Two Easy-to-Perform Diagnostic Tests for Gilbert’s Syndrome

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caloric restriction; 
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rifampicin

A straightforward diagnosis of Gilbert’s syndrome could be made by measuring the much-lowered activity of hepatic bilirubin-UDP-glucuronosyltransferase. How ever, the risk of liver biopsy procedure as well as the complex and high-cost laboratory facilities makes the obvious diagnostic approach prohibitive and calls for a direct assay of the hepatic enzyme activity. It has been reported elsewhere¹-⁷ that rifampicin test and caloric restriction test can be used to diagnose Gilbert’s syndrome with high accuracy. This article reported a 17-year-old male with only partial indication of indirect hyperbilirubinemia. Rifampicin test and caloric restriction test were applied to assure the patient had Gilbert’s syndrome. These two non-invasive diagnostic means, with the benefit of avoiding hazardous liver biopsy, are gaining popularity in our routine Gilbert’s syndrome examination.

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Case Report

A 17-year-old male student first visited a local clinic, complaining about his yellowish sclera and yellow discoloration of skin. He thought he had jaundice and anemia, but without definite diagnosis. About one year later in September 1999, he came to our hospital with the same complaints. Physical examination showed a well-nourished body with normal growth and development. Liver span was normal, but spleen was palpable. There were no signs of ecchymosis or bruising anywhere in the whole body. Hemogram showed a hemoglobin level at 11.2 g/dL (normal range: 14-18 g/dL) with MCV at 63.3 fl (normal range: 80-94 fl). The serum biochemistry is try re su lts

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revealed a LDH at 185 U/L (normal range: 95-213 U/L), a total bilirubin at 5.3 mg/dL (normal range: 0.2-1.6 mg/dL) with a direct bilirubin at 0.6 mg/dL (normal range: 0-0.3 mg/dL). Other liver function tests in cluding aminotransferases and alkaline phosphatase were normal.

After admission, abdominal sonogram disclosed a splenomegaly (13.6 cm). Hematologic survey gave negative findings in haptoglobin, urine hemosiderin, ferritin, Ham test, G6PD assay and Coomb test. Reticulocyte percentage was 2.94% (normal range: <4%). Hemoglobin electrophoresis showed a profile of the beta-thalassemia trait, which could explain the splenomegaly and low MCV. But the diagnosis itself could not tell what behind the indirect hyperbilirubinemia. Negative findings of LDH, haptoglobin, urine hemosiderin and Coomb test made the hemolytic anemia unlikely. Gilbert’s syndrome, which causes direct hyperbilirubinemia, was then suspected. First, a rifampicin test according to Velilla was carried out. Bilirubin level was first checked when the subject was fasting and rechecked three times, one, two, four hours later respectively, following intake of a dose of 900 mg rifampicin. The total bilirubin level elevated markedly whereas the direct bilirubin level gained slightly. A caloric restriction test based on Owens method was then performed. Daily caloric intake was restricted to less than 400 kcal, and both the total and the direct bilirubin levels were checked in two days. All the data are showed in Table 1. Liver biopsy was performed and revealed a rather normal picture (Fig. 1). Finally, his UGT1A1 gene screening revealed heterozygous 211 G to A, the same as his younger brother. So Gilbert’s syn drome was diagnosed. The patient was thus treated with phenobarbital at 30 mg po qid at the clinic with rather good response as far as his condition was concerned. His total serum bilirubin level dropped down to 2 mg/dL in one month of the phenobarbital treatment.

**Discussion**

Jaundice can be evident when the serum bilirubin level exceeds the range of 2.0 to 2.5 mg/dL. In healthy people, serum bilirubin level represents a balance between the production and hepatic clearance of the pigment. The majority of patients with unconjugated hyperbilirubinemia are anicteric and asymptomatic. The incidence of unconjugated hyperbilirubinemia was reported to be as high as 5% in Westerners, although only a very modest proportion of these sufferers would show up at clinics looking for help.\(^1\)

Crigler-Najjar syndrome and Gilbert’s syndrome are both familial unconjugated hyperbilirubinemias caused by genetic lesions and mutations in volving a single complex locus encoding for bilirubin-UDP-glucuronosyltransferase which is involved in the detoxification of bilirubin by conjugation with...
Di ag nos tic Tests for Gilbert’s Syn drome

Diagnosis of Gilbert’s syndrome is often made by a way of exclusion. The syndrome is suspected in a patient displaying some mild or moderate unconjugated hyperbilirubinemia. The patient may have no systemic symptoms, no overt or clinically recognizable hemolysis, nor mal read ings from routine liver function tests, and a regularly ap pear ance through light microscopy in case a liver biopsy (though it is not necessary) is performed. Other studies establish the diagnosis of Gilbert’s syndrome by a liver biopsy (though it is not necessary) is performed. Other studies establish the diagnosis of Gilbert’s syndrome through separation of the unconjugated serum bilirubin by alkaline methanolysis, thin-layer chromatography or an immuno-histochemical staining method. But these methods are not easy to perform in an average hospital laboratory. Fortunately, Gilbert’s syn drome can be diagnosed by other more specific means in cluding a rifampicin test and a calor ic restriction test, instead of the more invasive liver biopsy and aforementioned immunohistochemical tests.

The antibacterial drug rifampicin fulfills the requirements of an ideal probe for hepatic or ganic anion transport and metabolism. Besides, it is readily available and quite safe. Bilirubin levels are determined in the middle of a fast, be fore and then one, two and four hours after the subject has taken 900 mg of rifampicin. A patient who has Gilbert’s syndrome will present higher to tal and in direct bilirubin levels than a healthy per son, who would remain within the normal level range. The rifampicin test is very effective in the diagnosis of sus pected Gilbert’s syndrome and is possible for use in primary care.

One of the most popular noninvasive tests used in the diagnosis of Gilbert’s syndrome is the calor ic restriction test. Daily energy intake in take of the patient is restricted to under 400 kcal, and the total and direct bilirubin levels are checked before and in two days after such a calor ic restriction. This pro vocative test produces a significant increase in serum unconjugated bilirubin in the majority of Gilbert’s syndrome patients as compared with normal subjects. In the normal subjects the unconjugated bilirubin level does not exceed 1 mg/dL. The diagnostic role of the reduced caloric intake test for Gilbert’s syndrome becomes increasingly important. During a fast the unconjugated serum bilirubin concentration can increase significantly higher in the patient with Gilbert’s syndrome than in a normal subject, but not so high as observed in a patient with acute hepatic failure. However, a differential diagnosis between these two diseases can be easily made by a serum biochemical test and the liver biopsy. Our patient had normal serum bilirubin and liver function tests. His positive calor ic restriction test should be related to Gilbert’s syndrome.

Phenobarbital significantly reduces the level of unconjugated serum bilirubin in patients with Gilbert’s syndrome. In Crigler-Najjar type 2, the serum bilirubin ranges from 6 to 25 mg/dL and can be lowered by phenobarbital treatment just as Gilbert’s syndrome. In the case of Gilbert’s syndrome, the serum bilirubin level does not exceed 9 mg/dL and is usually in the range 2-6 mg/dL. Since our patient had a rather high serum bilirubin level (7.5 mg/dL), it became difficult to differentiate whether the patient was with Gilbert’s syndrome and beta-thalassemia or with Crigler-Najjar type 2. Crigler-Najjar type 2 usually presents in neonates, and who maybe susceptible to kernicterus under stress. The positive result in both

In patients with Gilbert’s syndrome, the hepatic activity of bilirubin-UDP-glucuronosyltransferase is cut down to about 30% of the normal. Nearly 80% of the patients with in creased bilirubin level were either heterozygous or homozygous for the UGT1A1 TA sub variant as so ciated with Gilbert’s syndrome. Among patients with thalassemia, significantly higher bilirubin levels are found in (TA)7/(TA)7 geno type than in (TA)7/(TA)6 or (TA)6/(TA)6 geno type. Long TATAA motif re sults in reduced expression of liver bilirubin-UDP-glucuronosyltransferase. The (TA)7/(TA)7 geno type is the configuration found in patients with Gilbert’s syndrome, and is an example of the role that co-inherited modifiers play in the extent of clinical heterogeneity. The marked hyperbilirubinemia may well be a reflection of the co-inheritance of Gilbert’s syndrome and beta-thalassemia trait. The marked hyperbilirubinemia may well be a reflection of the co-inheritance of Gilbert’s syndrome and beta-thalassemia trait.

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rifampicin test and caloric restriction test on this young adult who be gan to have jaun dice at a rather late age of 17 strongly sug gest Gilbert’s syn drome as it ex cludes the pos si bil ity of Crigler-Najjar syn drome.

In con clu sion, Gilbert’s syn drome should be sus -pected if the pa tient has a mild hyperbilirubinemia with a high frac tion of unconjugated bil i ru bin, nor mal liver func tion, and no overt signs of hemolysis. Liver biopsy is not mandatory in this assess ment. The rifampicin test and ca lo ric re stric tion test play an im -por tant role in di ag no sis of Gilbert’s syn drome, as illus -trated in this case study.

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