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Serum α-Glutathione S-Transferase as a Sensitive Marker of Hepatocellular Damage in Patients with Cystic Fibrosis

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Summary

The aim of the study was to evaluate serum α-glutathione S-transferase (s-GSTA) levels in patients with cystic fibrosis (CF) and to compare s-GSTA with other liver function tests and with a hepatic ultrasound scan (US). The cytosolic enzyme, α-glutathione S-transferase is predominantly found in the liver and is distributed uniformly in the liver tissue. In our study s-GSTA levels were measured in 37 CF patients aged 1 to 28 years (mean age 10.4 years, 24 males). The control group consisted of 27 patients aged 2 to 17 years (mean age 8.5 years, 18 males). The presence of hepatobiliary abnormalities was assessed by clinical examination, ultrasound scan, s-GSTA, and conventional liver enzymes: alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and γ-glutamyl transferase (GMT). The calculated 5-95 % range of s-GSTA for the control group was 0.098-2.54 μg/l, for the CF group 0.43-9.76 μg/l. Mean s-GSTA level in the control group was 1.55 μg/l (S.D.=1.57), and 2.05 μg/l (S.D.=2.60) in the CF group. In the group of CF patients, the serum levels were significantly higher than in the control group ($P<0.01$). No significant correlation existed in the CF group between s-GSTA and conventional liver tests (ALT, AST, ALP and GMT). Four patients in the CF group had hepatobiliary abnormalities detectable by conventional liver tests, s-GSTA and US. Four patients had abnormal s-GSTA, while conventional liver tests and US were normal. One other patient had abnormal hepatic US, but normal standard liver tests and s-GSTA. The study has suggested that a raised s-GSTA level might be a marker of possible pathological changes of the hepatobiliar system in CF patients. Serum GSTA seems to be a more sensitive marker than transaminases for the monitoring of hepatocellular integrity and as an early predictor of hepatic damage.

Key words
Cystic fibrosis-related liver disease • Liver tests • α-glutathione S-transferase

Introduction

Cystic fibrosis (CF) is the most common life-limiting, autosomal-recessive disorder. Commonly, the major clinical manifestations of the disorder are chronic sino-pulmonary disease, pancreatic exocrine insufficiency, and elevated electrolyte levels in the sweat (Kiesewetter *et al.* 1993).

Liver disease associated with CF develop in about 20 % of patients, usually before or at puberty, and

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display a slowly progressive course. Liver diseases and liver failure are the third most common cause of death after cardiorespiratory causes and complications after transplantation and account for 2.3 % of overall CF mortality. Focal biliary cirrhosis is considered the specific hepatic lesion of CF, resulting from biliary obstruction and progressive periportal fibrosis. This is due to impaired secretory function of the biliary epithelium. Studies of tissue localization of cystic fibrosis transmembrane receptor (CFTR) have shown that its expression at the hepatobiliary level occurs exclusively at the apical domain of epithelial cells lining intra- and extrahepatic bile ducts (cholangiocytes) and gallbladder. CFTR is not expressed in hepatocytes and other liver cells. Reduced bile fluidity and alkalinity plug the intrahepatic bile ducts, causing their obstruction and leading to ductular proliferation, inflammation, and focal biliary cirrhosis (Colombo 2000). The impairment in ductular bile flow may also increase the susceptibility of the biliary epithelium to the damage by cytotoxic compounds excreted into the bile and to the aggression by infectious agents. Retention of detergent endogenous bile acids may be responsible for the cell membrane injury, which may also occur through increased free radical production favored by decreased lipid-soluble antioxidant activity (Colombo 2000).

The conventional markers associated with hepatocellular injury and biliary tract disorders include aminotransferases (AST, ALT), ALP and GMT. Serum ALT and AST are increased, at least to some extent, in most liver disorders. In general, the serum aminotransferase increase reflects the relative extent of active hepatocellular damage, but not necessarily its aggregate severity. However, even when combined with markers of hepatic synthetic function, such as serum albumin and prothrombin time, ALT and AST are relatively poor indicators of centrilobular hepatocellular injury because of their uneven distribution. In common with ALP and GMT, ALT and AST are distributed mainly within the periportal area, and substantial centrilobular necrosis can occur without a concomitant increase in serum aminotransferases (Beckett and Hayes 1993, Loguercio *et al.* 1998). An additional limitation of using aminotransferases as markers for hepatocellular injury is their relatively long plasma half-lives (17 h for AST, 47 h for ALT). Thus, during acute liver damage, abnormalities in serum aminotransferase concentrations often lag behind the changes in hepatocellular integrity.

The cytosolic enzyme, α-glutathione S-transferase (GSTA), may offer significant clinical advantages over conventional transaminases for monitoring hepatocellular integrity. This enzyme is predominantly found in the liver. Serum concentrations of GSTA have been found to ensure more sensitive indication of hepatocellular damage than convention transaminase activities in a variety of clinical conditions (Hayes *et al.* 1988, Beckett *et al.* 1989). The group of glutathione transferase enzymes catalyze the conjugation of glutathione with a large number of compounds and this family is divided into four main classes: α, μ, π, and θ, each subdivided into one or more isoenzymes (Rees *et al.* 1995). GSTA is a relatively small enzyme (MW 50 000) present in high concentrations in the hepatocyte cytosol. In contrast to ALT that has a predominately periportal distribution, GSTA has been shown by immunohistochemical studies to be distributed uniformly in the liver lobule (Nelson *et al.* 1995). GSTA measurements also appear to correlate better with histological abnormalities. It was concluded that the measurement of GSTA in the serum or plasma is probably the most sensitive biochemical test available for monitoring the effects of acute hepatic injury (Beckett and Hayes 1993).

The usefulness of GSTA as an indicator of acute and chronic liver damage in patients has been investigated in several clinical studies involving pregnant women with hemolysis, increased liver enzymes, and low platelet counts syndrome, neonates of women with hemolysis, increased liver enzymes, and low platelet counts syndrome, birth asphyxia, liver transplant rejection, acute alcohol intoxication, alcoholic cirrhosis, acetaminophen (paracetamol)-induced liver damage, and acute and chronic hepatitis.

Unlike transaminases, GSTA has been found to be unaffected by muscle damage, extrahepatic inflammation, and hemolysis, and is therefore presumed to be more specific than transaminases (Narkewicz 2001).

Methods

Serum levels of GSTA were measured in 27 control subjects. Their ages ranged from 2 to 17 years (mean age 8.5 years, 18 males and 9 females). Thirty-seven patients with cystic fibrosis (age ranging from 1 to 28 years, mean age 10.4 year; 24 males and 13 females) were selected for our study. The diagnosis of CF was confirmed by the sweat test and genotyping. The assessments were carried out during the stable phase of their illness and no other causes of liver disease were suspected before or during the assessment period.

The presence of hepatobiliary abnormalities was assessed by clinical examination, ultrasound examination, serum GSTA, and conventional liver enzymes: alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and γ-glutamyl transferase (GMT). The hepatic ultrasound scan was carried out by an experienced pediatric radiologist. The findings, which were considered abnormal, were parenchymal irregularity, periportal fibrosis and irregularity of the liver edge consistent with the macronodular pattern of cirrhosis.

Serum was collected within two weeks of clinical and ultrasound examination and was stored at –25 °C prior to analysis.

The GSTA enzyme immunometric assay procedure was carried out according to the factory instructions (Biotrin HEPKIT-Alpha, Biotrin Int., Stillorgan, Dublin, Ireland).

Conventional liver biochemical tests (AST, ALT, ALP, GMT) were performed on a clinical chemistry automatic analyzer ADVIA 1650 (Bayer Diagnostics, Tarrytown, New York, USA). AST, ALT, ALP and GMT were measured by commercial AST kit, ALT kit, ALP kit and GMT kit (Bayer Diagnostics, Tarrytown, NY, USA).

The results of measured biochemical tests were assessed as mean concentrations ± S.D. ANOVA statistical analysis was used for statistical analysis.

Results

The calculated 5-95 % range of GSTA in the control group was 0.098-2.54 μg/l, therefore abnormally high level of serum GSTA was defined as >2.55 μg/l. Mean concentration of GSTA in control group was 1.55± 1.57 μg/l. For the group with CF 5-95 % GSTA range was 0.43-9.76 μg/l, mean concentration was 2.05±2.60 μg/l. In the CF group, the serum levels were statistically significantly higher than in the control group ($P<0.01$).

Mean levels of the liver conventional biochemical tests measured in our 37 patients with CF were: AST (0.54±0.22 μkat/l), ALT (0.43±0.25 μkat/l), ALP (3.94 ± 1.38 μkat/l) and GMT (0.55 ± 0.76 μkat/l).

No significant correlations existed in the CF group between GSTA and our measured conventional liver tests: AST ($r = 0.072$, $p = 0.67$), ALT ($r = 0.087$, $p = 0.61$, Fig. 1.), ALP ($r = –0.067$, $p = 0.70$) and GMT ($r = –0.197$, $p = 0.26$, Fig. 2).

Four patients with CF had hepatobiliary abnormalities detectable by either conventional liver tests, serum GSTA and on the ultrasound scan. In four patients, serum GSTA level was abnormal, but standard liver tests and the ultrasound scan were normal. One patient had an abnormal ultrasound liver scan, but normal standard liver tests and serum GSTA level. Abnormal liver tests occurred only in those children, who already had raised GSTA level or ultrasound abnormalities.

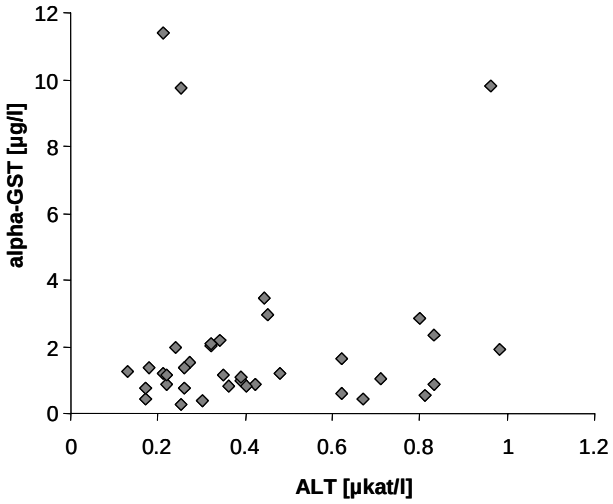

Fig. 1. *Correlation between alpha-GSTA and ALT (r=0.087, p=0.61).*

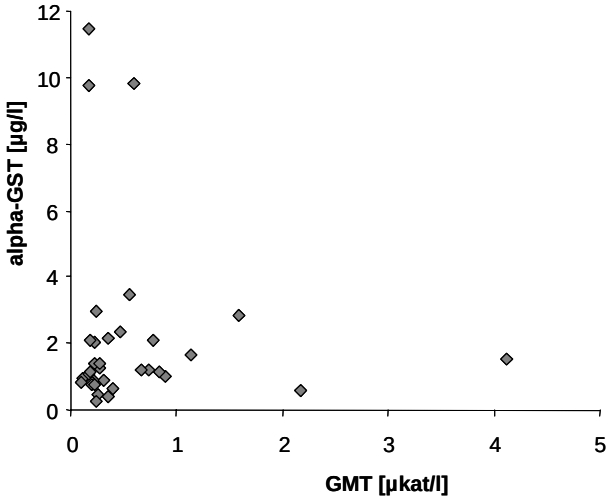

Fig. 2. *Correlation between alpha-GSTA and GMT (r= -0.19, p=0.26).*

Discussion

We found significantly higher GSTA levels in patients with cystic fibrosis. In four patients GSTA levels

were abnormal, but standard liver tests and ultrasound scan were still normal. It seems that the determination of serum GSTA level is a clinically useful marker for the assessment of hepatocellular integrity monitoring and an early predictor of hepatic damage. Finding of elevated GSTA level requires monitoring patient to predict liver disease in children with cystic fibrosis.

We found that the serum GSTA level was normal in one child with an abnormal hepatic ultrasound scan and normal liver tests. The normal serum GSTA could be explained by the same factors, which produce a fall in transaminases in established liver cirrhosis, namely, a low degree of ongoing hepatocyte injury, reduction of enzyme synthesis in hepatocytes and malnutrition (Balistrei and Rej 1994). The ultrasound scanning is therefore very useful for determination of CF-related liver disease (CFLD) with parenchymal changes.

The absence of correlation between GSTA and standard liver tests in the CF group seems to be of interest. This could be due to the fact that four patients from CF group had higher levels of GSTA and normal standard liver tests.

Several other causes could be responsible for biochemical abnormalities of liver function in patients with CF other than CFLD, including such insults as medication, infection, hypoxemia and malnutrition. Conventional liver tests become elevated relatively late in the pathological process.

Pathological changes of hepatobiliar system can develop in children without any abnormalities in standard liver biochemical parameters (Narkewicz 2001). Thus, there is a need for sensitive, specific, and minimally invasive markers of early CF-related liver disease. GSTA may provide important additional information on assessment of early CFLD together with conventional liver tests and ultrasound scan of the liver, but it could be normal in advanced cases of cirrhosis as the transaminases. Serum insulin-like growth factor binding protein-3 (IGFBP-3) is a very useful marker for the assessment of hepatic dysfunction in patients with cirrhosis (Šídlová *et al.* 2002). Liver biopsy can give direct evidence of fibrosis (Potter *et al.* 1997), but the risks of the procedure may outweigh the potential benefits of early identification.

The high intracellular concentration of GSTA and its small size lead to a rapid release of high amounts of the enzyme into the plasma following even minor hepatocellular damage. When the enzyme is released into the plasma, hepatocytes are more sensitive to damage (Murray *et al.* 1998).

In conclusion, we have carried out a study to determine the usefulness of serum GSTA levels in detecting cystic fibrosis-related liver disease (CFLD) in our patients with cystic fibrosis. Our study suggests that serum GSTA seems to be a more sensitive marker than transaminases for detection of CFLD and that further monitoring of patients with elevated serum GSTA is essential.

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Reprint requests

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