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Plasma bilirubin and gamma-glutamyltransferase activity are inversely related in dyslipidemic patients with metabolic syndrome: Relevance to oxidative stress

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ABSTRACT

Background: Subnormal levels of plasma bilirubin levels are associated with premature coronary artery disease and cardiovascular morbidity. Plasma gamma-glutamyltransferase (GGT) activity is linked to bilirubin level in hepatic disease and elevated GGT is equally associated with hepatic steatosis, a frequent feature of metabolic syndrome (MS). In order to assess the potential relationship between GGT activity and bilirubin levels in subjects exhibiting features of the metabolic syndrome, we determined circulating bilirubin levels and GGT activity in a cohort of dyslipidemic patients.
Methods and results: This cross-sectional study involved patients ($n = 1433$) displaying atherogenic dyslipidemia in primary prevention referred to our Prevention Center. Among these patients, 25% presented with MS as defined by recent NCEP ATP III criteria. Circulating levels of transaminases, as well as GGT activity, were elevated in MS patients; by contrast, bilirubin concentrations were significantly lower in such patients as compared to those lacking this syndrome ($p < 10–4$ for all comparisons). Comparisons of patient groups on the basis of the number of MS criteria which were concomitantly present revealed a progressive decrease in mean bilirubin levels; this reduction paralleled a progressive increase in mean GGT activity as a function of the number of MS components in the overall population (p value for trend < 10–4).
Conclusion: Elevation in systemic GGT activity, which is characterized by extended generation of ROS, together with potentially deficient bilirubin-mediated antioxidative capacity of plasma, may therefore constitute key components of the systemic oxidative stress typical of metabolic syndrome.

1. Introduction

Prospective cohort studies have revealed that low levels of bilirubin, the major hepatic breakdown product of haemoglobin, are closely correlated to premature coronary artery disease (CAD) and cardiovascular diseases (CVD) [1]. Equally, elevated plasma gamma-glutamyltransferase (GGT) activity, a biomarker of hepatic function, is intimately associated with coronary artery disease (CAD) and cardiovascular mortality [2].

Oxidative stress is an emerging cardiovascular (CV) risk factor [3] and interestingly, GGT activity is a major determinant of redox state on the one hand [4], while on the other, bilirubin is an effective antioxidant [5].

Our earlier studies established that the incidence of elevation of circulating hepatic enzymes in a cohort of dyslipidemic patients at high CV risk is frequent [6] and in addition revealed that elevation of GGT and subnormal levels of circulating thiol compounds, including glutathione, were significantly associated with features of the metabolic syndrome [7]. Moreover, metabolic syndrome (MS) is typically associated with a subclinical inflammatory state and oxidative stress [8].

In order to evaluate the potential relationship between GGT activity and bilirubin level as a function of the nature and the number of criteria of MS as defined by NCEP ATP III, we evaluated circulating levels of bilirubin and GGT activity in a cohort

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of patients displaying atherogenic dyslipidemia. Our data indicate that subnormal levels of bilirubin and elevated GGT activity may act synergistically to enhance the systemic oxidative stress typical of metabolic syndrome.

2. Methods

2.1. Patients

All patients were referred to our Prevention Center for Dyslipidemia and Cardiovascular Disease by their general practitioner and uniformly exhibited a personal history of dyslipidemia. All patients were requested to complete a questionnaire on their personal and familial medical history, smoking habits, lifestyle and diet and clinical status.

Routine medical examination included weight, height (BMI was calculated as weight/(height)2), waist circumference and repetitive blood pressure monitoring of the right arm for at least 30 min. Subjects with persistent systolic blood pressure ≥140 mmHg and/or diastolic pressure ≥90 mmHg and/or who were under treatment with antihypertensive drugs were considered as hypertensive. All patients underwent an electrocardiographic examination at rest. Patients included in the study displayed either hypercholesterolemia (serum LDL-cholesterol >160 mg/dL), or hypertriglyceridemia (serum triglycerides >150 mg/dL), or low serum HDL-cholesterol levels (<35 mg/dL), or a combination of these features.

Details of alcohol consumption were requested in a sample of patients ($n = 767$) who were classified as regular drinkers when estimated alcohol consumption was equal to/or greater than 10 g/day and as heavy drinkers when estimated alcohol consumption was equal to/or greater than 40 g/day.

Smokers were current smokers, but equally included patients who had smoked cigarettes within 1-year of inclusion in the study.

Patients were classified as displaying metabolic syndrome on the basis of the modified Adult Treatment Panel III (ATPIII) criteria [9].

All patients underwent systematic measurement of thyroid stimulating hormone (TSH), creatinine and serology for hepatitis B and C when plasma transaminases activities were elevated above the normal range; in this way, patients with hypothyroidism, severe renal insufficiency (creatinine level ≥140 μmol/L) or active liver disease due to viral infection (positive serology for virus hepatitis B or C) were excluded. Furthermore patients with clinically evident or previously diagnosed malignant disease, jaundice or cirrhosis and those with Gilbert's disease were excluded. Patients in secondary cardiovascular prevention in addition to those presenting diabetes were excluded.

All patients gave written informed consent for clinical studies and for future analysis of biological specimens.

2.2. Blood samples and analytical methods

Blood samples were withdrawn by venipuncture between 8:00 and 9:30 AM after an overnight fast.

Routine biochemical measurements, including creatinine, were performed on each serum sample using an autoanalyser in the 2 h following sampling. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and (GGT) activities were determined at 37 °C according to the procedure of Klauke et al. [10]. In a large group of healthy blood donors [11], the upper limit of normal values for men was established as 35, 32 and 42, for ALT, AST and GGT, respectively, and for women as 26, 27 and 32 respectively.

Total cholesterol and triglyceride concentrations were determined by an automated enzymatic method (Bio Merieux, Marcy l'Etoile, France) and HDL-cholesterol by an enzymatic procedure after phosphotungstic acid/magnesium chloride precipitation of apoB-containing lipoproteins. Low density lipoprotein–cholesterol concentration was calculated according to the Friedewald formula only when triglyceride levels were <400 mg/dl.

2.3. Statistical methodology

Results are expressed as mean (SD) or mean (CI) for continuous variables and as number and percentage for qualitative variables. Skewed variables (plasma triglycerides and GGT activity) were logarithmically transformed to improve normality prior to analysis and then back-transformed to their natural units for presentation in the text and tables. We evaluated the relationships of each metabolic syndrome component relative to levels of bilirubin and to GGT activity and compared patient groups on the basis of the number of metabolic syndrome criteria (0, 1, 2, 3, 4 and 5 criteria) which were concomitantly present. We performed ANOVA followed by post hoc test for linear trend to determine whether the means of the column increased systematically with progressive increment in the number of metabolic syndrome components [12]. In order to discriminate among the metabolic syndrome components and to determine whether their effect on GGT and bilirubin might be dominated by one or two specific MS components, we performed a multivariate logistic analysis. The increase in GGT activity above the normal range was the dependent dichotomic variable (0/1: normal/elevated) and each of the five components in the multivariate analysis was entered in the analysis as a dichotomic variable (0/1: absent/present); age was also included in this multivariate analysis. Furthermore as it is well established that alcohol consumption is associated with GGT activity and as Breitling et al. [13] recently showed that there is a detrimental interaction between alcohol consumption and cigarette smoking in a European population, we included alcohol consumption and cigarette smoking in the multivariate analysis. For bilirubin, we created a dichotomic variable with median as the cutoff (0/1: below or equal/above the median) as almost all patients were within the normal range; the cutoff point was different between men (8 μmol/L) and women (7 μmol/L); age was also included in this multivariate analysis. Statistical analyses were carried out with the use of JMP (SAS Institute, Cary, NY) software.

3. Results

The major biological and clinical characteristics of the study population ($n = 1433$) as a function of patients with ($n = 356$; 25%) or without metabolic syndrome are presented in Table 1. Plasma total cholesterol did not differ between the two groups. LDL-cholesterol was higher in patients in whom metabolic syndrome was absent. Non-HDL-cholesterol levels were higher in metabolic syndrome patients as compared to subjects lacking the syndrome. The proportion of patients treated with lipid-lowering drugs differed between the two groups (50% in patients with MS vs. 42% in patients without MS; $p = 0.01$). Patients with metabolic syndrome exhibited higher systolic and diastolic blood pressure and displayed hypertension more frequently than patients without metabolic syndrome.

Circulating levels of both transaminase and GGT activities were higher in metabolic syndrome patients as compared to patients lacking the syndrome. Several patients exhibited active liver disease with increased transaminases activities; they were however maintained in the study as their viral serology (hepatitis B and C) was negative. Surprisingly and in contrast with the elevation of GGT and transaminases activities, bilirubin levels were lower in patients with metabolic syndrome as compared to those who did not present this syndrome, although GGT activity was weakly