

CASE REPORT

## 유전성 구상적혈구증과 길버트 증후군 1예

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### A Case of Hereditary Spherocytosis Coexisting with Gilbert's Syndrome

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We recently encountered a case of hereditary spherocytosis coexisting with Gilbert's syndrome. Patient was initially diagnosed with Gilbert's syndrome and observed, but other findings suggestive of concurrent hemolysis, such as splenomegaly and gallstones were noted during the follow-up period. Therefore, further evaluations, including a peripheral blood smear, osmotic fragility test, autohemolysis test, and red blood cell membrane protein test were performed, and coexisting hereditary spherocytosis was diagnosed. Genotyping of the conjugation enzyme uridine diphosphate-glucuronosyltransferase was used to confirm Gilbert's syndrome. Because of the high prevalence rates and similar symptoms of these 2 diseases, hereditary spherocytosis can be masked in patients with Gilbert's syndrome. In review of a case and other article, the possibility of the coexistence of these 2 diseases should be considered, especially in patients with unconjugated hyperbilirubinemia who also have splenomegaly and gallstones. (*Korean J Gastroenterol* 2013;61:166-169)

**Key Words:** Hereditary spherocytosis; Gilbert disease; Anemia; Jaundice; Splenomegaly

#### INTRODUCTION

Hereditary spherocytosis (HS), the most common form of inherited hemolytic anemia, is caused by a defect in one of the proteins that couples the red cell membrane skeleton to the plasma membrane. Its prevalence in Europe and North America is 1 per 2,000 persons.<sup>1</sup> The primary lesion in HS is the loss of membrane surface area, leading to reduced membrane deformity, due to defects in the membrane proteins ankyrin, band 3,  $\alpha$ -spectrin,  $\beta$ -spectrin, or protein 4.2.<sup>1</sup> The clinical symptoms are variable but most often include anemia, jaundice, and splenomegaly. Most patients have well-compensated anemia but exhibit icterus and cholelithiasis due to

chronic hemolysis.<sup>2</sup>

Gilbert's syndrome (GS) is a common cause of unconjugated hyperbilirubinemia due to diminished activity of the conjugating enzyme uridine diphosphate-glucuronyl transferase (*UGT1A1*). The prevalence of GS is approximately 3-10% in the West and 3% in the East.<sup>3</sup>

HS and GS can coexist,<sup>4,6</sup> but only one such case has been reported in Korea.<sup>7</sup> Herein, a case of HS combined with GS that we recently encountered is presented.

#### CASE REPORT

The patient was a 41-year-old man who exhibited icteric

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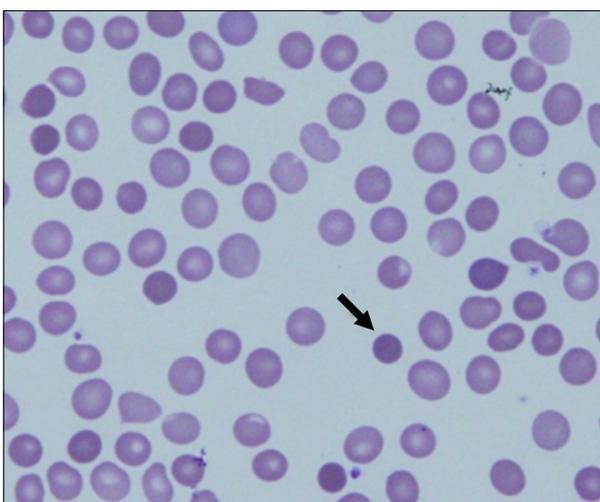
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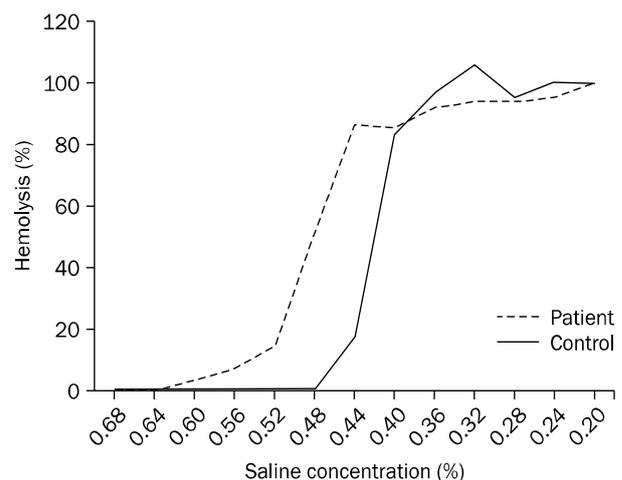
sclera and hyperbilirubinemia. His jaundice has been detected when he was 22 years old but had been observed without treatment. He had recently noticed a feeling of increased fatigue and therefore visited a gastroenterologist of Korea Cancer Center Hospital via local clinic with laboratory test which shows high total bilirubin level (10.4 mg/dL). He has 3 brothers and 2 sisters. One of his brothers had undergone cholecystectomy due to cholecystitis with multiple pigmented gallbladder (GB) stones. The patient's height and weight were 178 cm and 58 kg, respectively. His body temperature was 36.7°C and blood pressure was 120/80 mmHg. Heart rate and respiration rate were in normal ranges. Physical examination revealed icteric sclera, but there were no abdominal tenderness and organomegaly. Bowel sound was normoactive. There were no specific finding in physical examination.

Routine laboratory tests were performed and the following results were obtained: white blood cell count, 7,580/ $\mu$ L; hemoglobin, 13.0 g/dL; platelet count, 250,000/ $\mu$ L; hematocrit, 37.2%; mean corpuscular volume, 86.9 fL; mean corpuscular volume concentration, 34.8 g/dL; AST, 14 IU/L; ALT, 11 IU/L; total bilirubin, 4.0 mg/dL; direct bilirubin, 0.5 mg/dL; and ALP, 62 IU/L. Abdominal ultrasonography showed several 6 mm GB stones and wall thickening of the GB, thereby suggesting adenomyomatosis. Splenomegaly with the largest dimension of 13 cm was also noted. The patient was initially diagnosed as GS because of the elevated

indirect bilirubin level with hemoglobin levels within the normal range. However, after the detection of the GB stones and splenomegaly by abdominal ultrasonography, the patient was supposed to have concurrent hemolysis. To evaluate the possibility of hemolysis, the following laboratory examinations were performed. The haptoglobin level was found to be below 10 mg/dL (normal range, 30-200 mg/dL), and direct and indirect Coombs' tests were negative. The peripheral blood smear (PBS) showed normocytic normochromic red blood cells (RBCs) with poikilocytosis, including spherocytosis and schistocytosis (Fig. 1). The HS was suspected due to above findings. The osmotic fragility test and autohemolysis test were performed to diagnose HS (Fig. 2). The patient's osmotic fragility test showed increased fragility (beginning, 0.56%; ending, 0.36% NaCl), and the autohemolysis test was positive (saline, 7.0%; glucose, 1.6%). The findings in the RBC membrane protein analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were normal. Thus, the diagnosis of HS was confirmed and concurrent of GS was still suspected. Therefore, to confirm the diagnosis of GS, *UGT1A1* genotyping was performed and it found *UGT1A1* gene polymorphism (c.3279T > G, c.53[TA]<sub>7</sub> and c.211G > A[G71R]). While splenectomy was not indicated in this patient, cholecystectomy for treatment of the adenomyomatosis of the GB was recommended, but the patient



**Fig. 1.** Peripheral blood smear (Wright-Giemsa stain,  $\times 1,000$ ). Normochromic normocytic red blood cells and characteristic spherocytes (arrow) lacking central pallor, which was specific to hereditary spherocytosis. Some schistocytes were present.



**Fig. 2.** Osmotic fragility test. Increased osmotic fragility of red blood cells (RBCs) was an important diagnostic feature of hereditary spherocytosis. The patient's RBCs began to lyse at 0.56% NaCl and were completely lysed at 0.36% NaCl. Because the RBCs of patients with hereditary spherocytosis have a limited capacity to expand, they lyse at higher concentrations of NaCl than normal control RBCs (beginning, 0.44% and end, 0.32%).

refused.

## DISCUSSION

GS is the most common form of familial unconjugated hyperbilirubinemia. Plasma bilirubin concentrations are most often less than 3 mg/dL, with 2-3-fold increases commonly occurring during fasting or intercurrent illness.<sup>8</sup> The clinical presentation is nonspecific, but some patients experience weakness or upper abdominal discomfort. GS does not lead to hepatic damage and therefore usually requires no treatment. Decreased conjugation of bilirubin due to diminished activity of the conjugation enzyme *UGT1A1* is associated with increased production of monoconjugated bilirubin. The disorder is usually due to polymorphism in a TA-repeat element, A(TA)<sub>5-8</sub>TAA, of the *UGT1A1* promoter.<sup>9</sup> Maruo et al.<sup>9</sup> reported linkage of GS to A(TA)<sub>7</sub>TAA (*UGT1A1* 28) and T-3279G (*UGT1A1* 60). An *in-vitro* expression study revealed that the transcriptional activity of the A (TA)<sub>7</sub>TAA allele is one-third of the normal value and that T-3279G reduces the transcriptional activity to 60% of the normal value. The prevalence of GS is usually estimated as approximately 3-10% in the West and 3% in the East.<sup>3</sup> The prevalence of GS in Korea specifically has not been studied but is predicted to be similar to that of other East Asian populations.<sup>3</sup>

HS is the most common congenital hemolytic anemia in Caucasians, with an estimated prevalence of 1 in 2,000.<sup>1</sup> The main clinical features of HS are hemolytic anemia, which can range from compensated to severe, variable jaundice, splenomegaly, gallstones, and may sometimes require exchange transfusion at birth and/or repeated blood transfusions. The molecular defect is highly heterogeneous and may involve the genes encoding spectrin, ankyrin, band 3, or protein 4.2.<sup>1</sup> Deficiency or dysfunction of any of these proteins, all of which are involved in the attachment of the cytoskeleton to the membrane integral domain, results in a loss of surface area and leads to spheroidal, osmotically fragile RBCs that are selectively trapped in the spleen. The increased osmotic fragility is an important diagnostic feature of HS. The more spherical HS-affected RBCs with lower surface area-to-volume ratios have a limited capacity to expand in hypotonic solutions and lyse at a higher concentration of NaCl than do normal biconcave RBCs.<sup>10</sup> The autohemolysis test is also helpful to diagnose HS. The RBCs lose membrane

and become more spherocytic after incubating sterile defibrinated blood at 37°C for 48 hours. In normal blood, the amount of autohemolysis at 48 hours is 0.2-2.0% when incubated without added glucose and less than 0.9% when incubated with added glucose.<sup>10</sup> Autohemolysis is virtually always increased in blood from patients with HS, while the addition of glucose decreases the lysis to a variable extent. Rarely, patients with strong clinical and laboratory evidence of HS will exhibit normal incubated osmotic fragility.<sup>10</sup>

There is no specific biological correlation between HS and GS, but the high prevalence rates of both diseases suggest that their coexistence should occur more frequently than it is actually diagnosed. The prevalence of the coexistence of HS and GS has not studied in Korea, but from the individual prevalence rates (GS, 3-10%; HS, 1 per 2,000 persons), it can be predicted almost 15-50 per million births. The diagnosis of HS with coexisting GS is complicated, and the HS is usually masked or misdiagnosed. The simultaneous presence of GS has been described as a possible cause of HS misdiagnosis.<sup>6</sup> The co-inheritance of GS in patients with HS is associated with a higher bilirubin level and a tendency to form gallstones almost 5-fold greater than that of normal HS patients.<sup>11</sup> Therefore, the possibility of the coexistence of GS and HS should be considered in GS patients who have Gallstones, because of high prevalence.

The patient in this report were initially suspected to have GS alone and were observed for some time. During follow-up, evidences of hemolysis was detected, which led to their being diagnosed with coexisting GS and HS. But the patient had splenomegaly during their initial work-ups for hyperbilirubinemia. In addition, he had a bilirubin level higher than usual for GS or HS alone, even it was checked local clinic and decreased spontaneously, and had GB stones. In other words, we should suspect that HS was present due to unusual hyperbilirubinemia, splenomegaly and GB stones. Familial history is important for finding clues to this diagnosis.

SDS-PAGE analysis was performed for diagnosis, and the result was normal. However, this is not unusual, because 20-30% of HS patients show no membrane protein abnormalities.<sup>12</sup> Therefore, diagnosing of the HS was on the basis of compatible PBS findings and the results of the osmotic fragility and autohemolysis tests. And GS was diagnosed by *UGT1A1* genotyping.

No treatment for HS coexisting with GS has been

established. According to the treatments for the individual disorders, splenectomy could be considered if indicated by the clinical manifestations and complications such as anemia and gallstones.<sup>2</sup> Cholecystectomy for the treatment of asymptomatic gallstones in HS patients is a debatable matter; therefore, close ultrasonographic follow-up is recommended.<sup>2</sup> However, coexisting GS increases the risk of developing gallstones 5-fold.<sup>11</sup> The patient who had gallstones was considered a candidate for cholecystectomy. However, there have been no specific recommendations or guidelines for this situation, and further evaluation is therefore needed.

In summary, we encountered a patient with coexisting GS and HS, initially it was considered only GS, but their splenomegaly and gallstones suggested HS. After then HS was diagnosed by a PBS, osmotic fragility test, and autohemolysis test, and the GS was confirmed by *UGT1A1* genotyping. Because of the high prevalence rates and similar symptoms of GS and HS, HS can be masked in GS patients. Therefore, the possibility of the coexistence of GS and HS should be considered, especially in patients with splenomegaly and gallstones. No treatment for patients with coexisting GS and HS has yet been established.

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