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Original Article

Genetics Section

The Association between Prolonged Jaundice and TATA Box Dinucleotide Repeats in Gilbert's Syndrome

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ABSTRACT

Introduction: Jaundice is a common condition during the neonatal period. Prolonged jaundice occurs in a large number of breastfed infants, considering the impact of genetic factors on the incidence of jaundice.

Aim: To determine the association between prolonged jaundice and TATA box dinucleotide repeats in Gilbert's Syndrome (GS).

Materials and Methods: In this case-control study, the case group consisted of 51 neonates with jaundice, aged more than two weeks with indirect bilirubin level higher than 10 mg/dl. Acute diseases, mother's use of phenobarbital and other medications were the exclusion criteria. The control group consisted of 54 newborns without jaundice. The two groups were matched in terms of age and sex. TATA box polymorphisms in the promoter region of UGT1A1 gene were evaluated using Polymerase Chain Reaction (PCR) in order to determine TATA box dinucleotide repeats.

Results: Overall, 64.7% and 50% of subjects in the case and control groups were male, respectively (p=0.168). The mean age of neonates in the case and control groups was 20.1±7.1days and 18.8±4.1 days, respectively. The distribution of Gilbert genome was not significantly different between the two groups. In the case group, 13.7% of the subjects were homozygous, 37.3% were heterozygous and 49% were normal. In the control group, 7.4% of the participants were homozygous, 35.2% were heterozygous and 57.4% were normal.

Conclusion: The results of this study showed an association between TATA box polymorphism and prolonged jaundice in neonates which revealed that TATA box polymorphism is an important risk to increase and extend icterus.

Keywords: Indirect bilirubin level, Mutation, Neonatal, Polymorphism, Prolonged icter

INTRODUCTION

Inherited deficiency in the glucuronidation of endogenous bilirubin results in GS in 5%-7% person of the human population, leading to bilirubin accumulation, hyperbilirubinemia and jaundice [1,2]. Previous genetic studies have shown that the clinical phenotype, reported in 3% of Asians, can be caused by dinucleotide polymorphisms in the TATA box promoter of bilirubin uridinediphosphate-glucuronosyltransferase gene (UGT1A1). Although other heterozygous mutations in the coding exon 1 of UGT1A1 gene (G71R) can also provide the Gilbert phenotype in Japanese and Asian populations, this syndrome is a contributing factor for prolonged hyperbilirubinemia in infants with jaundice and may accelerate jaundice when associated with other inherited blood disorders [3].

Jaundice in infants and older children may represent as conjugated or non conjugated bilirubin or can be associated with inherited disorders. GS is caused by a homozygous mutation in the TATA element (TA Repeat 7) of UGT1A1, reduced gene level expression. The relationship between this syndrome and haemolytic anaemia increases the risk of hyperbilirubinemia and cholestasis [4]. Neonatal hyperbilirubinemia (bilirubin level > 223 mmol/L during the first seven days of life) could be a result of GS and its genotype frequencies have been investigated before [4].

In infants with GS, it takes longer to reach the normal bilirubin level. This confirms that GS is one of the contributing factors for neonatal hyperbilirubinemia, although other factors are also involved in the diagnosis of jaundice. Slower decrease of bilirubin level for A(TA)7TAA in homozygous neonates indicates that GS is an important factor for determining prolonged jaundice [4,5].

Jaundice is a preventable cause of brain damage, mental retardation and death in early infancy. Prolonged jaundice causes anxiety in families, promotes the use of synthetic drugs, and reduces breastfeeding in infants. On the other hand, GS is a cause of neonatal jaundice.

Since no previous study has determined the relationship between GS and prolonged neonatal hyperbilirubinemia in Iran, the aim of this study was to assess the relationship between prolonged neonatal jaundice and TATA box dinucleotide repeats in GS.

MATERIALS AND METHODS

In this cross-sectional case-control study, during January 2012 to February 2014, 105 patients were enrolled. This study was approved by Regional Ethical Committee for research of Babol University of Medical Sciences. And written consent was obtained from parents of all neonates recruited in this study.

The case group consisted of patients with these inclusion criteria: term neonates with prolonged jaundice, aged over two weeks with indirect bilirubin level >10 mg/dl [6]. The exclusion criteria were: severe underlying diseases and use of phenobarbital and other medications by mothers. The control group consisted of infants without jaundice, referred to Amirkola Hospital for routine neonatal care such as weight gain and growth chart. The two groups were matched in terms of age and sex.

For sample size estimation, the formula for comparing the prevalence between two groups was used then the prevalence from the literature, with prevalence of 0.32 and 0.1 was retrieved from the literature [7], the significance level of 95% and power of 80% were used. Subjects were recruited with convenient method.

Primers characteristics	Forward	Reverse
b1 and b2	5'-ATT AAC TTG GTG TCG ATT GG-3	5'-AGC CAT GGC GGC CTT TGC TC-3

[Table/Fig-1]: Characteristics of primers used in this study.

Polymorphisms of UGT1A1 gene promoter for determining the number of TATA box di-nucleotide repeats were prepared as follows [8]:

1. Five cc (cubic centimeters) of blood was taken from each infant, using EDTA-containing tubes;
2. DNA was extracted via alkaline lysis method;
3. DNA was amplified by PCR (Siemens, Germany) using these specific primers as shown in [Table/Fig-1].

In each reaction, the following materials [Table/Fig-2] were used for DNA amplification. Temperature conditions for PCR reactions are shown in [Table/Fig-3]. After DNA amplification by PCR, 5 µl-10 µl of the final product was placed on a 20% polyacrylamide gel electrophoresis and stained with silver nitrate [8].

Material	Measures
dH_2O	39.45 µl
10 mM dNTP	0.5 ml
Primer F	10 pmol
Primer R	10 pmol
50 mM $MgCl_2$	1.25 ml
Buffer 10X	5 µl
DNA	100-500 ng

[Table/Fig-2]: PCR reaction materials and methods.

Temperature	Time
94 °C	2 minutes
94°C	30 seconds
60 °C	30 seconds (15 cycles)
72 °C	30 seconds
94 °C	30 seconds
58 °C	30 seconds (25 cycles)
72 °C	30 seconds
72°C	4 minutes (the final elongation)

[**Table/Fig-3]:** Temperature conditions for PCR reactions.

STATISTICAL ANALYSIS

Statistical analysis was performed using Chi-square test, t-test and One-way ANOVA test through SPSS software version 15.0. A p-value less than 0.05 were considered statistically significant.

RESULTS

In this study, cases and controls were not significantly different with regards to the gestational age (38.5±0.3 versus 38.6±0.4, p=0.32) and birth weight (3.2±0.4 versus 3.0±0.7, p=0.21).

Prevalence of GS was higher in the case group. There was no significant difference in genotype of people with hyperbilirubinemia.

In the case group, 33 subjects (64.7%) were male and 18 (35.3%) were female. In the control group, 27 cases (50%) were male and 27 cases (50%) were female (p=0.168). The mean age of the infants in the case and control groups was 20.1±7.1 days and 18.8±4.1 days, respectively (p=0.284).

The mean bilirubin level in icteric infants was 13.7±2.1 mg/dl. Distribution of G6PD status and blood group incompatibility are shown in [Table/Fig-4]. As shown in [Table/Fig-4], the distribution of blood group incompatibility and G6PD in the case and control groups showed no significant difference (p>0.05).

Frequency distribution of Gilbert mutations (TATA box dinucleotide repeats) in the case and control groups is shown in [Table/Fig-5]. The frequency distribution of Gilbert mutations (dinucleotide repeats in TATA box) in the case and control groups showed no significant difference (p<0.05) [Table/Fig-5].

Groups / Variables		Case group Frequency (%)	Control group Frequency (%)	p-value*
Blood Group incompatibility	Yes	10 (19.6)	7(13)	0.431
	No	41 (80.4)	47 (87)	
G6PD status	Yes	11 (21.6)	8 (14.8)	0.45
	No	40 (78.4)	46 (85.2)	

[Table/Fig-4]: Distribution of G6PD status and blood group incompatibility in the case and control groups.
*Chi-square test.

Groups / Mutations	Study group Frequency (%)	Control group Frequency (%)	p-value*
Homozygous	7 (13.7)	4 (7.4)	0.502
Heterozygous	19 (37.3)	19 (35.2)	
Normal	25 (49)	31 (57.4)	
Total	51 (100)	54 (100)	

[Table/Fig-5]: Distribution of Gilbert mutations (TATA box di-nucleotide repeats) in the case and control groups.
*t-test.

Considering the Gilbert mutations shown in [Table/Fig-6], mean±SD of serum bilirubin (mg/dl) in icteric neonates was not significantly correlated with Gilbert mutations (p>0.05) [Table/Fig-6].

Gilbert mutation	Number	Bilirubin serum level mg/dl (Mean±SD)	p-value*
Homozygous	7	14.4±3.1	0.416
Heterozygous	19	13.2±1.5	
Normal	25	13.8±2.2	

[Table/Fig-6]: The mean and standard deviation of bilirubin in icteric neonates, according to Gilbert mutations.
*One-way ANOVA.

DISCUSSION

This study determined the relationship between TATA box di-nucleotide repeats in GS and prolonged neonatal jaundice. Results showed that homozygous mutations of Gilbert, associated with 7TA repeat was higher in infants with prolonged jaundice than infants without jaundice.

The prevalence of jaundice was 13.7% in homozygous infants with prolonged jaundice and 7.4% in non-icteric neonates, with no significant difference between the two groups, probably due to the small sample size (p>0.05).

It seems that additional homozygous TA repeats associated with GS decrease the protein adhesion of TATA box to TATA and changes promoter activity since the coding region of the gene is intact. Enzyme production is reduced by 30% although the structure remains normal. The reduction of enzyme diffusion leads to reducing conjugation and increasing serum bilirubin concentration [7]; those with TA7 mutations have less TA6 conjugation. In fact, conjugating enzyme activity is inversely correlated with repeated TA [8].

However, in a study by Muslu N et al., conducted in Taiwan, 107 infants with jaundice and 55 normal infants were compared. Overall, 11% of icteric infants and 13% of non-icteric infants had UGT1A1 gene mutations [9]. The results of the present study were in agreement with the previously mentioned findings. However, other studies have found a significant association between gene mutation and neonatal jaundice.

Monaghan G et al., performed a study to examine the association between UGT1A1 gene mutations and prolonged jaundice [10]. Blood samples were collected and DNAs were prepared from 85 term infants with unexplained hyperbilirubinemia. The genetic

analysis of UGT1A1 TATA (DNA test for GS) was performed on all patients. The results showed that in addition to common UGT1A1 TATA alleles (TA6 and TA7), a new TATA allele (TA5) was present in infants with prolonged jaundice.

In another study, the incidence of familial hyperbilirubinemia genotype in patients with prolonged hyperbilirubinemia was five times higher than those with acute hyperbilirubinemia. The results showed that genetic predisposition to prolonged neonatal hyperbilirubinemia are associated with TATA box polymorphisms of UGT1A1 gene in breastfed infants [11].

Similarly, in a study by Akaba K et al., conducted in Japan, 159 Japanese term infants were evaluated, and the relationship between UGT1A1 gene and jaundice was investigated. G71R mutation frequency was 19% in infants. Also, infants who were carriers of G71R mutation had significantly higher bilirubin levels on days two to four. The frequency of G71R mutation was 47% in infants requiring phototherapy and 16% in infants not requiring phototherapy. In this study, G71R mutation was considered a risk factor for neonatal hyperbilirubinemia [12].

In a study by Huang CS et al., in Taiwan, the effect of coding region variation in UGT1A1 on the risk of hyperbilirubinemia was evaluated. In this study, 123 full-term and healthy neonates with hyperbilirubinemia were selected as the case group with no risk factors for hyperbilirubinemia, and 218 healthy term infants were selected as the control group [13].

In another similar study by Alaee E et al., which included 87 term infants with jaundice and 81 infants without jaundice, G71R polymorphism was evaluated by PCR-CCTP. They found no association between G71R polymorphism and Jaundice [14]. In all the studied subjects, exons 1-4 and exon 5 coding regions of UGT1A1 gene were investigated by PCR method. Overall, this study showed that nucleotide 211 variation (G71R) in UGT1A1 gene was a risk factor for the development of neonatal hyperbilirubinemia. Therefore, pediatricians should closely follow homozygous infants with hyperbilirubinemia as carriers of G71R variations in UGT1A1 gene [13].

Maruo Y et al., examined the relationship between neonatal hyperbilirubinemia and UGT1A1 gene mutations. They evaluated 25 infants with non-physiologic hyperbilirubinemia (7 mmol/L) and 25 infants with no obvious cause. The control group consisted of 50 Japanese infants whose jaundice index was controlled during the first week of life; mutations were identified by PCR. The frequency of the mutant allele in infants with hyperbilirubinemia (0.34) was significantly higher than that of the control group (0.16) [15].

In a study conducted by Seco ML et al., using distributed TA7, the correlation between GS and jaundice was studied in a group of infants [16]. In this study, 136 icteric and non-icteric infants were assessed. DNA was obtained from patients using PCR, and the promoter region of the UGT1A1 gene, located next to the TATA box, was determined. Overall, 7% and 14% of genotypes were homozygous in the non-icteric and icteric groups, respectively. Comparison between these groups showed that the prevalence of UGT1A1 polymorphisms was higher in infants with jaundice.

CONCLUSION

Our findings showed that the distribution of homozygous Gilbert mutations, associated with 7TA mode, was greater in infants with prolonged jaundice, compared to those without jaundice. However, this difference was not statistically significant, perhaps due to the small sample size. To confirm our results, studies on a larger population is required. Taken together, differences observed in our study could be clarified by the genetic background of the Iranian population, such as consanguineous marriages.

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