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Original Article

Haematology Section

Relation Between the Uridine Diphosphate Glucuronosyltransferase 1A1 Polymorphism and the Bilirubin Levels in Sickle Cell Disease

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ABSTRACT

Background: Genetic variations in the promoter of uridine diphosphate (UDP)–glucuronosyltransferase 1A1 (UGT1A1) may be associated with hyperbilirubinaemia and it appears to be a risk factor for gallstone formation.

Aims: Our aim was to detect the correlation between the UGT 1A1 (TA)n repeats and hyperbilirubinaemia and gall stone formation in Indian sickle cell patients.

Settings and Design: This was a cross-sectional study; which was carried in an autonomous tertiary care hospital.

Materials and Methods: The study subjects were 50 sickle cell anaemia and 70 sickle cell β-thalassaemia patients who were diagnosed by HPLC. The haemogram of the patients was measured by using an automated cell analyzer, while the serum bilrubin measurement was done by using a Beckman- CX-9 auto analyzer. The presence of gall stones was detected by ultra sound examination.

Statistical Analysis: ANOVA and the T-test were applied to compare the means of the groups. The allele frequencies were calculated according to the Hardy-Weinberg equilibrium.

Results: The allele, 7/7 TA of the UGT1A1 genotype was more frequent in the sickle cell patients and it was associated with hyperbilirubinaemia and gall stone formation.

Conclusions: The allele, 7/7 TA of the UGT1A1 polymorphism affects the bilirubin levels and the development of gallbladder stone in the Indian sickle cell patients.

Key Words: Haemoglobinopathies, Sickle cell anaemia, UDP-Glucuronosyltransferase1A1

INTRODUCTION

UGT is encoded by the UGT1A1(uridine diphosphate glucuronosyltransferase 1A1) gene at chromosome 2q37.1, whose genotypes with a reduced enzyme activity, are known to lead to the Crigler-Najjar syndrome type 1 and type 2 and the Gilbert's syndrome [1]. Bilirubin UDP-glucuronosyltransferase 1A1 (UGT1A1) is the hepatic enzyme that catalyzes the glucuronidation of bilirubin into a water-soluble conjugated form to facilitate its efficient excretion [2]. The variation in a (TA) tandem repeat sequence within the promoter region affects the UGT1A1 gene expression. The wild type UGT1A1 promoter region contains a (TA)nTAA sequence with 6 (TA) tandem repeats, although variant alleles with 5, 7, and 8 (TA) repeats have been identified. Persons with the homozygous 7/7 UGT1A1 genotype have the Gilbert syndrome with a phenotype of mild unconjugated hyperbilirubinaemia from the decreased UGT1A1 enzyme activity [2-4]. Children with sickle cell anaemia have a variability in the clinical disease expression, which include a wide range in the steady-state laboratory parameters. Almost every child with homozygous HbSS has an elevated serum bilirubin concentration [5]. Associations among the UGT1A1 promoter variation, high indirect bilirubin levels, and gallstone formation have also been reported in β-thalassaemia, haemoglobin E/β-thalassaemia, and hereditary spherocytosis [6-8]. Bilirubin monoglucuronide triggers bilirubin gallstone initiation, as has been demonstrated in patients with many forms of haemolytic anaemia [8-10]. Many children with HbSS and hyperbilirubinaemia develop gallstones with a cumulative incidence of 50%, by adulthood [11,12].The laboratory and clinical variability in hyperbilirubinaemia and gallstone formation may reflect environmental factors [13]. There is a paucity of data in terms of the UGT1A1 variation and the gall stone formation with hyperbilirubinaemia in Indian sicklers. Thus, our aim was to detect the UGT1A1 (TA)n repeats with hyperbilirubinaemia and gall stone formation in Indian sickle cell patients.

MATERIALS AND METHODS

The subjects were sickle cell patients who attended the out patients department; All India Institute of Medical Sciences (AIIMS), New Delhi, India. This study was done in the Department of Haematology and it was approved by the institutional ethical committee. The gallbladder examinations were done by ultrasound. About 5 ml blood sample was collected from the patients after taken their informed consent. The complete blood count and the red cell indices were measured by an automated cell analyzer (SYSMEX K-4500, Kobe Japan). The quantitative assessment of Hb F, Hb A, Hb A2 and Hb S and the diagnosis of the sickle homozygous and the sickle beta thalassaemia patients was done by high performance liquid chromatography (HPLC-Bio-Rad-Variant™Bio Rad, CA, USA). DNA extraction was performed by the phenol-chloroform method. The UGT1A1 (TA)n promoter length variability was genotyped according to the method of Eden et al., [14] (2005). The measurement of serum bilrubin was done by a Beckman- CX-9 auto analyzer by using the Randox diagnostic kit. The allele frequency was calculated according to the Hardy-Weinberg equilibrium. The ANOVA test were applied to compare the means between the three groups, while the T- test was applied to compare the means of two groups on the GraphPad (version 3.06) software. A p-value of <0.05 was considered as statistically significant.

RESULT

The study subjects were 50 sickle cell anaemia (32 males and 18 females with a mean age of 11.2±5.4 years) and seventy sickle cell β-thalassaemia patients (46 males and 24 females with a mean age of 11.8±5.5 years). The total bilirubin was 3.2±1.3 mg% in the sickle cell homozygous patients and it was 2.5±1.4 mg% in the sickle beta thalassaemia patients. The total bilirubun value was higher in the sickle cell anaemia patients than in the sickle beta thalassaemia patients and it was statistically significant (p-value 0.006), while the unconjugated bilrubin concentration was 2.1±0.45 mg% in the sickle homozygous patients and it was 1.9±0.38 mg% in the sickle beta thalassaemia patients (p-value 0.009). The TA repeats, 6/6, 6/7 and 7/7 of the UGT1A1 genotype promoter polymorphism were identified in our cases. The allele 7/7(TA) repeats were more frequent in sickle beta thalassaemia (54.28%) as well as in sickle homozygous (52%) patients, while the 6/6 TA repeats were 18% in the sickle homozygous patients and they were 15.71% in the sickle beta thalassaemia patients. The 6/7 TA repeats were 30% in the sickle homozygous as well as in the sickle beta thalassaemia patients. The allele frequencies of the 6/6, 6/7 and the 7/7 (TA)n repeats in the sickle homozygous patients was 0.104, 0.436 and 0.456 respectively, while they were 0.094, 0.424 and 0.478 respectively in the sickle β-thalassemia patients . The bilirubin levels were higher in the 7/7 (TA) genotype in the sickle cell anaemia patients as well as in the sickle beta thalasseamia patients and all the values were statistically significant (p-value <0.05). All the details of the bilirubin levels with the (TA)n genotypes in the sickle homozygous and in the sickle β-thalassaemia patients are given in [Table/Fig-1 and 2] respectively. The bilirubin levels in the sickle homozygous and in the sickle β-thalassaemia patients with and without the presence of gall stones had a great variability with significant differences (p-value <0.05). Details are given in [Table/Fig-3]. A higher frequency of the gall stones was present in male patients than in the female patients, while the patients of the 12-17 years age group presented a higher frequency of gall stone formation. Details of the age and sex wise presentation of the gall stones in the sickle homozygous and the sickle β-thalassaemia patients are given in [Table/Fig-4].

Bilirubin	Mean± SD			
	6/6 (TA) N=9	6/7 (TA) N=15	7/7 (TA) N=26	P-value
Total bilirubin mg%	2.97±0.34	3.17±0.59	3.21±0.45	0.042
Unconjugated bilirubin mg%	1.83±0.12	2.13±0.48	2.43±0.35	0.037
M/F ratio	6/3	9/6	15/11	–
Gall stone	0	1	7	–

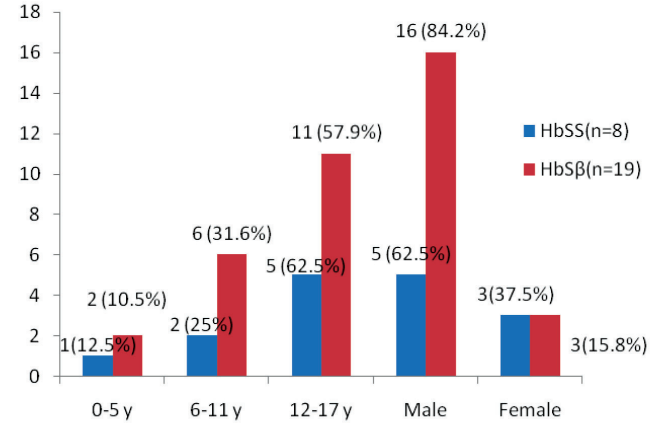
[Table/Fig-1]: Total bilirubin and unconjugated bilirubin with (TA)n repeats in HbSS patients

Bilirubin	Mean± SD			
	6/6 (TA) N=11	6/7 (TA) N=21	7/7 (TA) N=38	P-value
Total bilirubin mg%	2.91±0.27	3.15±0.44	3.22±0.54	0.036
Unconjugated bilirubin mg%	1.77±0.12	1.92±0.26	2.02±0.44	0.021
M/F ratio	8/3	12/9	23/15	-
Gall stone	0	5	14	-

[Table/Fig-2]: Total bilirubin and unconjugated bilirubin with (TA)n repeats in HbSβ thalassemia patients

DISCUSSION

The UGT1A1 promoter polymorphism exerts a powerful influence on the bilirubin levels and the development of gallbladder disease in sickle cell anaemia [15-19]. Few studies have stated that the UGT1A1 gene promoter polymorphism and the age-at-onset of cholelithiasis were strongly correlated [17,20]. A study also proved that the (TA)7/(TA)(7) genotype may be a risk factor for the symptomatic gallstones in older people with sickle cell disease [14]. A study with hydroxyurea in relation to the UGT1A1 gene promoter polymorphism in Indian patients with different haemoglobinopathies concluded that higher bilirubin levels were associated with the (TA)(7)/(TA)(7) [21]. The serum bilirubin levels are elevated in individuals who have a TA repeat polymorphism in the promoter region or a Gly71Arg polymorphism in the first exon of the gene [3,20-24]. Individuals with seven TA (7-TA) repeats in the UGT1A1 promoter have elevated serum bilirubin levels that cause a mild hyperbilirubinaemia (Gilbert's syndrome), but individuals with the more common six TA (6-TA) repeats do not have this condition [3,4]. In some cases, the (7)-TA polymorphism as well as other mutations have also been associated with severe (Crigler-Najjar type II disease) hyperbilirubinaemia [22,24-26]. In our cases, the (TA)7 alleles were more frequent in the sickle homozygous as well as in the sickle β-thalassemia patients. The (TA)7 alleles of the UGT1A1 genotype were significantly associated with hyperbilirubinaemia in both the groups of patients (p-value <0.05). Gall stones were more frequent with the 7/7 alleles with hyperbilirubinaemia in the sickle cell anaemia and the sickle β-thalassaemia patients. Over the past several years, two new UGT1A1 TA repeat promoter polymorphisms,



[Table/Fig-4]: Age and sex wise presentation of gall stone in HbSS and HbSβ-thalassemia patients.

Bilirubin	Mean±SD HbSS			Mean±SD HbSβ		
	Patients with gall stone N=8	Patients without gall stone N=42	P-value	Patients with gall stone N=19	Patients without gall stone N=56	P-value
Total Bilirubin mg%	3.2±0.54	2.7±0.63	0.015	2.83±0.31	2.3±0.42	<0.001
Unconjugated Bilirubin mg%	1.76±0.84	1.32±0.43	0.03	1.62±0.63	1.23±0.53	0.01

[Table/Fig-3]: Bilirubin status in HbSS and HbSβ patients with and without presence of gall stone

5-TA and 8-TA, have been identified primarily in individuals of African descent. A recent experimental work demonstrated that the four different promoters, (5-TA, 6-TA, 7-TA, 8-TA) had a transient transcriptional activity that was inversely related to the number of the TA repeats. In contrast, the concentrations of unconjugated serum bilirubin which were found in the circulation have been found to be increase directly with the number of the TA repeat elements [27-31]. Hence, the UGT1A1 repeat polymorphisms were predictive of the serum bilirubin concentration and subsequently, the UGT1A1 genotypes could be indicative of the susceptibility to or of the protection against the oxidative damage which was mediated through the modulation of the bilirubin levels. Gallstone formation was thought to develop from the increased bilirubin monoglucuronide in comparison to the usually more predominant diglucuronide form [9]. The primary bilirubin catabolizing hepatic enzyme, uridine diphosphate (UDP) glucuronosyltransferase 1A1 (UGT1A1) mediates the conjugation of bilirubin into a water-soluble form that is excreted in bile [32]. A simple dinucleotide repeat polymorphism, (TA)5-8, in the TATA box of the UGT1A1 gene is associated with a reduced expression of the hepatic enzyme and this results in the chronic unconjugated hyperbilirubinaemia that occurs in Gilbert's syndrome [3,4,22,33,34]. The coinheritance of the Gilbert's syndrome with disorders that increase the turnover of the red blood cells or their precursors, has been reported to elevate the bilirubin levels in β-thalassaemia [35,36]. The G6PD deficiency and the hydroxyurea therapy response in sickle cell (SS) disease [37-41]. The variant (TA)7 UGT1A1 allele was reported to influence the serum bilirubin levels in patients with chronic haemolytic anaemia, such as a β-thalassaemia trait or the G6PD deficiency and it was found to be associated with gallstone formation in hereditary spherocytosis or homozygous β-thalassaemia [6,8, 42]. Among our cases, adolescents and male patients were found to be more susceptible to gall stone formation. (TA)6 was less frequent while (TA)7 more frequent in the sickle homozygous as well as in the sickle β-thalassaemia patients. Total bilirubin and unconjugated bilirubin with the frequency of the gall stone were significantly higher in patients with the (TA)7/(TA)7 genotypes than in those with other genotypes. These observations of our study concluded that the 7/7 TA alleles of the UGT1A1 genotype promoter polymorphism influenced the bilirubin production and the development of gallbladder stones in Indian sickle cell patients. This study provided us the clinical phenotype and the genotype information and it helped us in producing good preliminary results to explore the clinical trends and the genetic associations of the UGT1A1 polymorphism in sickle cell patients. Further studies with a large sample size are required to elucidate the role of the UGT1A1 polymorphism in gallstone formation and in the symptom manifestations in sickle cell patients.

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REFERENCE

[1] Huang YY, Huang MJ, Yang SS, Teng H-C, Huang C-S. Variations in the UDPglucuronosyltransferase 1A1 gene for the development of unconjugated hyperbilirubinemia in the Taiwanese. *Pharmacogenomics*, 2008; 9: 1229–35.

[2] Kadakol A, Ghosh SS, Sappal BS, Sharma G, Chowdhury JR, Chowdhury N R. Genetic lesions of bilirubin uridinediphospho-glucuronate glucuronosyltransferase (UGT1A1) which cause the Crigler-Najjar and the Gilbert's syndromes: Correlation of the genotype to the phenotype. *Hum Mutat* 2000; 16:297–306.

[3] Bosma PJ, Chowdhury JR, Bakker C, Gantla S, de Boer A, Oostra BA, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyl-transferase 1 in Gilbert's syndrome. *N Engl J Med* 1995;333:1171–75.

[4] Monaghan G, Ryan M, Seddon R, Hume R, Burchell B. Genetic variation in the bilirubin UDPglucuronosyltransferase gene promoter and the Gilbert's syndrome. *Lancet* 1996; 347:578–81.

[5] West MS, Wethers D, Smith J, Steinberg M. Laboratory profile of sickle cell disease: A cross-sectional analysis. *J Clin Epidemiol* 1992; 45:893–909.

[6] Galanello R, Piras S, Barella S, et al. Cholelithiasis and Gilbert's syndrome in homozygous betathalassaemia. *Br J Haematol*. 2001;115:926-28.

[7] Premawardhena A, Fisher CA, Fathiu F, et al. Genetic determinants of jaundice and gallstones in haemoglobin E beta thalassaemia. Lancet. 2001;357:1945-46.

[8] Del Giudice EM, Perrotta S, Nobili B, Specchia C,d'Urzo G, Iolascon A. Coinheritance of the Gilbert's syndrome increases the risk for developing gallstones in patients with hereditary spherocytosis. *Blood*. 1999;94:2259-62.

[9] Duvaldestin P, Mahu JL, Metreau JM, Arondel J, Preaux AM, Berthelot P. Possible role of a defect in hepatic bilirubin glucuronidation in the initiation of cholesterol gallstones. *Gut* 1980; 21:650–55.

[10] Perotta S, del Giudice EM, Carbone R, Servedio V, Schettini F, Nobili B, et al. Gilbert's syndrome accounts for the phenotypic variability of congenital dyserythropoietic anemia type II (CDAII). *J Peds* 2000;136:556–59.

[11] Lachman BS, Lazerson J, Starshak RJ, Vaughters FM, Werlin SL. The prevalence of cholelithiasis in sickle cell disease as was diagnosed by ultrasound and cholecystography. *Paediatrics* 1979; 64:601–03.

[12] Sarnaik S, Slovis TL, Corbett DP, Emami A, Whitten CF. Incidence of cholelithiasis in sickle cell anemia which was assessed by using the ultrasonic gray-scale technique. *J Pediatr* 1980; 96:1005–08.

[13] Werlin SL, Scott JP. Is biliary sludge a stone-in-waiting? *J Pediatr* 1996; 129: 321–22.

[14] Haverfield E V, McKenzie CA., Forrester T, Bouzekri N, Harding R, et.al. The UGT1A1 variation and gallstone formation in sickle cell disease. *Blood*. 2005; 105:968-72.

[15] Carpenter SL, Lieff S, Howard TA, Eggleston B, Ware REE. The UGT1A1 promoter polymorphisms and the development of hyperbilirubinemia and gallbladder disease in children with sickle cell anemia. *Am J Hematol*. 2008; 83(10):800-3.

[16] Passon RG, Howard TA, Zimmerman SA, Schultz WH, Ware RE. Influence of the bilirubin uridine diphosphate-glucuronosyltransferase 1A promoter polymorphisms on the serum bilirubin levels and cholelithiasis in children with sickle cell anemia. *J Pediatr Hematol Oncol*. 2001; 23(7):448-51.

[17] Chaar V, Kéclard L, Diara JP, Leturdu C, Elion J, Krishnamoorthy R, et al. Association of the UGT1A1 polymorphism with the prevalence and the age at onset of cholelithiasis in sickle cell anemia. *Haematologica*. 2005; 90(2):188-99.

[18] Vasavda N, Menzel S, Kondaveeti S, Maytham E, Awogbade M, Bannister S, et al. The linear effects of alpha-thalassaemia and the UGT1A1 and the HMOX1 polymorphisms on cholelithiasis in sickle cell disease. *Br J Haematol*. 2007; 138(2):263-70.

[19] Carpenter SL, Lieff S, Howard TA, Eggleston B, Ware RE. The UGT1A1 promoter polymorphisms and the development of hyperbilirubinemia and gallbladder disease in children with sickle cell anemia. *Am. J. Hematol*. 2008; 83:800–03.

[20] Martins R, Morais A, Dias A, Soares I, Rolão C, Ducla-Soares JL, et al. Early modification of the clinical course of the sickle cell disease by the UDP-glucuronosyltransferase 1A1 gene promoter polymorphism. *J Hum Genet* 2008; 53(6): 524-28.

[21] Italia KY, Jijina FF, Jain D, Merchant R, Nadkarni AH, Mukherjee M, et al. The effect of the UGT1A1 promoter polymorphism on the bilirubin response to the hydroxyurea therapy in hemoglobinopathies. *Clin Biochem*. 2010; 43(16-17):1329-32.

[22] Aono S, Adachi Y, Uyama E, Yamada Y, Keino H, Nanno T, et al. Analysis of the genes for bilirubin UDP-glucuronosyltransferase in Gilbert's syndrome. *Lancet* 1995;345:958-59.

[23] Koiwai O, Nishizawa M, Hasada K, Aono S, Adachi Y, Mamiya N, et al. Gilbert's syndrome is caused by a heterozygous missense mutation in the gene for bilirubin UDP-glucuronosyltransferase. *Hum. Mol. Genet*. 1995; 1183(4):1186-12.

[24] Akaba K, Kimura T, Sasaki A, Tanabe S, Ikegami T, Hashimoto M, et al. Neonatal hyperbilirubinemia and mutation of the bilirubin urdine diphosphateglucuronosyltranserase gene: a common missense mutation among the Japanese, Koreans and the Chinese. *Biochem. Mol. Biol. Int*. 1998; 46: 21-26.
[25] Koiwai O, Aono S, Adachi Y, Kamisako T, Yasui Y, Nishizama M, et al. The Crigler-Najjar syndrome type II is inherited both as a dominant and as a recessive trait. *Hum. Mol. Genet*. 1996; 5: 645-47.
[26] Ciotti M, Chen F, Rubaltelli FF, Owens IS. A coding defect and a TATA box mutation at the bilirubin UDP-glucuronosyltransferase gene cause the Crigler-Najjar type I disease. *Biochem. Biophys. Acta*. 1998; 1407: 40-50.
[27] Grant DJ, Bell DA. Genotyping of the UGT1A1 gene in four ethnic populations, In: P. H. Byers, (ed.), *The American Society of Human Genetics*. University of Chicago Press. 1998; 63: A212.
[28] Beutler E, Gelbart T, Demina A. Racial variability in the UDP glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for the regulation of the bilirubin metabolism? *Proc. Natl. Acad. Sci. USA*. 1998; 95: 8170-74.
[29] Guillemette C, Millikan RC, Newman B, Housman D. Genetic polymorphisms in Uridine Diphospho-Glucuronosyltransferase 1A1 and their association with breast cancer among African Americans. *Cancer Res*. 2000;60: 950-56.
[30] Lampe JW, Bigler J, Horner NK, Potter JD. UDP-glucuronosyltransferase (UGT1A1*28 and UGT1A6*2) polymorphisms in Caucasians and Asians: Relationships to the serum bilirubin concentrations. *Pharmacogenetics* 1999; 9:341-49.
[31] Ishihara T, Kaito M, Takeuchi K, Gabazza EC, Tanaka Y, Higuchi K, et al. Role of the UGT1A1 mutation in fasting hyperbilirubinemia. Gastroenterol. *Hepatol*. 2001; 16:678-82.
[32] Crawford J, Ransil B, Narciso J, Gollan J. Hepatic microsomal bilirubin UDP-glucuronosyltransferase. *J Biol Chem*. 1992; 267:16943-950.
[33] Burchell B, Hume R. Molecular genetic basis of Gilbert's syndrome. *J Gastroenterol Hepatol*. 1999; 14:960-66.
[34] Miners JO, McKinnon RA, Mackenzie PI. Genetic polymorphisms of the UDP-lucuronosyltransferases and their functional significance. *Toxicology*. 2002; 181-182:453-56.
[35] Galanello R, Perseu L, Melis MA, Cipollina L, Barella S, Giagu N, et al. Hyperbilirubinaemia in heterozygous beta-thalassemia is related to the co-inherited Gilbert's syndrome. *Br J Haematol*. 1997; 99:433-36.
[36] Galanello R, Cipollina M, Dessi C, Giagu N, Lai E, Cao A. Co-inherited Gilbert's syndrome: a factor determining hyperbilirubinemia in homozygous beta-thalassemia. *Haematologica*. 1999; 84:103- 5.
[37] Bancroft JD, Kreamer B, Gourley GR. Gilbert's syndrome accelerates the development of neonatal jaundice. *J Paediatr*. 1998; 132:656-60.
[38] Galanello R, Cipollina M, Carboni G, et al. Hyperbilirubinemia, the glucose-6-phosphate dehydrogenase deficiency and Gilbert's syndrome. *Eur J Paediatr*. 1999;158:914-16.
[39] Iolascon A, Faienza MF, Perrotta S, Meloni GF, Ruggie G, del Giudice EM. Gilbert's syndrome and jaundice in glucose-6-phosphate dehydrogenase deficient neonates. *Haematologica*. 1999; 84:99-102.
[40] Kaplan M, Renbaum P, Levy-Lahad E, Hammerman C, Lahad A, Beutler E. Gilbert's syndrome and the glucose-6-phosphate dehydrogenase activity: a dose-dependent genetic interaction which is crucial for neonatal hyperbilirubinemia. *Proc Natl Acad Sci U S A*. 1997; 94:12128-32.
[41] Heeney M, Howard T, Zimmerman S, Ware R. The UGT1A promoter polymorphisms influence the bilirubin response to the hydroxyurea therapy in sickle cell anemia. *J Lab Clin Med*. 2003; 141:279-82.
[42] Sampietro M, Lupica L, Perrero L, et al. The expression of the uridine diphosphate glucuronosyl-transferase gene is a major determinant of the bilirubin level in heterozygous b-thalassaemia and in glucose-6-phosphate dehydrogenase deficiency. *Br J Haematol* 1997;99:437–39.

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