JGH Journal of Gastroenterology and Hepatology

JGHF

doi:10.1111/jgh.12375

EDITORIAL

Unconjugated hyperbilirubinemia: A blessing in disguise?

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See article in *J. Gastroenterol. Hepatol.* 2013; 28: 1643–1647.

Gilbert's syndrome (GS) is an inherited condition associated with reduced activity of the enzyme uridine diphosphate-glucuronosyltransferase (UGT1A1), involved in conjugation of bilirubin, in the liver. The condition is identified in around 6% of healthy population,[1] being somewhat more frequent in people of African ancestry than in European and Asian populations.[2] The most common genetic alteration underlying GS is the presence in homozygous form of a $(TA)_7$-TAA variant, also known as the UGT1A1*28 variant, in place of the usual $(TA)_6$-TAA allele in promoter region of the UGT1A1 gene;[3] some other genetic polymorphisms are also associated with GS, but their relative contribution is smaller.[4]

The condition is characterized by persistent, mild elevation of blood levels of unconjugated bilirubin, which gets accentuated during fasting, illnesses including systemic infections, or following administration of certain drugs. Other tests of liver function are essentially normal. Though the initial detection of hyperbilirubinemia, often during a health check or investigation for another condition, may raise an alarm, the condition is universally benign. Thus, the affected persons have no evidence of liver injury or progression to serious illness, except for a slightly increased risk of developing gallstones[5] and of occurrence of dose-dependent adverse effects following administration of certain drugs, such as irinotecan,[6,7] that use UGT1A1 for their elimination from the body. The persons with GS are believed to have a normal life span, and need no treatment or follow-up.

In the previous issue of the *Journal*, Horsfall *et al*.[8] report data that show a markedly reduced overall risk of death from all causes in persons with GS than those without this condition. Their study with a "cohort" design included 4266 "patients" with GS and 21 968 matched controls from a primary-care database in the United Kingdom, who had been "followed-up" for a median of 9 years. Their data showed that all-cause mortality in the GS cohort was almost half that of the control group. This effect remained largely unchanged after adjustment for various comorbidities. The stark difference observed would make one envy the people with GS. However, it also begs an important question—is this difference real?

This is not the first study to show that GS patients are endowed with health benefits. Several previous studies have looked at the relationship of GS with the risk of cardiovascular diseases (CVD), including coronary artery disease,[9] peripheral arterial disease,[10] and ischemic stroke.[11] Whereas the initial studies on the health effects of GS looked at the relationship of CVD with serum bilirubin levels, subsequent studies have assessed the relationship of these diseases with UGT1A1 alleles associated with increased serum bilirubin levels. Of these studies, several have shown a protective effect of high bilirubin levels or of the genetic changes associated with GS on various CVDs.[9,12,13]

The most convincing evidence supporting an inverse relationship between the GS genotype and the risk of CVD in healthy people came from the Framingham Offspring Cohort Study.[9] In this cohort study of 1780 unrelated individuals, homozygous carriers of UGT1A1*28 allele had higher serum bilirubin levels and nearly one third the risk of CVD and ischemic heart disease during a 24-year follow-up than those with either one or no such allele; in addition, the risk of myocardial infarction was reduced to nearly half, though this did not reach statistical significance. Further, an analysis of 13 214 adult participants in the National Health and Nutrition Examination Survey 1999 to 2004 in the United States showed reduced stroke prevalence and improved stroke outcomes in persons with a higher serum total bilirubin level.[11] In another large cohort study, patients undergoing chronic hemodialysis and serum bilirubin levels in the upper tertile had an adjusted hazard ratio of 0.32 for cardiovascular events (CVEs) and 0.48 for all-cause mortality during a 12-year follow-up than those with bilirubin in the lower tertile; further, in this study, individuals homozygous for UGT1A1*28 variant had approximately one-tenth the risk for CVEs and one-fourth the risk for all-cause mortality than in those with the major allelic form of the gene.[13] Carotid artery intima-media thickness, a marker of atherosclerosis, has also been found to be inversely related to serum bilirubin levels.[14] These data indicate that the presence of increased bilirubin or of homozygous UPT1A1*28 genotype protects against CVEs.

However, the news has not been uniformly positive. Several other studies on the subject have failed to detect a significant association between GS and CVD.[10,15–20] A recent, large meta-analysis of 11 studies with 14 711 cases and 60 324 controls, including eight previously published studies and three Mendelian randomization studies undertaken by the authors of this meta-analysis, examined the relationship of risk of various CVDs with three polymorphism sites related to the UGT1A1 gene.[21] In this analysis, homozygous carriers of the variants associated with increased bilirubin (UGT1A1*28/UGT1A1*28 genotype, rs887829 AA, or rs6742078 TT) showed no reduction in risk of various ischemic CVDs taken together (odds ratio = 1.01, 95% CI = 0.88–1.16) compared to heterozygotes and noncarriers.[21] Moreover, in another study, GS genotype has shown no association with the severity of CAD.[22]

Interestingly, our search in the database of human genome-wide association studies[23] failed to show any association of coronary heart disease with genetic markers located on the segment of the long arm of chromosome 2, where UGT1A1 gene is located. This provides an important piece of evidence against association of GS with CAD, since the genome-wide studies on the subject have been quite large.

Relationship of GS with other diseases besides CVD, such as cancers and chronic diseases, has also been studied. These studies have found a negative association between the presence of UGT1A1*28 allele or high serum bilirubin and risk of endometrial[24] and colon cancers.[25] Protective effects of GS against the

Accepted for publication 5 August 2013.

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occurrence of diabetes[26] and rheumatoid arthritis[27] have also been reported.

The study by Horsfall *et al*. in the previous of the *Journal*, had several positive features, including a "cohort" study design and a large sample size. Further, the outcome assessed was all-cause death rate, whereas most of the previous studies among healthy persons had focused on CVD. All-cause death rate has the advantage of aggregating the effects of the factor under study on several disease processes (e.g. on CVD, malignant diseases, cerebrovascular accidents, infections, etc.). This obviates the possibility of the factor providing benefit against one disease while simultaneously increasing the risk of other diseases, wherein the harms may outweigh the benefits. Also, mortality is a hard endpoint that does not suffer from ascertainment bias. Thus, all-cause mortality provides a clinically relevant, reliable and preferred endpoint. However, it would have been useful if the authors had, in addition, provided data on cause-specific and age-specific mortality rates in the GS and non-GS groups. Such disaggregated data would have helped us identify the causes of death that are most influenced by the presence of GS.

The study design did have some limitations. Most importantly, the assignment of each study subject as case or control was based primarily on whether a primary physician had recorded the person as having GS or not. Because of the limited clinical consequences of the "diagnosis" of GS, the clinicians may not always add this term to their patients' case records, leading to underdiagnosis. We need to consider the likely impact of this phenomenon on the study results. A nonselective failure to diagnosis GS would merely reduce the power of the study, making it harder to detect a difference between "cases" and "controls"; this is not a consideration in the current study since the authors did find a difference between the two groups. Of greater concern is a possibility of selective assignment of diagnosis of "GS" in persons who were healthier. A physician encountering a patient in whom no other diagnosis has been reached may be more likely to add the diagnosis of GS to the electronic records than for a patient in whom another diagnosis has been made. The resultant selection bias would be expected to result in unmatched groups, with the GS cases being healthier and with a lower all-cause death rate. Given the structure of the database used, it is difficult to exclude this possibility.

If the association between GS and overall death rate were indeed true, what could be the underlying mechanisms? Given our current understanding about bilirubin, it may be reasonable to suspect the involvement of pathways related to free radicals and oxidative stress.

A free radical is an atom, ion, or molecule with unpaired valence electrons. These particles are inherently unstable, and try to attain stability by reacting with other molecules. Biological systems contain several molecules such as superoxide ions, hydroxyl and hydroperoxyl radicals, hydrogen peroxide, pernitric oxide, nitrogen dioxide, peroxynitrite, and ozone, which contain oxygen molecules and act as free radicals, and are collectively referred to as reactive oxygen species. The reactive oxygen species can induce oxidative injury in body tissues by interaction with various intracellular biomolecules. The body cells are able to counteract these oxidative radicals with an array of natural antioxidant systems, such as glutathione, superoxide dismutase, glutathione peroxidase, catalase, vitamins C and E, and beta-carotene. An imbalance between the pro-oxidant and antioxidant activities, known as oxidative stress, is widely implicated in the pathogenesis of ageing, diabetes mellitus, atherosclerosis, coronary artery disease, reperfusion injury, oncogenesis, and age-related neurodegeneration.

Bilirubin is the breakdown product of heme. Heme is oxidized by heme oxygenase enzyme into biliverdin, a nontoxic substance, which is in turn reduced by biliverdin reductase into bilirubin. Though both bilirubin and biliverdin possess antioxidant properties, bilirubin is at least three times more potent in this regard.[28–30] While acting as an antioxidant, bilirubin is oxidized to biliverdin, which is then efficiently reduced back into bilirubin; this amplifies the antioxidant effect of bilirubin. The antioxidant effect of bilirubin is one of the likely pathways for its beneficial effects on human health. However, additional protective mechanisms may exist and need to be looked for.

In conclusion, the demonstration of reduced overall mortality in persons with GS in the study by Horsfall *et al*. should be of interest to a variety of medical specialists, even though the data are not necessarily conclusive. One hopes that large, prospective, long-term follow-up cohort studies will soon follow to confirm or refute its findings. If these studies confirm the protective effect of GS on the overall risk of death, it would be important to determine the mechanisms underlying this association. Such information may open a vista of newer interventions to improve human health and survival.

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