Gamma-Glutamyltransferase and Its Isoenzymes: Progress and Problems

Elmer Nemesanszky and John A. Lott

Because of its sensitivity to disturbances of excretory liver function, gamma-glutamyltransferase (GGT; EC 2.3.2.2) assay has become one of the most important diagnostic tests for hepatobiliary disorders. However, its value in the differential diagnosis of liver diseases is limited. It is present in kidney, liver, pancreas, and intestine in electrophoretically distinct but not necessarily organ-specific forms. Much effort has been expended to relate diseases of these organs to isoenzyme findings in serum. Although the activities of GGT isoenzymes in disease differ from those in health, the existence of disease-specific patterns is controversial. Much is known about the biochemistry, structure, and immunochromistry of GGT, but more work is needed on methods, standardization of terminology, and correlation of isoenzyme findings with diseases. Further progress in the last area should improve the clinical applicability of the isoenzyme activities.

Additional Keyphrases: liver disease • alcoholism • cholestasis • cell destruction • cancer • tumor markers

Interest in gamma-glutamyltransferase (GGT, glutamyl:gamma-glutamyl-peptide 5-glutamyltransferase, EC 2.3.2.2) isoenzymes has grown significantly in the recent past, leading to new areas of research in liver pathology. GGT is highly sensitive to pathological impairment of the liver's excretory capacity. Hanes et al. (1) were the first to describe GGT in human tissue, and the diagnostic usefulness of GGT was first reported by Szczeklik et al. (2). Within a few years, this test was being performed routinely and frequently in clinical laboratories around the world, primarily for the diagnosis of hepatobiliary disorders (3-6). The test can detect alcohol use and abuse in "social drinkers," and, even in minimal liver injury, such that biopsy findings are normal, GGT activities in serum are often abnormal (3, 7, 8). Workers desiring to improve the specificity of the GGT test have been intrigued with analyzing the "isoenzymes," in the hope of finding correlations between specific patterns and diseases.

We review here the clinical relevance of GGT isoenzymes in patients having abnormal serum activities of total GGT, focusing on the diagnostic usefulness of GGT isoenzyme assay, current problems in associating GGT isoenzyme patterns with disease, and developments in methodology.

Biochemistry and Physiology

The glycoprotein GGT is one of the key enzymes in the gamma-glutamyl cycle (9). Its primary catalytic functions appear to be the cleavage of glutathione into glutamic acid and cysteinylglycine and, acting as a transpeptidase, catalysis of the transfer of the gamma-glutamyl moiety to acceptor amino acids. The amino acid so formed enter the cell's amino acid pool for re-utilization. Reduced glutathione is reformed in the gamma-glutamyl cycle, and acts as a coenzyme in several intra-cellular enzymic reactions (10, 11).

Distribution in Tissues

GGT is found mainly in the membranes of cells that show high secretory or absorptive capacity: the epithelial cells lining the biliary tract, hepatic canaliculii, proximal renal tubules, pancreatic acinar tissue, pancreatic ductules, and intestinal brush border cells (9-13). In order of decreasing activity per gram of tissue, GGT occurs in kidney, liver, pancreas, and intestinal brush border cells (3, 4, 13-15). In adults, liver cells have significant GGT activity only in the peripheral zones of the liver lobules, which are rich in biliary epithelial cells. Fetal liver, however, contains high GGT activity throughout (16, 17). Measurable GGT activity has not been found in skeletal muscle or myocardium (17, 18). The serum of healthy persons contains only trace amounts of GGT as compared with the tremendous activity in kidney.

Isoenzymes of GGT

In tissue. In an effort to understand the isoenzyme patterns of GGT seen in serum, tissues (primarily from kidney and liver) have been extensively analyzed for GGT and its isoenzymes. GGT extracted from tissues shows considerable heterogeneity as to molecular mass and charge. It is membrane bound and amphiphilic, i.e., it has hydrophilic and hydrophobic moieties. The molecular mass of GGT isolated from liver is reported as 90 000 to 120 000 Da, depending on the isolation technique (12, 19-22).

GGT consists of light and heavy fragments, 22 000 and 47 000 Da. The catalytic site is located on the light, hydrophilic fragment. The hydrophobic domain is part of the heavy fragment by which the enzyme is anchored to the membrane via its amino terminal. When detergents such as bile acids or Triton X-100 are used to extract the enzyme from tissues, the hydrophobic, membrane-bound fraction of GGT can be brought into solution. If proteases such as papain (EC 3.4.22.2) or trypsin (EC 3.4.21.4) are included in the extractant, the hydrophobic domain is removed, producing a soluble, hydrophilic form of GGT (19, 23, 24).

Variants of GGT have been obtained that show the same molecular mass and catalytic behavior but marked electrophoretic heterogeneity, presumably because of differences in...

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1 Ist. Department of Medicine, Postgraduate Medical School, 1989-Budapest, P.O. Box 113, Hungary. To whom correspondence should be addressed.
2 Department of Pathology, The Ohio State University, Columbus, OH 43210.

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their sialylation (12, 22, 25, 26). Matsuda et al. (27), working with papain-solubilized GGT purified by chromatography on DEAE-cellulose, found a positive correlation between the isoelectric point of the various GGT forms and their sialic acid content. GGT from malignant tumors is more heavily sialylated than GGT from normal tissue; GGT isolated from hepatoma contained 291 nmol of sialic acid per gram of protein, about threefold the amount for GGT from normal kidney or liver (26–29).

GGT isoenzymes from liver, kidney, and pancreas differ in their lectin-binding properties, suggesting dissimilarities in their glycosylation (23, 30, 31), and GGT solubilized with detergent or bile acids changes its electrophoretic mobility if treated with sialidase (EC 3.2.1.18) (26, 27). Purified GGT readily forms aggregates and complexes with lipids and proteins, altering its electrophoretic pattern (24, 27, 32–34).

The light polypeptide fragments of GGT isolated from either liver and kidney are immunologically similar, as are the heavy fragments of the enzyme isolated from either organ (4, 14, 31, 35–37). Because GGT is believed to arise from a single gene locus, heterogeneity of the purified forms is most probably related to post-synthetic changes in sialylation, glycosylation (26, 28), or overall structure (4, 19, 20, 22, 24, 34).

In serum. Numerous studies agree that the liver is the source of most GGT activity in serum; kidney, pancreas, and the intestines contribute little to the normal activity in serum (3, 4, 23, 24, 38, 39). Liver and serum GGT have the same kinetic characteristics and the same hydrophilic and hydrophobic fragments (20). GGT is cleared by the liver and excreted in bile; a small amount is catabolized by the kidneys (15, 40–48).

GGT isolated from kidney, pancreas, and intestine has less electrophoretic mobility than that from liver. Although some have suggested that the slower-moving fractions in serum are organ specific, so that diseases of liver, kidney, and pancreas could be identified with these isoenzymes, this unfortunately has not been the case (23, 31, 46–48). The electrophoretic heterogeneity of the GGT isoenzymes in serum are the result of differences in sialic acid residues, carbohydrate content, and formation of complexes with small peptides or lipid compounds (32, 49–54), facilitated by the enzyme’s hydrophobic domain (19, 24, 50–55). Unlike creatine kinase (EC 2.7.3.2) or lactate dehydrogenase (EC 1.1.1.27), the isoenzymes of GGT show no tissue-specific characteristics.

The antigenic sites of GGT in serum and liver must be the same, because antisera raised against purified, papain-treated liver GGT showed the same reactivity with the slow- and fast-moving isoforms of GGT in serum and with the hydrophobic and hydrophilic forms of GGT extracted from liver (23, 24, 31, 35, 38).

Separation methods. Zonal electrophoresis followed by staining for enzymatic activity is the most useful technique. The supports most commonly used are agar, agarose, and cellulose acetate gels. Use of polyacrylamide gels is associated with a loss of 70 to 80% of GGT activity during electrophoresis (56, 57). Chromatography appears to be very tedious and inappropriate for the clinical laboratory.

Four compounds most commonly used for staining electrophoretic media for GGT activity are γ-γ-glutamyl-p-nitroanilide, γ-glutamyl-α- (or β-) naphthylamide, and γ-glutamyl-7-amino-4-methylcoumarin. The first should be abandoned because of its poor solubility and the faintness of the bands that are obtained for samples from healthy persons. The naphthylamide derivatives are more soluble and sensitive, but reportedly are carcinogenic (54, 56). The coumarin derivative is the most desirable, because its fluorescent reaction products provide the greatest analytical sensitivity (58).

Patterns of GGT isoenzymes in serum from healthy persons. The electrophoretic pattern obtained depends on the method; seemingly negligible changes can significantly alter the number and position of the bands for GGT. No uniform numbering scheme is in widespread use, which makes intercomparison of studies difficult. Attempts to describe the electrophoretic mobility of the GGT isoenzymes relative to that of the common serum proteins failed to provide accurate identification points. In healthy persons, two or three GGT bands are found in the α1- and α2-globulin regions (Figure 1). Table 1 summarizes the normal patterns for GGT isoenzyme as obtained with various methods (32, 33, 58–65).

Chemical Pathology of GGT and GGT Isoenzymes

Measurement of total GGT in serum is an extraordinarily sensitive test for disorders that affect the excretory capacity or structural integrity of the liver. The enzyme is the basis of tests for diseases of the liver and biliary tract. The low specificity, however, limits its use in the differential diagno-
sis of hepatobiliary diseases, and other tests and procedures are necessary before a diagnosis is made. Persistent cholestasis, caused by primary biliary cirrhosis, drugs, or malignancies, can produce enormous increases in serum GGT (3, 4, 6, 20, 45, 66–68). What is lacking at present is knowledge of the quantitative relationships between GGT activities and degrees of organ injury. Table 2 lists hepatobiliary disorders that increase serum GGT, and the approximate increases observed. Nonhepatobiliary conditions associated with abnormal GGT activities in serum are summarized in Table 3 (69–95).

Causes of Increased GGT in Serum

Why GGT is abnormally increased in serum of patients with liver and biliary tract disorders is not completely understood, although several theories have been proposed.

Induction of de novo synthesis. There is considerable evidence that in the face of impairment of biliary excretion, the GGT content of hepatocytes increases (66). The hepatocytes appear to adapt to stress by reverting to become like those in the fetus (16). A similar finding obtains in malignant liver cells, in liver cells compressed by a liver tumor, and in newly regenerated areas of cirrhotic liver (16, 96).

Table 2. Serum GGT Activity in Hepatobiliary Diseases

<table>
<thead>
<tr>
<th>Disorder</th>
<th>No. patients</th>
<th>% with abnormal GGT</th>
<th>Average increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary liver cancer</td>
<td>27</td>
<td>100</td>
<td>21.0</td>
</tr>
<tr>
<td>Tumor, metastatic to liver</td>
<td>138</td>
<td>100</td>
<td>14.3</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>26</td>
<td>100</td>
<td>13.6</td>
</tr>
<tr>
<td>Chronic active hepatitis</td>
<td>38</td>
<td>96</td>
<td>7.5</td>
</tr>
<tr>
<td>Intrahepatic cholestasis</td>
<td>46</td>
<td>94</td>
<td>8.2</td>
</tr>
<tr>
<td>Alcoholic hepatitis</td>
<td>144</td>
<td>84</td>
<td>3.8</td>
</tr>
<tr>
<td>Extrahepatic obstruction</td>
<td>41</td>
<td>82</td>
<td>5.1</td>
</tr>
<tr>
<td>Inactive cirrhosis</td>
<td>136</td>
<td>77</td>
<td>4.2</td>
</tr>
</tbody>
</table>

*Unpublished data (E.N.).

Table 3. Nonhepatobiliary Causes of Increased Serum GGT

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Magnitude of Increase*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticoagulants (e.g., coumarin)</td>
<td>Slight</td>
<td>3, 70–77</td>
</tr>
<tr>
<td>Antihyperlipidemics (clofibrate)</td>
<td>Slight</td>
<td></td>
</tr>
<tr>
<td>Oral contraceptives (estrogen)</td>
<td>Slight</td>
<td></td>
</tr>
<tr>
<td>Analgesics (e.g., acetaminophen)</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Anticonvulsants (e.g., phenytoin)</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Antihypertensives (tricyclics)</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Barbiturates</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>Moderate to marked</td>
<td>3, 5, 6, 78, 79</td>
</tr>
<tr>
<td>Disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Slight</td>
<td>61, 82</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>Slight</td>
<td>83</td>
</tr>
<tr>
<td>Kidney diseases</td>
<td>Slight</td>
<td>3, 88, 89</td>
</tr>
<tr>
<td>Neurological disorders</td>
<td>Slight</td>
<td>72</td>
</tr>
<tr>
<td>Obesity</td>
<td>Slight</td>
<td>75</td>
</tr>
<tr>
<td>Pulmonary diseases</td>
<td>Slight</td>
<td>85</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Slight</td>
<td>84</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>Slight to moderate</td>
<td>80</td>
</tr>
<tr>
<td>Myocardial injury</td>
<td>Slight to moderate</td>
<td>4, 61, 90, 102</td>
</tr>
<tr>
<td>Exocrine pancreatic diseases</td>
<td>Moderate to marked</td>
<td>81, 86, 87</td>
</tr>
<tr>
<td>Malignancy</td>
<td>Slight to marked</td>
<td>3, 92–95</td>
</tr>
</tbody>
</table>

* Slight increase, serum GGT < 2 × upper reference limit (URL); moderate, >2 and <5 × URL; marked, >5 × URL.

Membrane-bound GGT is induced in liver during cholestasis (17, 44). Why cholestasis stimulates the synthesis of GGT is not known. Ethanol and many other chemicals and drugs increase the GGT content of hepatocytes; the mechanism is probably microsomal induction of GGT synthesis (49, 97–102).

Release of membrane-bound GGT by detergent effects. Bile salts are surface active, and their detergent action can liberate GGT from its membrane-bound sites. Micellar-like structures form, and the hydrophobic enzyme is solubilized (60). Ethanol may solubilize membrane-bound GGT and so increase GGT in serum (86, 101, 102).

Regurgitation of bile. The GGT activity in bile is about 10-fold that in normal serum. The entry of bile into blood would be expected to increase serum GGT (45, 103, 104). The finding of biliary alkaline phosphatase and lipid-aggregated GGT in the blood of jaundiced patients points to regurgitation of bile (45, 103–105).

Change in permeability and destruction of cells. Increased GGT in serum and the presence of cell-membrane fragments in blood suggest cell destruction, as is the case in both hypoxic and inflammatory diseases of liver (66).

GGT Isoenzyme Patterns in Disease

Ever since Kokot and Kuaska (106) reported the presence of GGT isoenzymes in serum, there has been considerable interest in the relationship of diseases and isoenzyme patterns. It was hoped that assay of GGT isoenzymes would provide more specific information than assay of total serum GGT. Intensive work on the analysis, characterization, and purification of GGT isoenzymes has gone on in the last decade, but there is still considerable disagreement as to the clinical significance of various GGT isoenzyme patterns.

Cholestasis. Many authors (59, 62, 68) believe that GGT isoenzymes show sensitive and specific changes in cholestasis, where increased production of GGT and destruction of cells may be operative, both of which possibly leading to increases in GGT in serum. Cell destruction is supported by the finding of liver cell-membrane fragments in serum in patients with cholestatic disorders (24, 105).

There is no general consensus as to the GGT isoenzyme pattern that is pathognomonic for cholestasis. Several reports suggest that a GGT isoenzyme with beta mobility—i.e., GGT-5—is a sensitive test for cholestasis (59, 69, 107, 108). Baines found that increases in the beta-migrating band and a simultaneous decrease in the alpha-1-migrating isoenzyme were present in patients with cholangitis and liver malignancies (109). Both these disorders generally cause cholestasis. Kruiswijk et al. claimed that an increase of the GGT isoenzyme with an alpha-1 mobility, i.e., GGT-2, is a specific sign for cholestasis (67). Degenaar et al. (110) found that serum GGT-2 has higher activity in extrahepatic than in intrahepatic obstruction. This was not confirmed by Park et al. (33), who observed increased GGT activity at the alpha-1, and pre-alpha-2 positions, and a new GGT band at the gamma position in several patients with impaired biliary excretion.

Some workers have suggested that an electrophoretically identifiable GGT of intermediate molecular mass (250 000 to 500 000 Da) is present in the serum from most patients with liver diseases (54, 64). In a recent report, Wenham et al. (111) demonstrated that a band with an electrophoretic mobility of 0.45 to 0.55 relative to albumin, designated "GGT-IIIB," is prevalent in the sera of patients with extrahepatic obstruction. Determination and visual interpretation of GGT-IIIB was possible when polycrylamide gel was the solid support used. A laboratory marker of extrahepatic obstruction is an important finding, and these workers
found GGT-IIB in 88% of the 16 such patients they examined. Independent confirmation of this finding is needed. If it is validated, an important new tool for the diagnosis of extrahepatic obstruction would be available.

It is generally agreed that the GGT isoenzyme pattern in patients with cholestatic diseases differs from that observed in the serum of healthy individuals. At the present time, it is not possible to use GGT isoenzymes to distinguish intrahepatic from extrahepatic obstruction, and no cholestasis-specific isoenzyme pattern appears to exist (46, 52–54, 108); however, not everyone shares this view (43, 67, 110–113).

Changes in serum lipid concentration in patients with cholestasis can alter the GGT isoenzyme pattern. The mechanism is not completely understood, but chylomicrons, cholesterol, low-density lipoproteins, and triglycerides can complex with GGT, altering its size and electrophoretic mobility. This effect of lipids is unpredictable, mitigating against the usefulness of examining GGT isoenzymes in cholestatic disorders (51–53, 65, 105, 115, 116). Electrophoretically different GGT isoenzymes can be produced in vitro by incubating liver slices in serum; the GGT is eluted from the liver slices by serum, and appears to complex with serum lipids (50, 54, 63). Indeed, lipoprotein-X, a complex of GGT and phospholipids not normally found in serum, is commonly present in the serum of patients with cholestasis (24, 113, 114).

Alcoholic liver disease. In alcoholic liver disease, there is augmentation of the more anodically migrating, alpha-1 form of GGT, which may be produced by an abnormally increased sialylation (101, 102, 117). Whether early and late alcoholic liver disease can be differentiated on the basis of the GGT isoenzyme findings remains to be proved (118, 119). Treschke et al. (101, 102) claimed that a clear-cut distinction can be made between patients with alcoholic fatty liver and cirrhosis based on the pattern for GGT isoenzymes; this finding also needs confirmation. The increased GGT in alcoholic liver disease may be attributed to the induction of enzyme synthesis by alcohol, because an increased GGT-2 is often observed in the serum of these patients. But an abnormal GGT-2 is not specific for alcoholic liver disease, nor does it necessarily mean that the enzyme has been induced by alcohol or drugs (61, 67, 107, 108).

Liver malignancies. There are several studies on the value of GGT isoenzymes in liver malignancies; most reported an additional slow-moving, hydrophobic GGT band—i.e., GGT-5—in the beta region (54, 59, 60, 62). In primary liver cancer, an additional fast-moving GGT band in the alpha-1/albumin region, GGT-1, is often observed. This isoenzyme, a hydrophilic fragment, is probably cleaved from the beta hydrophobic enzyme by circulating proteolytic enzymes. Patients with liver malignancies often show increased serum proteolytic activity, possibly explaining the origin of the fast-moving GGT band (32, 54, 107, 109). Neither GGT-1 nor GGT-5 are sensitive or specific for liver malignancies; these bands can hardly be considered as tumor markers, because the GGT-1 band has also been observed in patients with non-malignant liver diseases (39, 47, 51). Other speculation as to the origin of GGT-1 in liver cancer has centered on the enzyme being a novel form, modified liver GGT, or a variant of GGT (28, 97, 120, 122–125). Hepatomas show much higher GGT activity than does normal liver tissue (120, 121).

Clinical Utility of GGT Isoenzymes

A GGT isoenzyme assay has limited clinical use, because there is no clear relationship between diagnostic entities and GGT isoenzyme patterns. The multiple methodologies and numbering systems in use for GGT isoenzymes have beclouded the relationship between laboratory findings and diseases.

But it is too early to abandon GGT isoenzymes, because current knowledge in the following areas should permit maturation of this field: The molecular heterogeneity of GGT has been explored, and the chemical nature of the GGT forms are now fairly well understood. What remains to be studied are interactions between GGT and various blood constituents such as lipids, peptides, bile salts, cations, and immunoglobulins. All these appear to affect the electrophoretic mobility and chemical characteristics of GGT in serum. Methodological research should be promoted to standardize the procedure of choice for clinical laboratories and the nomenclature of the GGT isoenzymes. Then detailed laboratory and diagnostic correlations can be sought by clinicians and laboratory scientists. Although the multiple forms of GGT are not genetically distinct isoenzymes, it is useful to speak of isoenzyme patterns from a practical point of view. The patterns observed for healthy persons are nearly always different from those for patients with liver diseases.

References
