Interpreting the Profile of Liver-Function Tests in Pediatric Liver Transplants

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Results of traditional laboratory tests of liver function were correlated with the clinical course in 26 pediatric patients after liver transplantation. On the basis of clinical outcome after transplantation, the patients were divided into two groups: (a) uncomplicated course with short hospital stay, and (b) post-transplantation course complicated by multiple clinical problems. The patterns of results for tests reflecting liver function—bilirubin (total and conjugated), aspartate (EC 2.6.1.1) and alanine (EC 2.6.1.2) aminotransferases, and y-glutamyltransferase (yGT, EC 2.3.2.2)—were consistent with the clinical findings in these patients. Values for alkaline phosphatase (EC 3.1.3.1), however, were only rarely increased, even when there was clinical evidence of biliary obstruction. Not only was serum yGT increased in obstructive jaundice, but this sometimes was the only test giving results outside the normal limits. We suggest that the persistent and marked increases of yGT observed in half of the patients may have resulted from immune-mediated damage to the transplanted liver.

Additional Keyphrases: y-glutamyltransferase · alkaline phosphatase · bilirubin · aspartate and alanine aminotransferases · immune-mediated damage · biochemical indicator of immune rejection

Liver transplantation is still an experimental surgical procedure performed at a few health centers and reserved primarily for severely ill patients with hepatic disease, for whom other therapeutic approaches have proven ineffective (1–4). Immune-rejection of the transplanted organ and complications resulting from immunosuppressive treatment are two major determinants of post-transplant survival in these patients (5). Drugs routinely used to suppress rejection are not specific; they also inhibit immune defenses against microbial infections (5). A more selective immunosuppressant, cyclosporin A, is replacing the other less selective drugs (7–9), with concomitant improvement in patient survival. Thus, liver transplantation is now being performed more often in children (10, 11) and is also being considered for introduction at other medical centers.

In clinical follow-up of patients after transplantation, many ancillary services are used, including tests on serum, to assess liver function. The group of these tests that are followed serially on a daily basis are bilirubin, total and conjugated; the aminotransferases, ALT, AST, and yGT; and ALP. These routine laboratory tests are useful in following the course of hepatic recovery from other diseases, such as hepatitis, but their usefulness in monitoring the functional status of the transplanted human liver has not been reported. Some experimental studies have been done (12–14), but these animal transplant recipients were previously healthy; there was relatively little delay in transplanting the liver, thus minimizing the anoxic period; and the tests were not done daily. This differs markedly from the human situation, where the recipients are very ill and where also there may be an anoxic period as long as 6 h between the time the liver is obtained and transplanted, even though it is transported in a chilled electrolyte solution (11).

In this study we have attempted to correlate the clinical course after transplantation and the results of liver-function tests in a group of pediatric liver-transplant recipients.

Subjects and Methods

We followed the progress of 26 patients during hospitalization after the transplantation, 14 males and 12 females, ages seven months to 18 years. The diagnoses and the number of patients were: biliary atresia, 13; a1-antitrypsin deficiency, 6; intrahepatic cholestasis (Byler disease), 2; and one each with Wilson's disease, tyrosinemia, neonatal hepatitis, post-viral cirrhosis, and cirrhosis of unknown etiology.

Serum tests of liver function, as mentioned above, were done daily on blood samples from the patients. (The tests were a part of the clinical follow-up protocol and no samples were obtained simply for the purpose of this study.) All the patients received appropriate infusions at various stages during hospitalization to correct electrolyte and coagulation imbalances. The tests of liver that we selected to follow were those least likely to be affected by these manipulations.

A few patients required retransplantation. One patient had three separate transplants, and three had two transplants each, one of them almost 18 months after the first transplantation.

The serum samples were analyzed the same day the specimens were obtained, by standard laboratory methods, in a centrifugal analyzer (Cobas-Bio; Roche Analytical Instruments, Nutley, NJ) (15). The reagents used were from the following kits: bilirubin (American Monitor Corp., Indianapolis, IN); ALT, AST, and ALP (Beckman Instruments, Inc., Carlsbad, CA); and yGT (Bio-Dynamics, Indianapolis, IN). Appropriate serum controls were run concurrently; samples giving results outside the range of linearity were diluted and re-analyzed. To facilitate comparisons, we expressed results as multiples of the upper limit of the normal reference range for each assay, these upper limits being: total bilirubin, 15 mg/L; conjugated bilirubin, 4 mg/L; ALT, 37 U/L; AST, 34 U/L; yGT, 44 U/L; and ALP, 300 U/L. We took into account that the ranges for ALP and yGT are age related and, for the latter, also sex related.

Results

The clinical course after transplantation differed for each patient, but the patients could be categorized into two groups, as follows. Eleven patients (42%) had a relatively short hospital stay (<30 days) and their recovery was uneventful. In contrast, 15 patients (58%) had complica-

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⁴ Nonstandard abbreviations: ALT, alanine aminotransferase (EC 2.6.1.2); AST, aspartate aminotransferase (EC 2.6.1.1); yGT, gamma-glutamyltransferase (EC 2.3.2.2); and ALP, alkaline phosphatase, EC 3.1.3.1.
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tions, hepatic and systemic, and were hospitalized up to 120 days. The test results for these two groups were different, which we illustrate by the biochemical and the relevant clinical findings for a representative case from each group.

**Group I: Uncomplicated post-transplantation course with a short hospital stay.** This 12-year-old boy, who had developed hyperbilirubinemia soon after birth, was diagnosed as having α1-antitrypsin deficiency. At the time of evaluation for transplantation, his serum bilirubin was 181 mg/dL (total) and 105 mg/dL (conjugated). Clinical findings included liver cirrhosis, splenomegaly, and esophageal varices, and he had experienced several episodes of upper gastrointestinal bleeding in the preceding year. There was rapid clinical improvement after the transplant. There were no complications and the patient was discharged from the hospital 18 days after surgery. The pattern of serum liver-function tests (Figure 1) shows increased values for all tests except ALP at the time of transplantation, with a rapid subsequent decline. All test results returned to normal by day 8 and remained within the normal range until the patient was discharged from the hospital. In the pretransplant period the serum enzyme activities were not increased, but immediately after the transplant the ALT and AST activities were greatly increased.

**Group II: Complicated post-transplantation course with prolonged hospital stay.** A two-year-old boy was diagnosed as having biliary atresia at age two months. Despite palliative surgery (Kasai procedure), his condition gradually worsened. At the time of evaluation for transplant, he had liver cirrhosis, splenomegaly, and ascites. Results of his liver-function tests after the transplant are profiled in Figure 2, which also indicates the days when the major complications occurred. In the immediate post-surgical period, the patient developed hypertension, which was controlled by sodium nitroprusside administration; this resolved by day 4. On day 11 he had a sharp increase in temperature; blood cultures showed *Escherichia coli*. Treatment with specific antibiotics did not improve the patient's condition, and he became jaundiced by day 13. Three days later, transhepatic cholangiography revealed a defect in the liver, confirmed by liver scan to be due to an infarction. A follow-up scan showed that the defect had enlarged to involve a substantial portion of the liver. The liver was removed on day 21 and a second liver was transplanted. The extirpated allograft had multiple focal infarctions and abscesses scattered throughout the liver; *E. coli, Klebsiella sp.,* and *Candida albicans* were cultured from the abscesses. Subsequent recovery was slow without clinical complications until day 42, when the patient developed peritonitis with *E. coli*, which resolved with treatment. Two separate septicemic episodes ensued, due to *C. albicans* and *Staphylococcus aureus*, both of which responded to antibiotic therapy. A subsequent liver biopsy (day 56) showed pericholangitis with infiltration of polymorphonuclear and plasma cells.

The liver-function profile showed that immediately after both transplantations both total and conjugated bilirubin and ALT and AST increased transiently, declining gradually during the subsequent week. γGT, but not ALP, had similar increases following the surgical procedure. The first episode of sepsis with abscesses and the liver infarctions were reflected by a marked increase in AST and ALT by the 13th day, a smaller though persistent increase in γGT, and a gradual increase in bilirubin (total and conjugated). There was, however, no alteration in ALP activity. As these values started declining, the patient had the second transplant and a transient increase to the previous activity, followed by a slow decline to normal. The γGT activity remained increased for much longer. With the subsequent episodes of peritonitis or sepsisemia, the concentrations of ALT and AST activity and of bilirubin showed no change. Thus the increases in the values of these analyses of serum correlated with pathologic alteration affecting the liver directly, but not when systemic infection was present without direct involvement of the liver. The transient increases in the values of the liver functions, immediately after the transplant and lasting a few days, have been observed in all these patients. With the re-establishment of the vascular connections, these increases may result from release into circulation of the enzymes and bilirubin accumulated in the liver during the anoxic noncirculatory phase of the few hours between harvesting the liver from the donor and placing it in the recipient. However, subsequent to this transitory phase of increases in results for liver-function tests immediately after transplantation, the fluctuations in these values reflected complications affecting the liver.

In only one patient of this group was there no correlation between the pattern of results for serum liver-function tests and the clinical findings, including complications related directly to the transplanted liver.

**Observations common to the two groups:** Besides the characteristics of the liver enzyme patterns peculiar to these groups individually, we noticed three features that occur commonly in the liver-transplant patients, irrespective of whether the post-operative clinical course was uneventful or complicated:

- **Immediately after transplantation,** all the patients had
increases in the enzymes and of bilirubin much above the preoperative values. This rise was transient and lasted four to seven days, if there were no complications affecting the liver. However, in the presence of complications such as blockage of the bile-duct anastomotic site from post-surgical edema, these increases persisted until the problem was resolved.

- γGT increased without any accompanying increase in ALP, even when there was evidence of bile-flow obstruction. In only one patient were parallel increases in both the enzymes observed.
- In five (46%) patients in group I and 10 (67%) of group II, γGT activity remained increased after all other analytes tested had returned to basal values. These increases ranged from 10- to 150-fold above the upper limit of the normal reference interval. In most of these patients, this was the only biochemical abnormality of liver function at the time of discharge from the hospital, and the increase had no obvious clinical correlate. ALP activity was invariably normal.

Figure 3 shows the pattern of increases in γGT activity and the lack of response in serum ALP for three patients. Two of these had complicated clinical courses (upper and middle panels) and one had an uncomplicated course after the transplant (lower panel). Though the complications resolved two weeks before discharge from the hospital in those two patients, the increase in γGT activity persisted. In the patient with the uneventful post-operative recovery, γGT activity was initially normal, but increased and remained high at the time of discharge. In subsequent outpatient follow-up of the patients for up to 18 months, these increases have been seen to persist.

Discussion

This study shows the pattern of results for the traditionally performed liver-function tests in pediatric liver transplant patients. Abnormal results reflect clinical complications in these patients after transplantation. Although, from previous experience, this would have been the expected findings with these biochemical variables in various liver diseases (16, 17), this is the first study documenting these findings in patients with human liver transplants. The tests were done daily; however, from the patterns observed in the pediatric patients, apparently they can be done less frequently, or even as indicated by the clinical situation, without loss of important clinical information.

Certain observations were unique to this study. First was the finding that immediately after the surgical procedure the values of all the tests increase markedly but in the absence of clinical complications gradually return to normal within a week. A possible cause for this may be the alterations in the transplanted organ during the anoxic phase. Up to 5 h may elapse between the time the liver is obtained from the donor and circulation through the organ is re-established in the recipient. Though the liver to be transplanted is kept bathed in a chilled electrolyte solution, cellular metabolites may accumulate in the noncirculatory interval, and may be reflected by the increases in the serum test values immediately post-transplantation. This hypothesis is supported by the fact that the time for the decline and return to baseline concentrations in plasma approximates the reported half-life of these enzymes (18, 19).

The second observation concerns the almost total lack of ALP fluctuations except for the initial increases in the immediate post-transplant period. In only one patient did the serum ALP activity parallel the γGT activity, when there was evidence of biliary obstruction with increases in total and conjugated bilirubin. In all other cases, no such parallel increases in ALP and γGT were observed. Increases in γGT corresponding to biliary obstructive episodes and increasing concurrently with bilirubin concentrations were seen in all cases. This lack of sensitivity of ALP vs γGT in children with liver diseases has been observed previously (20); γGT in liver-transplant patients may thus provide information that is more specific than ALP and correlates better with the other clinical and laboratory observations.

The third observation in this group of transplant patients was the isolated increase of γGT. Although γGT increase occurred transiently in the immediate post-transplant period or in the obstruction of bile flow, the activity of this enzyme also increased in the subsequent weeks in many patients from both groups. The mechanism(s) for these persistently high activities of γGT remains unclear, though several possibilities exist:

- The serum enzyme γGT activity increases in response to some drugs that induce enzyme production from liver (21). The transplant recipients were treated with cyclosporin A and steroids for immunosuppression (7-9), which were the only drugs common to all the patients. Steroids are known inducers of the liver enzyme activity, although the effect of cyclosporin A on serum enzymes is unknown. However, only some of the transplant patients exhibit increases in γGT.
- Additionally, the serum enzyme and bilirubin patterns of patients from a group undergoing kidney or heart transplantation procedures (n = 5 each), who were on similar immunosuppressive treatment, did not exhibit γGT increases (unpublished observations). This may preclude drug induction as a mechanism for γGT increases.
- The increases may be from a viral infection of the liver. Immunosuppression predisposes patients to various viral infections that can involve the liver (7) and lead to serum enzyme increases. Isolated increases of γTTP would be unlikely, and viral cultures in these patients performed as part of the clinical protocol identified only one case of viral infection with generalized herpes simplex. This patient did not have the isolated increases of γGT activity.
- γGT may be increased as a result of immune-mediated liver damage. In normal liver, γGT is localized in the canalicular portion of hepatocyte membranes and in the bile-duct cells (22, 23). During fetal development, hepatocytes stain more intensely for γGT; and following chemical or mechanical injury (22-26), the γGT content in hepatocytes increases and the cellular distribution also changes, with foci of regenerative cells showing abundant staining for γGT. These foci may originate from progenitor cells in the portal bile ducts (23). The histopathologic criteria for immune rejection of transplanted liver are not defined, the only consistent histopathologic finding in the transplant...
associated with clinical rejection being the damage centered around the portal triads of the liver lobules. This is characterized by disappearance of bile ductules and accompanying foci of cellular proliferation (27-29). These regenerative foci may contain increased γGT, which would contribute to the increase of serum enzyme activity. No histochemical studies on transplanted livers have been reported, and for the group of patients we studied, liver biopsies were not a part of the clinical study protocol. Thus we cannot correlate the biochemical findings with liver histology. The serum γGT activity may possibly serve as a biochemical indicator in these patients and reflect immune damage to the transplanted organ. In subsequent studies, through correlation of clinical, immunopathological, and biopsy findings, the serum activity of γGT as a biochemical indicator of immune rejection in liver transplants can be investigated. Activities of γGT in pigs receiving experimental liver transplants have been shown to increase in chronic immune-rejection reaction (12-14), but the criteria, clinical or pathological, for rejection were not specified.

From the observations presented for this study we conclude that: (a) The routinely performed tests of liver function exhibit alterations consistent with clinical complications involving liver in the transplant patients, but daily monitoring is unnecessary. (b) Serum γGT activity increases without concomitant increases in results of other serum tests and may be related to chronic immune damage to the transplanted liver. (c) Serum ALP activity remains normal and does not provide any useful information in these patients.

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References