Association of G71R Mutation of the UGT1A1 Gen with Neonatal Hyper Bilirubinemia in the Iranian Population

Ebrahim Dastgerdy; M.D.[1], Gholamali Mamori; M.D.[2], Jalil Tavakoly Afshari; M.D.[2], Reza Saeedi; M.D.[2], Fatemeh Shahbazi; Ph.D.[3], Mahboobeh Shirazi; M.D.[4]

1 Neonatology Center of Semnan University of Medical Sciences, Emam Khomeini Hospital of Garmsar
2 Neonatology center of Mashhad University of Medical Sciences, Ghaem Hospital of Mashhad
3 Biology Department, Payame Noor University, Karaj Unit, Karaj, Iran
4 Department of Obstetrics and Gynecology, Tehran University of Medical Sciences, Tehran, Iran.

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Abstract

Objective: Jaundice with indirect hyperbilirubinemia is one of the most common neonatal problems that occur in 60% of term and 80% of preterm neonates but the causes are mostly unknown. It is suggested that race plays an important role in the prevalence of hyperbilirubinemia. It is a common problem in Iran that worries both parents and pediatricians. It has been found that a mutation in the UGT1A1 gene is responsible for structural changes in an encoded enzyme which reduces the function of the enzyme.

Materials and methods: This is a case-control study carried out in Ghaem Educational Hospital, Mashhad University of Medical Sciences from December 2007 for the period of one year. 26 healthy neonates tested for indirect hyperbilirubinemia within first week after full-term delivery and 53 healthy neonates without hyperbilirubinemia as a control group were included. Genomic DNA extracted using 2 cc blood sample followed by RFLP-PCR for detection of G71R mutation of UGT1A1 gene have been performed. SPSS software (version 16), t- test and chi square analysis have been used for statistical analysis of obtained data.

Results: 4.3% of the hyperbilirubinemic group was homozygotes for mutation in UGT1A1 and 26.1% were heterozygotes while 69.6% had no mutation. 21.3% of the control group had the mutation with 4.3% being homozygote and 17% being heterozygote.

Conclusion: Frequency of G71R mutation in the hyperbilirubinemia group was not significantly more than that in the control group among Iranian newborns. This finding suggests that G71R mutation may not contribute to the development of neonatal hyperbilirubinemia in Iranian newborns. It is recommended to establish further studies using well-designed inclusion criteria and more specialized mutation analysis techniques which cover all types of probable mutations in G71R gene.

Keywords: Hyperbilirubinemia, Newborn, UGT1A1 gene, Iran polymorphism

Introduction

Indirect hyperbilirubinemia related jaundice is one of the most common clinical problems that face the Iranian pediatrician in hospitals and their clinics. It seems that the incidence of indirect hyperbilirubinemia jaundice is higher among Iranian (Middle East) newborns than Caucasian newborns and is rather similar to that seen in East Asian population (1). The cause of such higher incidence is

Correspondence:
Dr. Mahboobeh Shirazi, Gynecology ward, Emam Hospital, Keshavarz blvd., Tehran, Iran.
Phone: + 98- 21- 66438674
E–mail: Mahboobeh.Shirazi@yahoo.in

unknown and no etiology has been found for it therefore we think this is due to differences between genotypes of Iranian and Caucasian populations which are similar to East Asian populations. We suggest genetic risk factors are partly involved in development of neonatal hyperbilirubinemia in Iranian newborns.

We hypothesized that this is caused by a mutation in the coding region of the UGT1A1gene which is responsible for the reduced function of the UGT1A1 enzyme and development of hyperbilirubinemia in Japanese, Chinese and Korean newborns (2). The most frequent hyperbilirubinemia-causing mutation in East Asian neonatal populations converts glycine (Gly) into arginin (Arg) at codon 71 (G71R) (3). This study is to determine the above mentioned mutation in Iranian newborns classified in two groups: with hyperbilirubinemia and without hyperbilirubinemia..

Materials and methods

Seventy nine neonates who have been born from December 2007 through December 2008 at Ghaem Hospital were recruited for this study after obtaining informed consent from their parents. Among them, 26 had hyperbilirubinemia. The definition of hyperbilirubinemia in this study is based on a total serum bilirubin level more than 18 mg/dl in the first 7 days of life. All neonates were born at Term (37-42 weeks gestation) and weighed more than 2500 g (mean=3061 ± 561g). They had no known risk factor including maternal diabetes, blood group and Rh incompatibility, polycythemia, infection, asphyxia hypothermia, hypoglycemia, drug therapy, cephalohematoma or liver dysfunction and other systemic diseases. They also had no clinically detectable pathology except for hyperbilirobinemia. All affected neonates received phototherapy and were fed with breast milk. There was no significant difference between the hyperbilirubinemia and non-hyperbilirubinemia group in terms of gestation age, birth weight sampling day and sex.

The neonates were monitored for serum bilirubin level, blood cell count and blood cultur C- reactive protein and all measures were within normal range, except for the serum bilirubin level.

The control group combined 53 full term Iranian neonates born in the same hospital from December 2007 to December 2008.

Jaundice of the control group was monitored during the first week of life with trans cutaneous bilirubinometer and their birth weight was greater than 2500 g. All of the neonates were fed with breast milk.

To our best knowledge the genetics of the conversion of indirect hyperbilirubinemia to direct hyperbilirubinemia among ichterus neonates has not been studied in Iran yet, therefore we carried out this study to find whether this condition correlates with mutations in the UGT1A1 gene in the Iranian population, as a part of South-West Asian population.

1) Sampling: blood samples were collected from neonates with icter as already defined.

2) DNA extraction: DNA was extracted using salting out methods (4).

3) Genotyping: PCR was performed using PCR-RFLP method.

Sense-primer:
5`TGACGCCTCGTTGTACATCAGAGCC3` and antisense-primer:
5`TCACACGCTGCAGGAAAGAA 3` were used for codon 71 to do sequence genotyping of the UGT1A1 gene. The sense-primer contains 1-bp mismatch (underlined) immediately proximal to the 3`end; and the 143-bp amplified product possesses a restriction site for endonuclease MSP1. MSP1 digestion of the PCR amplicon resulted in 119-bp and 24-bp fragments for the wild-type gly71 allele, but failed to cleave the 143-bp fragment containing the Gly71> Arg mutant allele.

PCR reaction was performed in 20 µl of 10X buffer (sinagen-Iran) using 250 mmol/µl Mgcl (sinagen-Iran) and 10 mmol/µl dNTP (gennetbio-korea) 5 IU/µl DNA Taq polymerase and 10 pmol/µl above-mentioned primers for each sample 200 ng/µl. Amplification was carried out using the biometra (UK) system at 940 for 5 minute and 30 cycles of denaturation at 940 for 30 seconds. Annealing was performed at 550 for 30 seconds followed by extension at 720 for 60 seconds. Finally the solution was left at 720 for 5 minutes. Gel electrophoresis was run on PCR products using 1.5% agarose gels with ethidium bromide staining. 5 µl of the amplified product was incubated together with 1 unit of MSP1 and the manufacture`s buffer for overnight at 370C. Finally, the digested products were size-separated utilizing acryl amid gel electrophoresis on 15% gels with silver staining.

Statistics

Clinical measures including total bilirubin level, gestational age, sampling day and patients' gender were compared between the hyperbilirubinemia and the control groups using student`s t – test.

Distributions of the genotypes and frequencies of

the G71R mutation were compared using chi-square test. Differences were considered significant when $P < 0.05$. The study was approved by the review board of MUMS neonatal department.

Results

The seventy nine neonate enrolled and 70 neonate completed the study 23 incase group and 47 in control group that genotypic distribution for G71R mutation among 70 Iranian neonates has been found as G/G 53 (75.7%), G/R14 (20%) and R/R 3 (4.3%) at a *p* value of 0.668, df=2 and x2= 0.806. Allele frequency of the G71R mutation was 14.3% (Table 1).

Analysis of UGT1A1 revealed that hyperbiliruinemia had the identical transition mutation in the codon (GGA to AGA) that caused Arg to replace Gly at position71 (G71R).

Among the case group one of the seven was homozygote and 6 cases were heterozygotes. In the control group, 10 out of 47 neonates (21.3%) had the transition mutation and two out of 10 cases were homozygotes while 8 of them were heterozygotes at position71 in exon 1 (G71R).

Analysis of frequency of the allele carrying mutation (G71R) in codon 71 indicates that the hyperbilirubinemic group (17.4%) is not significantly different (X2=1.409 df = 1 p= 0.235) from the control group (0.127), however the incidence of mutation in hyperbilirubinemic group was higher than the control group.

In the control group, the cases that carried mutation had a higher bilirubin level than the control group, although it was not statistically significant.

Discussion

The physiological condition of unconjugated hyperbilirubinemia that was seen in 60% of full term neonates during the first week of life is usually related to prematurity of UDPGT enzyme activity. Statistically, our study is unable to confirm that the higher levels of bilirubin is caused by mutations in UGT1A1 gene, as there was no significant increase in the frequency of the mutation (G71R) among hyperbilirubinemic group (17.4%) in comparison with the control group (12.7%). Our findings are consistent with the reports from Suomoto et al, 2004 (5). Akaba *et al*, 1999 (6), Marue *et al*, 1999 (7) and Yamata *et al* 2002 (8) have reported that the frequency of the G71R mutation in neonates with severe hyperbilirubinemia was higher than that in neonates without hyperbilirubinemia (2-7). Frequency of the G71R mutation has been reported to be extremely rare among Caucasian population (9).

We suggest that the frequency of the G71R in the Iranian population is between its frequency for East Asian and Caucasian population.

Conclusion

On the basis of our findings, we suggest that the factors other than the G71R mutation in the UGT1A1 gene such as environmental or nutritional factors may contribute to the development of neonatal hyperbilirubienema; however this needs further investigation on larger population of neonates.

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Table 1: Represents the genotypic distribution of G71R mutation in two groups

	Case			Control			P
	Num	Alle	Per	Num	Alle	Per	
Homozygote	1	2	4.3	2	4	4.3	
Heterozygote	6	6	26.1	8	8	17	
Total mutant	7	8	30.4	10	12	21.3	0.235
Nonmutant	16	38	69.6	37	82	78.7	
Total	23	46	100	47	94	100	

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