Liang Lijun, Akira Matsunaga, Kazuki Kijima, Emi Shirahata, Hiroko Izumino, Kiyoshi Hayasaka

Department of Pediatrics, Yamagata University School of Medicine, Yamagata, Japan
Department of Pediatrics, Affiliated Hospital of Ningxia Medical College, Yinchuan 750004, China
( Accepted September 26, 2003 )

ABSTRACT

We present two patients with idiopathic nephrotic syndrome who showed temporary indirect hyperbilirubinemia during the course of treatment with immunosuppressant drugs and/or steroids. We analyzed the gene of the bilirubin uridine-diphosphate glucuronosyltransferase (B-UGT) and found that patient 1 was a compound heterozygote for the G71R and T-3263G mutations and patient 2 was a heterozygote for the G71R mutation. Hyperbilirubinemia should be considered as one of the adverse effects of immunosuppressant drugs and/or steroids in patients carrying the polymorphic mutations of the B-UGT gene.

Key words: nephrotic syndrome, bilirubin uridine-diphosphate glucuronosyltransferase, hyperbilirubinemia

INTRODUCTION

Idiopathic nephrotic syndrome is a common renal disease in children. The overall survival of patients with idiopathic nephrotic syndrome has markedly improved since steroids have been introduced into its treatment. At present, steroids are recommended as the first choice for the treatment of idiopathic nephrotic syndrome in childhood. In spite of its effectiveness, long-term therapy sometimes causes adverse effects such as obesity, diabetes, peptic ulcers, osteoporosis, cataracts and glaucoma. In 1999, we reported that four patients with leukemia showed intermittent hyperbilirubinemia during combined therapy consisting of steroids and other anti-leukemic agents\(^1\). They had the common mutation of the bilirubin uridine-diphosphate glucuronosyltra-
nsferase (B-UGT) gene associated with Gilbert syndrome. Gilbert syndrome is common occurring in 2-12% of the population. It is clinically characterized by mild unconjugated hyperbilirubinaemia in the absence of structural liver disease or haemolysis. The G71R and (TA)7 mutations of the B-UGT gene are common among East Asians and Caucasians, respectively.

In this report, we present two unrelated patients with idiopathic nephrotic syndrome showing hyperbilirubinaemia during steroid therapy due to these common mutations of the B-UGT gene.

METHODS & MATERIALS

CASE REPORTS

Patient 1. A 12-year-old girl developed nephrotic syndrome and was admitted to our hospital. She was treated with prednisolone (60mg/day) and her urinary protein was negative by the second week of administration. She did not show any liver disease, and laboratory findings were normal, including ALT, AST, LDH, alkali phosphatase, GTP, LAP and cholinesterase. She did not have any hemolytic episodes and was serologically negative for hepatitis A, B and C. After 3 weeks of prednisolone therapy, she developed hyperbilirubinaemia (total bilirubin 1.4-2.2mg/dl, direct bilirubin 0.2-0.3 mg/dl), which gradually decreased to normal range over the following 2 weeks. Her parents and younger brother were healthy and had no history of jaundice.

Patient 2. A 22-year-old male was admitted to our hospital for the treatment of relapsed nephrotic syndrome. When the patient was 11 years of age, he developed nephrotic syndrome and was under prednisolone therapy. Two months later, he relapsed for the first time and underwent a percutaneous renal biopsy. The biopsied specimens showed nephropathy with minimal change. Over the following 4 years, he frequently relapsed and received combined therapies consisting of steroid and immunosuppressants (mizoribine, cyclophosphamide, cyclosporin and chlorambucil). When he was under prednisolone therapy (40 mg/day) at our hospital, he showed hyperbilirubinaemia (serum total bilirubin 1.7mg/dl), which reduced to normal range the following week. He did not have any liver disease and exhibited normal levels of serum ALT (20 IU/L) AST (14 IU/L), LDH (284 IU/L), alkali phosphatase (137 IU/L) and GTP (8 IU/L). He did not have any hemolytic episodes and was serologically negative for hepatitis A, B and C. There was no history of jaundice in his family.

Analysis of the UDPGT1 gene

Blood was collected after informed consent was obtained, and genomic DNA was extracted from the two patients by the standard procedure. Promoter and coding regions including exon-intron boundaries of the B-UGT gene were amplified as four fragments by PCR and their sequences were determined according to methods reported in previous papers.

RESULTS

Direct sequencing revealed that patients 1 and 2 were heterozygous for the Gly71Arg mutation (Fig.1A: data of patient 1 not shown). Patient 1 was also heterozygous for the T-3263G mutation (Fig.1B). They did not have any other mutations, including the (TA)7 mutation.
DISCUSSION

We reported two patients who presented temporary indirect hyperbilirubinemia by immunosuppresant drugs and/or prednisolone therapy for idiopathic nephrotic syndrome. They did not exhibit elevation of transaminases or hemolysis. Indirect hyperbilirubinemia has not been reported as a complication of nephrotic syndrome. Patients with nephrotic syndrome sometimes require intravenous albumin or plasma administration and have a risk for hepatitis viral infection. However, our patients did not receive those administrations and were negative for hepatitis A, B and C. As other possibilities, hepatic damage may be caused by chemical agents such as steroids or immunosuppresant drugs. Fatty liver and the elevation of transaminases are well known as adverse effects of steroids. Immunosuppresant drugs also cause liver dysfunction and hyperbilirubinemia. Our patients did not show liver dysfunction, however, these agents were probably associated with the hyperbilirubinemia in our patients. Not all patients who receive these drugs always present hyperbilirubinemia, and some additional factors, including genetic factors, are likely required. Polymorphisms of the genes encoding the enzymes involved in the drug metabolism are well known. In addition, people carrying polymorphic mutation of the B-UGT gene present intermittent hyperbilirubinemia under combined therapy for leukemia\(^5\). We studied the B-UGT gene and found that patient 2 was a heterozygote for the G71R mutation and patient 1 was a compound heterozygote for the

---

Fig. 1.
Sequence chromatography of the antisense chain of the patient 2 revealed a heterozygous mutation C-to-T at nucleotide 211 (arrow), resulting in a G71R substitution (A). Sequence chromatography of the sense chain of the patient 1 showed a heterozygous T-to-G mutation at nucleotide-3263 (arrow) (B).
G71R and T-3263G mutations.

The G71R mutation of the B-UGT gene is common among Japanese, Koreans and Chinese. Expression experiments showed that the G71R mutation had about 30% of the activity of wild type in homozygous state. Recently, Sugatani, et al. reported that the T-3263G mutation was associated with the majority of Gilbert's syndrome. The T-3263G mutation is located in the phenobarbital response enhancer module in the B-UGT gene and significantly decreases the transcriptional activity. Plasma total bilirubin levels in compound heterozygotes for the T-3263G and (TA)7 or G71R mutations are significantly higher than in those with one or other of these mutations singly. It is interesting to note that the plasma bilirubin level of the patient 1 was higher than that of patient 2.

Our patients did not have any episodes of hyperbilirubinemia. It is likely that the patients carrying these mutations can produce B-UGT enough to conjugate the bilirubin at steady state; however, they may not be able to metabolize the bilirubin under the stress of drug administrations. The drugs may inhibit B-UGT activity or may impair the synthesis of B-UGT or may increase in the loading of unconjugated bilirubin.

Hyperbilirubinemia should be noted as one of the adverse effects of immunosuppressant drugs and/or steroids in the patients carrying the polymorphic mutations of the B-UGT gene in the treatment of nephrotic syndrome.

REFERENCES


