperature (T) while measuring the sample fluorescence (F). Fluorescence was further expressed as the first negative derivative of temperature (-dF/dT). The examples

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
<td>3379±520.66 (2490–4875)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>40.6±2 (38–42)</td>
</tr>
<tr>
<td>Males (n)</td>
<td>91 (53.2%)</td>
</tr>
<tr>
<td>Females (n)</td>
<td>80 (46.3%)</td>
</tr>
<tr>
<td>Natural delivery (n)</td>
<td>142 (83.0%)</td>
</tr>
<tr>
<td>Cesarean section (n)</td>
<td>29 (17.0%)</td>
</tr>
<tr>
<td>Apgar score (points)</td>
<td>9.12 ±1.9 (7–10)</td>
</tr>
<tr>
<td>Total serum bilirubin (mg/dl, 2nd or 3rd day after birth)</td>
<td>8.6 ± 6.5 (0.8–19.7)</td>
</tr>
</tbody>
</table>

Data presented as arithmetic means ± S.D. (ranges) or numbers (%).
of melting curve analyses of the UGT1A1 genotypes are shown in Figs. 1 and 2.

Statistical analysis. Normal distribution of continuous variables was verified with the Kolmogorov-Smirnov test, and their statistical characteristics were presented as mean values and standard deviations (S.D.), or medians and ranges. Statistical characteristics of continuous variables of the neonates with hyperbilirubinemia and normal bilirubin level were compared with the Mann-Whitney U-test. The Pearson’s chi-square test and the exact Fisher test were used for the intergroup comparisons of qualitative variable distributions (i.e. presence of the studied polymorphisms and general characteristics of the neonates). The results of all tests were considered significant at \( p < 0.05 \). All analyses were performed with the Statistica 10 software (Stat Soft. Inc., USA).

RESULTS

Polymorphism of the UGT1A1 gene

Homozygous UGT1A1*28 and UGT1A1*60 genotypes were detected in 25/171 (14.6%) and 35/171
in boys than in girls ($p = 0.005$). We noted no impact of the mode of delivery or birth weight on the prevalence of hyperbilirubinemia ($p = 0.449$, $p = 0.07$ respectively) (Table 4).

**DISCUSSION**

Presence of the (TA)$_7$//(TA)$_6$ genotype was documented in 14.6% of the examined newborns. This value is similar to others previously reported in the European studies. Prevalence of the (TA)$_7$//(TA)$_6$ homozygous genotype in the Caucasian population is estimated at several percent, amounting to 9.7% in Sweden, 13.6% in Slovenia, 11.6% in Germany, and 12.6% in the Netherlands (Borlak et al., 2000; Mercke Odeberg et al., 2006; Ostanek et al., 2007; Peters et al., 2003). However, data from a Turkish study documented a markedly lower percentage, amounting to 7.5% prevalence of this genotype. This has underlined that $UGT1A1*28$ is the most common mutation causing the Gilbert's syndrome in Caucasians. In contrast, its prevalence in the Asian population was estimated at about 1–2%. The most common mutation of the UGT1A1 gene in this population was nt211 G>A, with the prevalence up to 10–20% (Azlin et al., 2011; Tiwari et al., 2014; Liu et al., 2013).

Frequency of the (TA)$_7$//(TA)$_6$ genotype in our group seems relatively high as compared to the abovementioned investigations, although even higher prevalence of this genotype (16.3%) was documented by an Italian study (Biondi et al., 1999). Moreover, it should be noted that we have analyzed newborns only from one hospital, and therefore our findings are not representative for the whole Polish population.

Presence of the G/G genotype of $UGT1A1*60$ was identified in 20.5% of the newborns in our group. This value is higher than the prevalence of the $UGT1A1*28$ polymorphism. The prevalence of the $UGT1A1*60$ polymorphism has not been studied extensively thus far (Bosma et al., 1995; Sugatani et al., 2002; Maruo et al., 2004).

Nevertheless, previous studies dealing with the problem in question documented a tendency to the co-existence of both analyzed genetic variants (e.g. $UGT1A1*28$ and $UGT1A1*60$) (Costa et al., 2005, Maruo et al., 2004). The co-existence of $UGT1A1*28$ and $UGT1A1*60$ polymorphisms was documented in our group of newborns as well. The homozygous form of $UGT1A1*28$ was detected in more than 70% of the neonates carrying $UGT1A1*60$. The co-existence of both mutated homozygous genotypes is postulated to decrease the translational activity of $UGT1A1$ down to 30% (Maruo et al., 2004).

It is worth to note, that the UDP-glucuronosyltransferase activity is only one of numerous mechanisms contributing to the pathophysiology of jaundice in newborns.

Recent studies had shown that 388G>A, 521 T>C and 463 C>G mutations of the $SCL01B1$ (solute carrier organic anions transporter family member 1B1) gene may predispose to neonatal hyperbilirubinemia by limiting the hepatic bilirubin uptake (Liu et al., 2013; Biondi et al., 1999).

Glucose-6-phosphate dehydrogenase deficiency has been also reported as a risk factor for severe neonatal hyperbilirubinemia. The prevalence of this defect in newborns with indirect hyperbilirubinemia is estimated at about 4% (0.5–20%, depending on geographical and ethnic group) (Watchko et al., 2010; Celik et al. 2013). These important risk factors for neonatal jaundice, not assessed in our study, need further investigation.
We have documented a significantly higher prevalence of hyperbilirubinemia in male newborns. This observation is consistent with the results of previous research, however, the mechanism of this phenomenon is not clear (Tioseco et al., 2005). We did not reveal a statistically significant association between presence of the UGT1A1*60 polymorphism and the prevalence of hyperbilirubinemia in our group. However, we have observed a significant relationship between presence of the UGT1A1*28 polymorphism and an elevated level of bilirubin in our samples. This finding is consistent with the results of previous studies conducted in the Caucasian population (Twari et al., 2014; Borlak et al., 2000; Mercke Odéberg et al., 2006; Ostanek et al., 2007; Peters et al., 2003). We have also analyzed the effect of birth weight on the prevalence of hyperbilirubinemia but did not find a significant association between these two parameters. According to some authors, low birth weight is a risk factor of jaundice (Scaffold et al., 2013; Tioseco et al., 2005; Okwundu et al., 2013; Yang et al., 2013). Indeed, the immaturity of enzymatic systems in preterm newborns constitutes a potent factor modulating the severity of jaundice, and most preterm babies are delivered with low birth weight (Huang et al., 2002).

To minimize the number of invasive procedures, we have determined the serum bilirubin level only once, on the 2nd or 3rd day after birth. Presently, transcutaneous measurement is suggested to be a potential non-invasive method for serum bilirubin level determination, not requiring an extra blood sample. This method enables multiple determination of bilirubin level and is optimal for monitoring of jaundice in newborns (Scaffold et al., 2013).

Interestingly, authors from Nepal had relied only on visual determination of skin and sclera color while defining risk factors of hyperbilirubinemia in newborns (Scaffold et al., 2013).

The use of dried blood spot testing used to determine the UGT1A1 polymorphism had enabled us to analyze very small amounts of biological material, without unnecessary exposure of the studied babies to additional venipuncture. Blood collected onto the Guthrie card can be stored for a long period of time and reused if any additional analysis is required. The quality and purity of genetic material isolated from the cards is sufficient to obtain melting temperature profiles of the fluorescent probes and the PCR products, and to identify genetic profile of the probands accurately.

CONCLUSION

In conclusion, our study revealed that assessment of the UGT1A1 gene polymorphism is an important test in neonatal patients.

We have found co-existence of the mutated homozygous genotypes of UGT1A1*28 and UGT1A1*60, UGT1A1*28, but not UGT1A1*60 polymorphism correlated with presence of hyperbilirubinemia. Neonatal hyperbilirubinemia occurs more frequently in the male gender.

These preliminary findings should be verified in a larger cohort of neonates. Furthermore, other potential modulators of bilirubin level should be included in the risk analysis.

Conflict of interest

The authors declare no conflict of interest.

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REFERENCES


