

Release of α -Glutathione S-Transferase (α -GST) and Hepatocellular Damage Induced by *Helicobacter pylori* and Eradication Treatment

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Abstract: *Helicobacter pylori* (*H. pylori*) is a gram negative, spiral, microaerophilic bacterium that infects the stomach of more than 50% of the human population worldwide. *H. pylori* is well recognized as a critical factor in the majority of patients with peptic ulcer disease and successful treatment results in cure of the disease. On the other hand, *H. pylori* infection has been associated with several extra-intestinal diseases such as hepatic encephalopathy. In this study, a triple treatment was used in management and eradication of *H. pylori* infection. We hypothesized that *H. pylori* infection and/or eradication treatment increased the releasing of α -glutathione S-transferase (α -GST). We also investigated whether α -GST is a more sensitive marker than aminotransferases (traditional liver function tests) for hepatocellular damage. However, we did not find any association between both *H. pylori* infection and eradication treatment and α -GST levels. According to our data, eradication treatment did not cause hepatocellular damage.

INTRODUCTION

Helicobacter (*H. pylori*) is a gram negative, spiral, microaerophilic bacterium that infects the stomach of more than 50% of the human population worldwide [1]. Treatment of this infection has proved difficult, although *H. pylori* is now well recognized and has usually involved complex treatment regimens [2]. Multiple drugs increase the risk of adverse reactions, diseases and death [3]. *H. pylori* infections can be asymptomatic, but some people do get acid reflux (heartburn), indigestion, or other symptoms. Quadruple therapy in eradication of *H. Pylori* infection causes mild to moderate side effects [4-6]. Hepatic encephalopathy (HE), which is a complication of cirrhosis of the liver, has been linked to *H. pylori* infection because of the ammonia produced by the organism in the stomach [7, 8]. The measurement of α -GST levels may be used as a diagnostic tool for HE in clinic settings.

Glutathione S-transferase (α -GST) is a sensitive and specific biomarker of cell permeability and forms an important high-content parameter in the cluster analysis of toxic responses. Higher α -GST levels seem to reflect more complex damage [9]. α -GST is found at high concentrations in the human liver and is quickly released and in large quantities into the bloodstream during hepatocellular damage. Because the half-life of α -GST in plasma is ~1 h, its concentration will follow changes in hepatocellular damage more rapidly than aspartate aminotransferase (AST) or alanine aminotransferase (ALT), which have plasma half-lives of ~17 and 47 h, respectively [10, 11]. α -GST and π -glutathione S-transferase (π -GST) are potential noninvasive markers of

hepatocyte and biliary epithelial cell injury. α -GST can reveal hepatic effects not detected by alternative testing. The high sensitivity and specificity of α -GST indicates that unchanging levels almost exclude acute hepatic effects [12].

The aims of our study were: [1] to investigate whether eradication treatment induced liver damage by measuring serum α -GST levels as a specific biomarker [2] to show whether there was any changes in the release of α -GST in healthy subjects (control group) and *H. pylori* positive subjects before and after eradication treatment [3] to define any association between *H. pylori* infection, eradication treatment and serum α -GST levels and compare the levels of serum α -GST to traditional liver function tests (ALT, AST, GGT and AP).

MATERIALS AND METHODS

Subjects

Peripheral blood was obtained aseptically from 32 untreated subjects infected with *H. pylori* (20 female, 12 male) between the ages of 18, and 74 treated subjects who received *H. pylori* eradication treatment (one proton-pump inhibitor (PPI) and two antimicrobials). 26 normal healthy subjects (14 female, 12 male) were used as controls and none of them had *H. pylori* eradication treatment at any time. Informed consent was obtained from each subject. In addition, all subjects were given a detailed questionnaire to provide as much information as possible about factors (chronic alcohol abusers, cirrhotic patients, subjects with known liver damage, etc.) that may potentially confound the measurement of serum α -GST levels. Subjects with any of those were not considered suitable for study due to increased serum α -GST levels. We used ELISA immunoassay to measure α -GST levels (an anti-human α -GST immunoassay; HEPKIT) (Alpha-Biotech, Biotrin International, and Dublin, Ireland). *H. pylori* status was assessed by the ¹³C-urea breath test.

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Triple treatment was used in management and eradication of infection. Short-term low-dose triple treatment, comprising one proton-pump inhibitor and two antimicrobials from the choice of clarithromycin, amoxicillin and ornidazole, largopene (LAC regimen), is currently considered a regimen. *H. pylori*-positive subjects received this treatment regimen for 2 weeks.

Determination of Serum α -GST Levels

Serum was diluted 1:5 using the assay buffer provided; 100 μ l of diluted sample and control were added in duplicate to a micro titer plate coated with antibody to α -GST and incubated at room temperature for 1 h with shaking. After washing to remove the unbound fraction, 100 μ l of anti-GST IgG conjugated to horseradish peroxidase were added to each well, incubated for 60 min at room temperature and washed, and 100 μ l of tetramethylbenzidine substrate were added. After final 15-min incubation at room temperature, the reaction was stopped by adding 100 μ l of 1 N H₂SO₄. Absorbances were read at 450 nm with a 630-nm reference filter on a Molecular Devices Spectramax microplate reader. Standards of known concentrations were included in every run, and the concentrations of the samples and controls were calculated from the standard curve.

Assessment of Major Liver Function Tests; ALT, AST, AP and GGT

Traditional liver function tests represent a broad range of normal functions performed by the liver. The diagnosis of liver disease depends upon a complete history, complete physical examination, and evaluation of liver function tests and further invasive and noninvasive tests. Enzyme activities of ALT, AST and Alkaline Phosphatase (AP) and γ -Glutamyl Transpeptidase (GGT) were assessed photometrically using commercial Standard kits (Boehringer, Mannheim, Germany).

RESULTS

Table 1 shows the mean \pm standard deviation (SD) concentrations of α -GST in serum of the total 32 subjects with infected *H. pylori* prior to eradication treatment and after treatment and control subjects. The lowest α -GST concentrations were observed in *H. pylori* negative (healthy control) subjects (18.0 [4.9] μ g/L). Mean (\pm SD) α -GST concentrations were higher in *H. pylori* positive subjects before treatment (26.0 [20.9] versus 18.0 [4.9] μ g/L in the control subjects). Although there was an average 70% increase in serum α -GST levels of *H. pylori* positive subjects, this difference was not statistically significant. However, these results indicated that *H. pylori* infection and its protein products might induce damage and α -GST release. Serum α -GST levels

were also increased in treated group compared to control subjects. In contrast to expected, there was a little decrease in serum α -GST levels of treated subjects (Table 1) compared to untreated. This decrease might be due to partially eradication of *H. pylori* infection. However, the mean α -GST levels were still not as low as in control group. On the other hand, there was no significant difference (less decrease) between treated and untreated groups (26.0 (\pm 20.9) μ g/L and 21.6 (\pm 11.9) μ g/L, respectively) in the mean α -GST levels. Although the mean ALT, AST, GGT and AP levels did not change among groups, α -GST levels were different. This suggested that α -GST was more sensitive than traditional liver function tests. *H. pylori* was eradicated in 6 patients, the mean α -GST level was 61.1 (\pm 24) μ g /L in untreated patients and 20.6 (\pm 6.0) μ g / L in treated subjects ($p < 0.05$, paired t test). It could be explained that it was decreased by the releasing of α -GST from damage cells induced by *H. pylori* infection.

Table 1. The Mean (\pm SD) α -GST Concentrations (μ g/l) in Control Subjects, *H. pylori* Positive Subjects Before and After Eradication Treatment

Groups	n	Mean \pm SD α -GST (μ g/l)	Min-Max	Median
Control (<i>H. pylori</i> negative)	26	18 \pm 4.9	6.0-31.3	16.7
Before Treatment (<i>H. pylori</i> positive)	32	26 \pm 20.9	3.4-107.5	20.9
After Treatment (<i>H. pylori</i> negative)	32	21.6 \pm 11.9	3.1-65.6	19.2

$p > 0.05$ (Student's t- test and paired t-test).

DISCUSSION

H. pylori infects the stomach of more than 50% of the human population worldwide. A triple treatment is commonly used in management and eradication of *H. pylori* infection, and in this study we investigate its effect on hepatocellular damage in treated patients. α -GST is a sensitive and specific biomarker of cell permeability and forms an important high-content parameter in the cluster analysis of toxic responses. Higher α -GST levels seem to reflect more complex damage [9].

H. pylori is now well recognized as a critical factor in the majority of patients with peptic ulcer disease and successful treatment of the infection results in cure of the disease. However, treatment of this infection has proved difficult, involving a combination of drugs, and has usually involved complex treatment regimens [13]. Most clinical studies in the field of hepatology, including the monitoring of response

Table 2. The Levels of ALT, AST, GGT and AP in *H. pylori* Positive Subjects Before and After Eradication Treatment

Groups	n	Mean \pm SD ALT	Mean \pm SD AST	Mean \pm SD GGT	Mean \pm SD AP
Before Treatment (<i>H. pylori</i> positive)	32	19 \pm 5.2	18.6 \pm 8.4	31.8 \pm 25.6	158.5 \pm 74.6
After Treatment (<i>H. pylori</i> negative)	32	19.6 \pm 3.6	21.1 \pm 7.5	19.2 \pm 7.6	161.0 \pm 61.1

$p > 0.05$ (paired t test).

in therapeutic trials, employ serum ALT (alanine aminotransferase) as a marker of hepatocellular damage. However, in some infections, such as chronic hepatitis C virus (HCV) infection, serum ALT has been shown not to be a satisfactory marker of histologic disease activity. Recently, α -GST has been proposed as an alternative marker of hepatocellular damage induced by several drugs [14]. *H. pylori* infection is common in cirrhotic patients with hepatic encephalopathy. Increased ammonia levels have been observed in the gastric juice and blood more frequently in cirrhotic patients with *H. pylori* infection than in those without. Though the amount of ammonia produced by *H. pylori* may be too small to contribute to hepatic encephalopathy, eradication of *H. pylori* has been shown to improve the blood ammonia levels and hepatic encephalopathy [15]. Moreover, *H. pylori* inoculated by oral route could arrive in the liver and cause inflammation as an independent etiological factor. The routes by which the microorganisms reach the liver may also involve hematogenous and/or biliary system dissemination [16]. α -GST protein and GST activity can be measured in serum. In healthy subjects, it most probably reflects enzyme release during normal hepatic cell turnover. GST- μ protein and activity can be measured in peripheral lymphocytes. Thus, these measurements provide noninvasive approaches to monitoring the effect of interventions on GST protein levels and activities in healthy individuals [17]. All of the α -GST isoenzymes appear to be expressed in human liver, but the proportions are variable. Because of this interindividual variation, we chose to develop an assay using a pair of monoclonal antibodies selected originally for their ability to bind to both α -GST subunits. The commercially available EIA (enzyme immunoassay) for measuring α -GST in biological fluids (Hepkit) is reported by the manufacturers to detect total α -GST [18]. The evaluation of individuals with liver and biliary tract disease includes serum assays for biochemical markers. Traditional markers associated with hepatocellular injury and biliary tract disorders include aminotransferases, AP, γ -GGT, prothrombin time and bilirubin. In general, the size of 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 [19]. In common with AP and γ -GGT, ALT and AST are distributed mainly within the periportal area and substantial centrilobular necrosis can occur without a concomitant increase in serum aminotransferases. An additional limitation of using aminotransferases as markers for hepatocellular injury is their comparatively 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 changes in hepatocellular integrity [19].

Miyaji *et al.* examined the effect of the eradication of *H. pylori* on hyperammonemia in patients with liver cirrhosis. They observed that the diffuse distribution of *H. pylori* in the stomach contributed partly to hyperammonemia in patients with liver cirrhosis, and its eradication was effective in patients with hyperammonemia with diffuse infection in the stomach [20]. Nandakumar *et al.* evaluated whether *H. pylori* eradication in patients with Chronic Liver Disease

(CLD) affected arterial ammonia levels. They found that levels of arterial blood ammonia were higher in CLD than in Acid-Peptic Disease (APD) and correlated with severity of liver disease. Their results indicated that *H. pylori* eradication was associated with reduction in arterial ammonia levels in patients with CLD [21]. Arabaci *et al.* measured gastric mucosal hexosamine content in cirrhotic patients. They concluded that *H. pylori* infection contributes to gastric mucosal vulnerability by further depressing hexosamine levels [22]. Coppola *et al.* tested whether *Helicobacter* species play a role in the enhancement of liver necro-inflammation and fibrosis and in the development of hepatocellular carcinoma (HCC). They sought DNA sequences of *Helicobacter* species in liver specimens from patients with viral-related chronic hepatitis, HCC or metastatic liver carcinoma. Their data suggested that *Helicobacter* species were not involved in the pathogenesis of virus-related HCC, chronic hepatitis or liver carcinoma metastasis [23]. Higuchi *et al.* evaluated the effect of low-flow sevoflurane anesthesia and isoflurane anesthesia on hepatocellular integrity using α -GST, which is a more sensitive marker of hepatocellular damage than is aminotransferase activity. They found that there was no significant difference in α -GST concentrations between the sevoflurane and isoflurane groups during or after anesthesia. Their results indicated that low-flow sevoflurane and isoflurane anesthesia had the same effect on hepatic function, as assessed by plasma α -GST levels [24].

Oijen *et al.* studied GST activity and isoenzyme levels, glutathione peroxidase (GPO) activity and GSH levels in antral biopsies of 38 *H. pylori*-positive patients, before and after eradication treatment. They found that eradication of *H. pylori* infection increased GST activity and GSH levels and antral GST enzyme activity. Glutathione was assayed by High Performance Liquid Chromatography after reaction with monobromobimane. It was 532 (465 - 598) nmol / mg protein min (mean and 95% confidence interval) before treatment and was 759 (682 - 836) nmol / mg protein min after treatment ($p < 0.001$) [25]. Clarke *et al.* investigated the use of α -GST as an early index for hepatotoxicity in carbon tetrachloride (CCl_4) toxicity as compared with a standard enzyme marker, AST. Their findings indicated that α -GST was a more sensitive and more accurate reflector of CCl_4 induced hepatotoxicity than AST [26]. Shrimali *et al.* investigated the prevalence of *H. pylori* in patients of liver disease with hepatic encephalopathy. They found that the prevalence of *H. pylori* was significantly higher and they suggested that *H. pylori* might have a role in the pathogenesis of hepatic encephalopathy [27]. Suttner *et al.* measured the cytosolic liver enzyme α -GST, the formation of the lidocaine metabolite monoethylglycinexylidide (MEGX), and gastric mucosal tonometry-derived variables as sensitive markers of hepatic function and splanchnic perfusion. They found that increased serum levels of α -GST and the changes of gastric tonometry-derived variables implied a reduction in splanchnic perfusion, leading to a temporary impairment of hepatocyte oxygenation [28]. Together with the previous results, our data suggests that α -GST was a sensitive and specific marker of hepatocellular damage, which might be caused by *H. pylori* and drugs used eradication treatment. Limitations for using aminotransferases as markers for hepatocellular injury include their comparatively long plasma half-lives, which causes that, during acute liver damage, abnormalities in se-

rum aminotransferase concentrations often lag behind changes in hepatocellular integrity. In the present study, *H. pylori* infection slightly increased the release of α -GST. According to our data this infection might be partially recovered by eradication treatment. Both *H. pylori* infection and eradication treatment did not statistically significant increase the releasing of α -GST, even though there was a slightly increase in treated and untreated subjects compared to control subjects. In the future, further analysis is needed with a larger sample size to verify whether the eradication treatment induce hepatotoxicity. It is the responsibility of all the clinicians treating *H. pylori* to choose the best sensible treatment option available to eradicate the organism and minimize complications. It is also important for us to ensure that we avoid secondary bacterial resistance to anti-microbial.

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