Association of Serum Bilirubin with Aging and Mortality

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Background and aims: **Bilirubin, a breakdown product of heme metabolism, has been shown to be protective against cardiovascular mortality; however, it is also a marker of liver function. There are limited data on the longitudinal changes in bilirubin with aging in a population-based cohort of older adults. This study was designed to determine whether serum bilirubin changes with age in older adults, and to evaluate whether age attenuates the association between bilirubin and mortality.** ***Methods:*** **This is a prospective cohort study of 2364 participants with a mean age of 70 years, who completed a research clinic visit from 1984 to 1987, and 1703 participants who returned for a second research visit approximately 8 years later. Cross-sectional and longitudinal multivariable-adjusted analyses were performed to examine the association between serum bilirubin, aging, and mortality.** ***Results:*** **In cross-sectional analyses, when the cohort was divided into quartiles of age, higher baseline serum bilirubin levels were associated with older age in analyses adjusted for sex, body mass index (BMI), alanine aminotransferase (ALT), albumin, and metabolic traits (*P*-value <0.001). In longitudinal analyses, among the subset of participants who had two research visits, aging remained significantly associated with an increase in bilirubin in multivariable-adjusted models (*P*-value <0.0001). When the longitudinal cohort was divided into bilirubin quartiles, Kaplan–Meier analysis showed an incremental reduction in survival with higher bilirubin levels (*P*-value = 0.002); however, this association between bilirubin quartile and mortality was no longer significant after adjusting for age (*P*-value 0.30), suggesting higher bilirubin in older age does not confer survival advantage.** ***Conclusions:*** **Serum bilirubin levels gradually increase with age in older adults. Elevated bilirubin in older individuals is not associated with improved survival as previously reported in middle-aged populations.** **(J Clin Exp Hepatol 2014;4:1–7)**

Bilirubin is a breakdown product of heme metabolism and is subsequently degraded by uridine diphosphate-glucuronosyltransferase (UGT). In vitro studies show that bilirubin acts as a potent antioxidant under physiologic conditions and may have anti-inflammatory effects.[1–8] Bilirubin inhibits low-density lipoprotein (LDL) and lipid oxidation, preventing oxidized LDL and lipids that would otherwise contribute to atherosclerotic plaque, opposing the development of cardiovascular disease.[4,9–13] Results from observational cohort studies as well as studies of Gilbert's disease have demonstrated that slightly elevated bilirubin levels are associated with a reduced risk of cardiovascular disease, supporting the concept of bilirubin as a protective antioxidant.[7,14–22] In contrast to the protective role in cardiovascular disease, bilirubin levels are directly related to liver-related mortality. The process of bilirubin catabolism is dependent upon liver function. Bilirubin must be taken up into hepatocytes where uridine diphosphate-glucuronosyltransferase (UGT) converts water-insoluble bilirubin into a conjugated, water-soluble form that can be excreted into bile. Bilirubin secretion is mediated by an ATP-dependent multiple drug resistance protein 2(MRP2).[23,24] Elevated levels of bilirubin indicate hepatocellular dysfunction. Bilirubin is a sensitive index of liver disease, serving as one of three variables used to calculate the MELD score that determines all-cause mortality and organ allocation for liver transplantation.[25–27]

While bilirubin levels have been characterized in middle-aged populations and individuals with liver disease, limited studies have reported the relationship between bilirubin and mortality in an older population.[28] Fleming et al was one of the first to describe the prevalence of abnormal liver tests in an older population. In a cohort of 13,276 individuals who were aged 75 years and older, there was a 5.4% prevalence of abnormal bilirubin levels. Elevated bilirubin levels were associated with a modest increase in mortality; however, the relationship between absolute bilirubin levels and mortality was not certain because laboratories with different standards performed the liver tests.[29]

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Abbreviations: UGT: uridine diphosphate-glucuronosyltransferase; LDL: low-density lipoprotein; MRP2: multi drug resistance protein 2; MELD: model for end stage liver disease; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma glutamyl transpeptidase
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This study aims, first, to determine whether bilirubin changes with age in a well-characterized population based cohort of older individuals and, second, to evaluate whether age alters the relationship between bilirubin and mortality.

METHODS

Study Cohort

The Rancho Bernardo Study (RBS) is a prospective cohort that was established in 1972 in order to characterize cardiovascular disease risk factors in an older population. The study enrolled 82% of the residents living in a geographically defined suburban neighborhood in Southern California. Participants completed a more extensive research study clinic visit between 1984 and 1987 at which time blood samples were obtained. A subset of the cohort returned for a second research study clinic visit between 1992 and 1996 at which time a second blood sample was obtained. The current analysis includes 2364 participants who completed the 1984–87 study visit, and the subset of 1073 participants who completed both study visits and had available longitudinal data. This cohort and its subsets have been well characterized and followed in a longitudinal manner for long term mortality. The initial objectives, inclusion criteria, and cohort characteristics have been described at length in other publications.[12,30] The institutional review board at the University of California, San Diego approved this study and all participants gave written consent.

Clinical and Laboratory Assessment

Qualified interviewers obtained complete medical histories at each research visit. Current medical problems, medical history, including history of chronic liver disease, and medication use were reviewed. Self-reported medications were validated by review of pills and prescriptions brought to the clinic for that purpose. Participants self-reported their alcohol use, including quantity and frequency. Comparison of self-reported use and quantitative responses to a separate nutrition interviewer yielded similar results, providing internal validation for alcohol responses. Morning fasting venous blood samples were obtained during each research study visit, and metabolic parameters were measured in a routine hospital laboratory. Total serum bilirubin level (milligram/deciliter) was measured by photometry after the addition of 3,5-dichlorophenyl diazonium. Height (meters) and weight (kilogram) were measured, and body mass index (BMI) was calculated as weight in kg divided by height in meter squared (kg/m^2).[12,30–33]

Follow Up

Rancho Bernardo Study participants were followed on an annual basis with a mailed questionnaire and a clinical research visit approximately every 4 years where health assessment and vitals were performed. All participants were followed for an average (±SD) of 13.7 (±6.2) years for the longitudinal analyses.

Statistical Analysis

The cohort was divided into quartiles of age: 30–62 (quartile 1), 63–71 (quartile 2), 72–77 (quartile 3), and 78–93 (quartile 4) and subsequently into quartiles of bilirubin (mg/dL): 0.1–0.3 (quartile 1), 0.4–0.5 (quartile 2), 0.6–0.6 (quartile 3), 0.7–2.3 (quartile 4). Descriptive statistics, including BMI, alcohol use, aminotransferases, and lipid levels, were defined for each quartile. Average bilirubin levels were calculated as geometric means, and least square means were used to evaluate trends in bilirubin. Mean ± standard deviation was reported for variables with a normal distribution, and geometric means with 95% confidence intervals were reported for variables with a skewed distribution. In the subset of the cohort who was seen twice, descriptive statistics for each individual were compared from the 1984–1987 and 1992–1997 visits using paired *t*-test, using logarithmic transformation of bilirubin. The hazard ratio for all-cause mortality based on bilirubin quartile was evaluated before and after adjustment for age. Kaplan–Meier survival curves for each bilirubin quartile were calculated using the log-rank test of equality. All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). Statistical significance was defined as a two-tailed *P*-value of less than 0.05.

RESULTS

Population Characteristics

This study included 2364 adults (55% women) with an average (±SD) age of 69.7 (±10.5) years. The average BMI for men and women was 25.8 (±3.3) kg/m^2 and 24.3 (±3.8) kg/m^2, respectively. The baseline total serum bilirubin level was 0.5 mg/dl (95% confidence interval, 0.49–0.51) with a range from 0.1 to 2.3. Table 1 shows the comprehensive baseline characteristics of the cohort. Analyses of the variables by age quartile revealed significant positive trends in systolic blood pressure, diabetes, and aspartate aminotransferase (AST) and negative trends in BMI, ALT, albumin, triglycerides, and cholesterol. Table 2 shows the baseline characteristics stratified by bilirubin quartile, revealing positive associations with systolic blood pressure, diabetes, AST, ALT, and gamma glutamyl transpeptidase (GGT) and negative associations with percent women, total cholesterol, HDL, and triglycerides.

In the subset of the cohort, comprised of 1073 participants who completed two research study visits, the mean age at study visit 1 (between 1984 and 1987) was 65.7 years (range 30–89), and the mean age at study visit 2 (between 1992 and 1997) was 74.1 years (range 37–96).

All participants were followed for an average (±SD) of 13.7 (±6.2) years for a total of 32,387 person-years of follow up. The cumulative mortality was 56.2%

