Role of overnight rifampin test in diagnosing Gilbert’s syndrome

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Background: Gilbert’s syndrome (GS) is the most common inherited disorder of hepatic bilirubin metabolism, occurring in about 3-7 percent of the population. It is a mild and benign disorder that presents as mild icterus in the second and third decades of life. It is characterized by unconjugated hyperbilirubinemia in the absence of hemolysis and hepatocellular disease. Decreased hepatic bilirubin uridine diphosphate glucuronosyltransferase (UGT) is the main pathogenic factor, though transport abnormality in hepatocytes and occult hemolysis may also contribute. Patients with GS have an additional TA dinucleotide in the promoter region of UGT, leading to reduced amount of this enzyme. Thus, these patients are homozygous for a longer version of the TATAA sequence, A[TA]7TAA; its inheritance is autosomal dominant with incomplete penetrance.

Confirmatory provocative tests include the fasting test and nicotinic acid administration test. Genetic testing is currently available only in research settings. Recent data show that the rifampin test can be used as a diagnostic test. It induces cytochrome P-450 isoenzyme to increase bilirubin level in patients, but there is controversy about its effect on bilirubin level in normal individuals. We studied the effect of administration of rifampin on serum bilirubin level in patients with GS and healthy individuals.

Methods: Serum total and unconjugated bilirubin levels were measured in 16 patients with GS and 15 healthy individuals before and after a single 600-mg oral dose of rifampin. In patients with GS, the mean (SD) serum total and unconjugated bilirubin level increased from 2.15 (0.49) and 1.56 (0.41) mg/dL, respectively to 3.23 (0.72) (p<0.001) and 2.52 (0.71) mg/dL (p<0.001), respectively after rifampin administration, and in healthy subjects from 0.69 (0.13) and 0.34 (0.09) mg/dL, respectively to 1.68 (0.56) (p<0.001) and 0.84 (0.23) mg/dL (p<0.001), respectively. Elevation of bilirubin above the normal cut-off levels had poor accuracy for the diagnosis of GS. However, elevation of total serum bilirubin after rifampin above 2.4 mg/dL was 93.8% sensitive and 93.3% specific for the diagnosis of GS, and elevation of unconjugated bilirubin above 1.3 mg/dL was 100% sensitive and 100% specific.

Conclusions: Rifampin elevates bilirubin level to above normal in GS and healthy subjects. Overnight rifampin test may be useful for the diagnosis of GS if cut-off levels for serum total and unconjugated bilirubin level of more than 2.4 and 1.3 mg/dL are used.

Sixteen patients with GS and 15 healthy controls were studied. The diagnosis of GS was made by unconjugated hyperbilirubinemia (total serum bilirubin >1.3 mg/dL) on at least two occasions in the previous two years with normal values of other liver function tests, normal hepatic imaging, and absence of hemolysis based on normal reticulocyte count and peripheral blood smear. Control subjects were selected randomly from among healthy medical students with normal liver function tests including total serum bilirubin (<1 mg/dL) on at least two occasions in the previous two years, which showed no rise after 24 hours’ fasting. The cases and controls were similar in age and gender.

Subjects were excluded if they had a history of liver disease, diabetes mellitus, alcoholism, cholelithiasis, valvular heart diseases, congestive heart failure, hemolysis, hemoglobinopathy, positive HBsAg or anti-HCV test, or had used any drugs in the last two weeks. Fasting blood was taken for liver function tests (albumin, AST, ALT, alkaline phosphatase, LDH, bilirubin), reticulocyte count and peripheral blood smear on two consecutive days. A single 600 mg oral dose of rifampin was administrated the previous night to all study subjects 12 hours before the second sampling. The study subjects consumed normal diet and were
prohibited from using any chemical or herbal medication. Bilirubin was measured by the diazo method (Pars Azmon, Teheran, Iran). Unconjugated bilirubin was estimated as total bilirubin minus conjugated bilirubin. Total serum bilirubin concentration using this technique is below 1 (range 0.2-1) mg/dL in 99 percent of the general population; cut-off of 1.2 mg/dL was selected for this study as the upper normal level of serum total bilirubin.

The study was approved by the Semnan Internal Medicine Research Center. All participants gave informed consent at enrollment.

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) program (Chicago version, 11.5). Student’s paired t-test was used for comparison. Sensitivity and specificity were measured by ROC curve. A p value below 0.05 was considered significant.

Results
Mean (SD) fasting total and unconjugated serum bilirubin levels in 16 cases (10 men; age 25 [3.2] y) were 2.15 (0.49) and 1.56 (0.41) mg/dL, respectively. The corresponding values in 15 controls (9 men; age 24 [2.8] y) were 0.69 (0.13) and 0.34 (0.09) mg/dL, respectively. After rifampin administration, mean serum total and unconjugated bilirubin level in cases increased to 3.23 (0.72) (p<0.001) and 2.52 (0.71) mg/dL (p<0.001), respectively (Figure), and those in controls increased to 1.68 (0.56) (p<0.001) and 0.84 (0.23) mg/dL (p<0.001), respectively. Other biochemical tests, reticulocyte count and peripheral blood smear showed no change after rifampin administration.

Increase in serum total bilirubin to more than 2.4 mg/dL was 93.8% sensitive and 93.3% specific for the diagnosis of GS. Rise in unconjugated bilirubin to more than 1.3 mg/dL was 100% sensitive and 100% specific for the diagnosis of GS.

Discussion
Our data show that rifampin administration raised serum total and unconjugated bilirubin levels in GS. However, it also raises serum bilirubin to higher than normal level in healthy individuals.

Previous studies had shown that rifampin can not increase serum bilirubin level to higher than normal limit in healthy individuals;11,13,14 however, in our study 80% of healthy persons showed this phenomenon.

Fasting test is the conventional diagnostic method for GS; serum bilirubin level increases after 24 hours’ fasting and prolonged fasting raises the sensitivity of the test. Caloric restriction to about 400 Kcal in 24 h raises the serum unconjugated bilirubin level two-fold in patients.11,15

Administration of intravenous nicotinic acid can also be used for diagnosis; however, this provocative test is seldom necessary in clinical practice.8 The most important aspect of the diagnosis of GS is recognition of the disorder without any invasive and unnecessary testing.16

Rifampin test was considered as a diagnostic test in Spanish literature,14,17 and recently in English papers.11,13 Rifampin induces cytochrome P-450 isoenzymes and competes for the excretory pathways in the liver at the cellular level. These properties of rifampin may elevate the bilirubin level in some normal individuals. Rifampin does not seem to have a direct...
effect on UGT levels; however, in patients with GS who have reduced levels of UGT, addition of rifampin causes an exaggerated elevation in total serum bilirubin levels.\textsuperscript{10,11} These studies have shown that rifampin increases serum bilirubin after 2 hours in the fasting and 4 hours in the non fasting state.

Therefore total bilirubin increase up to 1.9 mg/dL in the fasting and 1.5 mg/dL in the non fasting state 2-6 hours after oral rifampin has 90%-100% sensitivity for diagnosing GS.\textsuperscript{11} Its specificity for diagnosing GS is not very high, because it also causes an increase in unconjugated bilirubin levels in some patients with chronic liver diseases, although its use is not recommended in these patients.\textsuperscript{13} Although in patients with chronic liver diseases, although its use is not recommended in these patients,\textsuperscript{13} Although in healthy individuals rifampin increases serum bilirubin, the level remains within normal limits.\textsuperscript{11,14} Absolute increase of bilirubin to >1.9 mg/dL 2 to 6 hours after the administration of 900 mg of rifampin distinguishes patients with GS from those without it.\textsuperscript{11} Due to side effects of rifampin, it would be prudent to avoid using this test in pregnant women, breastfeeding mothers and in patients with liver disorders.\textsuperscript{11,18}

This study aimed to assess overnight rifampin test as a practical and simple test for diagnosis of GS; but our data show that rifampin elevates bilirubin level to upper than normal concentration in healthy subjects also, and the higher post-rifampin levels in patients may reflect the higher basal bilirubin levels in GS.

In conclusion, we found that absolute rise in serum bilirubin to above normal after overnight rifampin test is not accurate for the diagnosis of GS. However, rise in serum total and unconjugated bilirubin levels above 2.4 and 1.3 mg/dL, respectively, after overnight rifampin test are highly sensitive and specific for this purpose.

References


