The Gamma-Glutamyltransferase Isoenzyme Pattern in Serum as a Signal Discriminating between Hepatobiliary Diseases, Including Neoplasias

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We have used the gamma-glutamyltransferase (GGT) isoenzyme pattern in serum as a means for discriminating between hepatobiliary diseases, including neoplasias. The reference pattern, determined in 142 normal subjects with a simplified conventional cellulose acetate electrophoretic procedure, contained two GGT bands, alpha1-GGT and alpha2-GGT, in proportions of 60–80% and 20–40%, respectively. Sera from 95 hepatobiliary patients showed typical isoenzyme features: (a) a beta-migrating GGT form that was <10% of the total GGT in chronic hepatitis and cirrhosis, and ≤30% of the total GGT in cirrhosis with intrahepatic cholestasis and in cases of extra- and intrahepatic obstructive jaundice, including liver neoplasias; (b) a gamma-migrating GGT band and (or) a "dep-GGT" (nonmigrating) band in cases of extrahepatic jaundice; and (c) an albumin-migrating GGT band that had a diagnostic sensitivity of 75% for hepatic tumors. The diagnostic specificity of this last band is 92% toward other hepatic disorders and 91% toward nonhepatic neoplasias; we consider it a potential specific marker for primary or metastatic liver neoplasias.

The enzyme gamma-glutamyltransferase (GGT; EC 2.3.2.2) is a glycoprotein present in various human tissues and organs. It occurs in multiple forms that differ mainly in their glycosidic moieties. Each GGT form comprises a dimer of two different monomers, both glycosylated, that originate from a single "propeptide" transformed by proteolytic activity. Thus, the polypeptide chain is coded for by a single gene, as is also shown by cDNA sequence analysis of rat kidney enzyme. Consequently, differences between the various forms of dimeric GGT are due to post-translational modifications of the oligosaccharide moieties.

Serum GGT is prevalently of hepatic origin, and its activity has long been known to be increased in most patients with liver or hepatobiliary diseases (4–6). This suggests that the serum isoenzyme pattern might be useful to discriminate between various hepatobiliary and pancreatic disorders. However, there being as yet no standard procedure for determining the serum GGT isoenzyme pattern, and because of the interaction of the isoenzyme forms with various serum constituents (7–10), a precise relationship has not yet been established between laboratory findings and diseases.

The aim of this study was to clarify the role of the GGT isoenzyme pattern in serum in pathophysiological conditions. We approached the problem in two stages: first we improved the method for measuring isoenzyme GGT so that it can be widely and routinely used (11); then we used this procedure to standardize both the profile and the quantitative reference pattern in normal subjects, and to determine the correlation between various isoenzyme patterns and well-defined clinical situations.

The data presented in this paper support the existence of disease-specific patterns for GGT isoenzymes in serum in patients with hepatobiliary disorders, including neoplasias.

Materials and Methods

Subjects and samples. The reference population in this study consisted of 142 subjects (69 women and 73 men), ages 18 to 65 years. All underwent a clinical examination and conventional laboratory tests (glucose, urea, creatinine in serum) in addition to specific tests performed to exclude the presence of clinically asymptomatic hepatobiliary and pancreatic diseases: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, amylase, cholinesterase, cholesterol, and triglycerides in serum. All results were within reference limits. Therefore the blood samples used for evaluating the GGT isoenzyme pattern can be taken as representative of the healthy population of our area (Naples, Italy) with respect to hepatobiliary diseases or other interfering diseases.

Ninety-five other sera, with high values of GGT, were subjected to isoenzyme electrophoresis. The patients' clinical histories were carefully examined. The diagnosis was referred by clinical wards and had been made after liver biopsy, echography, scintigraphy, etc. The same analytes as were determined for the reference subjects (see above) were measured so we could classify the patients according to their specific hepatobiliary and (or) pancreatic disorder.

Procedures. Elsewhere we have detailed the procedure for separating the GGT isoenzymes in serum (11). We estimated the total GGT in serum by the method of Persijn et al. (12) at 25 °C (kit no. 415251; Boehringer-Mannheim, Mannheim, F.R.G.). Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, triglycerides, cholesterol, amylase, cholinesterase, and total bilirubin in serum were measured with the methods routinely used in our laboratory, in a Cobas-Bio (Roche, Milan, Italy) centrifugal analyzer. Statistical analysis for the evaluation of diagnostic sensitivity and specificity was done according to Galen and Gambino (13). Reference intervals (14) and distribution curves (15) were calculated with an HP 85 calculator (Hewlett Packard Co., Avondale, PA).

Results

GGT Isoenzyme Pattern in Normal Subjects

Total GGT activity was within the reference range (16) in all the 142 healthy subjects examined. The GGT isoenzyme patterns were obtained with the modified procedure recently developed in our laboratory (11). Figure 1 shows typical patterns for normal subjects and patients with hepatobiliary

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1 Nonstandard abbreviations: GGT, gamma-glutamyltransferase; or gamma-glutamyltranspeptidase; (5-glutamyl)-peptide:amino acid 5-glutamyltransferase, EC 2.3.2.2; alpha1-GGT, alpha2-GGT; beta-GGT, gamma-GGT, alpha-GGT; and dep-GGT refer to GGT isoenzyme fractions co-migrating with serum a1-globulin, a2-globulin, b-globulin, g-globulin, albumin, and the nonmigrating fraction, respectively.

Received March 19, 1987; accepted October 26, 1987.

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diseases. Because there is no definite nomenclature for the various GGT forms (6), we have identified them according to their migration with respect to serum proteins under the same experimental conditions (Figure 1, left lane). All the healthy subjects showed only two GGT isoenzyme fractions (alpha1-GGT and alpha2-GGT).

The concentration of alpha1-GGT was always from 1.5 to 4.0 times higher than alpha2-GGT, as determined with an ultraviolet densitometer. The distribution diagrams of the alpha1-GGT and alpha2-GGT percentage values and the relative skewness and kurtosis indexes are shown in Figure 2; both distributions were gaussian. The reference intervals obtained by both methods—i.e., parametric (mean ± 2 SD: alpha1-GGT: 57.4–80.7% and alpha2-GGT: 18.5–41.5%) and nonparametric (97.5–2.5 percentiles: alpha1-GGT: 59.5–81.5% and alpha2-GGT: 19.2–42.4%)—almost completely overlap.

Pathophysiology of the GGT Isoenzyme Pattern

We determined the GGT isoenzyme pattern in 95 cases of hepatobiliary disease. The pattern typical of each type of disease is shown in Figure 3. Alpha1-GGT and a small fraction (<10% of the total GGT) of beta-GGT were found in active chronic hepatitis and cirrhosis. The proportion of the beta-GGT form was much higher, ≤30% of the total GGT, in patients with cirrhosis with intrahepatic cholestasis. In extrahepatic obstructive jaundice, in addition to a prominent (average 10–30%) beta-GGT band, gamma-GGT and dep-GGT isoenzymes were also present. The diagnostic sensitivity of these forms for extrahepatic obstructive jaundice was respectively about 50% for the gamma-GGT and dep-GGT forms, and 100% for the beta-GGT band, whereas the first two forms showed a higher diagnostic specificity than did the beta-GGT (approximately 80% vs 10%). Table 1 presents a detailed association of disease and GGT isoenzyme patterns, showing the number and frequency of the single GGT isoenzyme bands in relation to the various hepatobiliary diseases examined.

In several instances, particularly extrahepatic obstructive jaundice and liver tumor, the alpha2-GGT band appears evident, and well distinct from the alpha1 band (see Table 1), whereas in most other instances a large alpha1 fraction covers the position where alpha2-GGT migrates (see Figure 3).

Cases of hepatic carcinoma, both primary and metastatic, showed an additional isoenzymatic band with a 75% diagnostic sensitivity. This fast-moving band co-migrated with albumin. The band was not precipitated by low-density and high-density lipoprotein-precipitating treatments and was pathognomonic for liver cancer, both primary and metastatic. This form showed a diagnostic specificity of 91% toward other hepatobiliary diseases, and, as expected, also toward neoplasias present in other organs and tissues (92%). In fact, the pattern of the latter group of diseases (see Figure 3, no. 8) coincided with that of normal reference subjects.

As shown in Table 1, some liver tumors contain gamma-GGT and dep-GGT bands: in all these cases the patients showed extrahepatic obstructive jaundice caused by a primary pancreatic tumor (among the cases of secondary liver tumor).

Discussion

Using the simplified procedure we developed for the routine determination of the GGT isoenzyme pattern (11), we determined the GGT isoenzyme pattern in a sample population of 142 normal subjects. The pattern was constant and showed only alpha1- and alpha2-GGT. Our data, even though obtained with a different procedure, confirm earlier reports (17–20) of the presence of only two GGT fractions in serum; however, the relative amounts of the two fractions are inverted under our conditions (see ref. 11, Figure 2). Furthermore, when only a single GGT band was present in the serum of a patient affected by cirrhosis, this band co-migrated with alpha1-GGT under the conditions of our method but migrated with alpha2-GGT on the electrophoresis strips usually recommended for isoenzyme separation (Figure 4). Rosalki et al. (21, 22) attributed a band migrating between alpha1 and alpha2 in a minority of "healthy" subjects to borderline disturbances of lipid metabolism and (or) above-average chronic consumption of alcohol.

All the liver diseases examined in this study showed a prevalence of alpha1-GGT, either by itself (in almost 50% of the cases of chronic persistent hepatitis) or associated with the beta-GGT band. The percentage of the latter tends to increase gradually from the values in patients with chronic persistent hepatitis and cirrhosis toward those in patients with cirrhosis with intrahepatic cholestasis. Beta-GGT was also increased in diseases characterized by intrahepatic and
(or) extrahepatic biliary obstruction. Extrahepatic obstruction also showed gamma-GGT and dep-GGT bands (diagnostic sensitivity, 50%) (Table 1). Thus, the presence of either gamma- or dep-GGT, or both, is strongly indicative of the presence of biliary extrahepatic obstructive jaundice, as opposed to intrahepatic jaundice.

In chronic hepatitis and cirrhosis a high percentage of serum GGT is complexed with high-density lipoproteins, as shown by previous data (7–9) and further confirmed in our laboratory (11). This complex could migrate in the alpha-globulin region, thus overlapping the band present in normal subjects. In cirrhosis associated with cholestasis, the association of the GGT form with low-density lipoprotein could result in the beta band. We have shown (11) that serum from subjects with cholestasis contains complexes of GGT and low-density lipoprotein corresponding to the beta-, gamma-, and dep-GGT fractions. Perhaps biliary salts determine the release of the enzyme molecule, including the hydrophobic domain, which would bind low-density lipoprotein.

The most striking aspect of the typical patterns we have obtained is an alb-GGT band in the serum of subjects...
affected by either primary or metastatic hepatic tumors. The diagnostic sensitivity of this signal was 75%. Although our sample population of liver tumor is not large, it is larger than another population (only seven cases) for which the diagnostic sensitivity was lower (22). This band has also been reported in other non-neoplastic diseases (17); however, at least for the cirrhotic subjects, the band could be reflecting the evolution of cirrhosis into neoplasia. We found that the presence of alb-GGT has a diagnostic specificity of 92% for non-neoplastic hepatic disease, and 91% for neoplasias of other organs.

As postulated by Nemesanszky and Lott (6), the presence of this fast-moving band in sera of patients with neoplasia could result from a proteolytic effect of circulating proteolytic enzymes, which are often increased in sera of patients with liver malignancy. Our data (11), which show the absence of any major class of lipoprotein in the alb-GGT fraction, indirectly support this hypothesis. However, this should be verified by direct analysis of the structural composition of the single isoenzyme fractions.

In conclusion, with the simple electrophoretic method we devised for the determination of GGT isoenzyme patterns, we have defined the normal reference pattern, which consists of two bands: alpha1-GGT and alpha2-GGT. The concentration of the former was always higher (from 1.5 to 4.0 times) than the latter under the experimental conditions we used. In addition, there was a clear correlation between several hepatic diseases and the respective GGT patterns. The high diagnostic efficiency of the alb-GGT band in liver neoplasias, as compared with other hepatobiliary diseases, suggests that the presence of the band could be a useful biochemical signal of hepatic tumors.

Grants from the MPI and CNR "Progetto finalizzato oncologia," Rome, Italy, are gratefully acknowledged.

References