Investigation of Ornithine Carbamoyltransferase as a Biomarker of Liver Cirrhosis

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Abstract

Objective Ornithine carbamoyltransferase (OCT) is a liver-specific mitochondrial matrix enzyme and potential biomarker of liver fibrosis. This study investigated the OCT levels in patients with chronic liver disease with or without cirrhosis in order to assess the usefulness of OCT as a biomarker of cirrhosis.

Methods The subjects included 440 Japanese patients with chronic liver disease and 80 control subjects. The patients were divided into two groups, those with and without cirrhosis, both of which were further stratified into high-OCT and low-OCT subgroups.

Results In the non-cirrhosis group, the patients with non-alcoholic steatohepatitis (NASH), alcoholic liver disease, primary biliary cirrhosis and primary sclerosing cholangitis (PSC) comprised the high-OCT subgroup, while the patients with hepatitis B, hepatitis C and autoimmune hepatitis formed the low-OCT subgroup. There were significant differences in the OCT levels, OCT/aspartate aminotransferase ratios and OCT/alanine transaminase (ALT) ratios between these two subgroups (p<0.001). The same findings were observed in the cirrhosis group. The OCT levels were markedly higher in the cirrhosis group than in the non-cirrhosis group, particularly among the patients with PSC (p<0.001). The most useful biomarker for predicting cirrhosis was the OCT/ALT ratio in the patients with hepatitis C and NASH and the OCT level in patients with PSC.

Conclusion The OCT level differs among patients with different chronic liver diseases. The role of OCT should be further evaluated in order to improve our understanding of the pathogenesis of these diseases. The OCT level is a useful surrogate marker of cirrhosis, particularly in PSC patients.

Key words: ornithine carbamoyltransferase (OCT), biomarker, liver cirrhosis, non-alcoholic steatohepatitis (NASH), primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC)

blood. Previous studies have demonstrated that the serum OCT level and OCT/AST and OCT/ALT ratios are significantly elevated in patients with chronic liver disease, especially those with alcoholic liver disease (ALD), followed by hepatocellular carcinoma (HCC) and liver cirrhosis (LC) (3-6).

Despite the discovery of OCT over 50 years ago, measurement of the OCT level has not become a common clinical test, although it is a highly liver-specific and abundant protein with a half-life of several hours (6).

A sensitive and reliable enzyme-linked immunosorbent assay (ELISA) for OCT was recently developed (7). Since then, we have been investigating this old biomarker of liver disease and have previously reported that the serum OCT level and OCT/ALT ratio both increase in parallel with the stage of fibrosis in patients with non-alcoholic steatohepatitis (NASH), with both parameters showing marked elevation in NASH patients with HCC (8). Such findings suggested to us that OCT is a potential serum marker of liver fibrosis.

Accordingly, the aims of the present study were to determine whether the serum OCT level differs among patients with various liver diseases (with or without LC) and to assess the usefulness of OCT as a biomarker for predicting the presence of LC.

**Materials and Methods**

**Patients**

Four hundred and forty Japanese patients who attended Tokyo Women’s Medical University Hospital between January 2010 and December 2011 were included in the present case-control study. These patients had chronic liver disease caused by infection with hepatitis B virus (HBV) (hepatitis B, n=31) or hepatitis C virus (HCV) (hepatitis C, n=60), as well as autoimmune hepatitis (AIH, n=33), NASH (n=182), ALD (n=26), primary biliary cirrhosis (PBC, n=68) or primary sclerosing cholangitis (PSC, n=40). Patients with HCC or other malignancies were excluded. The control subjects (n=80) were Japanese adults without a history of chronic liver disease who underwent liver function tests and ultrasonography and were confirmed to have normal laboratory data with no evidence of fatty liver. Informed consent was obtained from all of patients and controls before entry into the study. This study conformed to the ethical guidelines of the Declaration of Helsinki (2008 revision), and the protocol was approved by our institutional research ethics committee.

**Diagnosis of chronic hepatitis**

Hepatitis B was diagnosed in patients with chronic hepatitis who were positive for HBV surface antigens, while hepatitis C was diagnosed in patients with anti-HCV antibodies and HCV-RNA. Patients who had received interferon or nucleoside analogs to treat HBV/HCV infection were excluded. AIH was diagnosed according to the criteria of the International Autoimmune Hepatitis Group (9). NASH was diagnosed based on the following criteria: (a) the detection of steatohepatitis on a histologic examination and (b) the exclusion of all secondary causes of hepatic fat accumulation, such as significant alcohol consumption (10). The diagnosis of ALD was made based on a history of chronic excessive alcohol intake (>70 g daily for more than five years), clinical evidence of liver disease with typical laboratory abnormalities and the exclusion of other causes of chronic liver disease. Ethanol intake was assessed by interviewing the patients and their family members (11). PBC was diagnosed in patients who met any two of the following three criteria: chronic cholestatic liver disease, positivity for antimitochondrial antibodies or anti-M2 antibodies or diagnostic liver histology (granulomatous cholangitis, etc.) (12). PSC was diagnosed using endoscopic retrograde cholangiopancreatography, and all patients with PSC met the criteria of the American Association for the Study of Liver Diseases (13).

The presence of cirrhosis was determined based on the results of a histopathological examination or unequivocal clinical and laboratory evidence of cirrhosis, such as ultrasound and/or computed tomography findings indicating cirrhosis (an irregular liver surface, splenomegaly, etc.) and the detection of signs/symptoms consistent with decompensated cirrhosis (jaundice, varices due to portal hypertension, ascites or hepatic coma). Fibrosis was staged as follows: stage 1 included portal fibrosis or zone 3 fibrosis in patients with ALD or NASH, stage 2 included perportal fibrosis, stage 3 included bridging fibrosis and stage 4 included LC. The patients were divided into groups, those with and without LC (non-LC group and LC group, respectively), then category of chronic liver disease was stratified into high-OCT and low-OCT subgroups based on a median OCT level of 50 ng/mL in the non-LC group and 70 ng/mL in the LC group. It has been reported that the OCT levels differ among patients with various chronic liver diseases (6). We hypothesized that the OCT levels in patients with steatohepatitis (NASH and ALD) and cholestatic hepatitis (PBC and PSC) would be higher than those observed in patients with viral hepatitis or hepatocyte injury (hepatitis B, hepatitis C and AIH). Because the pathogenesis of each chronic liver disease is quite different, we attempted to validate this hypothesis by investigating the subgroups. We set the normal range of the OCT level (mean ± 1.96 SD in 80 control=43 ng/mL) based on data obtained from 80 healthy control subjects (8).

**Methods**

All patients underwent various laboratory tests, including measurement of the AST, ALT, total bilirubin (T-Bil), gamma-glutamyl transferase (GGT), albumin, platelet (Plt), immunoglobulin G and immunoglobulin M levels, as well as serology for HBV, HCV and the titers of autoantibodies. All patients also underwent ultrasonography.

The serum OCT levels were measured using ELISA, as previously reported (5, 6). In brief, 50μL of the horseradish peroxidase-conjugated F (ab’) fragment of a monoclonal
anti-OCT IgG antibody (secondary antibody: Mo5B11) and 50μL of standard solution or sample diluted 10-fold with assay buffer (250 mmol/L glycine buffer (pH 9.4) containing 0.1% bovine serum albumin, 50 mmol/L NaCl and 0.1% ProClin950) were added to the wells of an antibody-coated dish (primary antibody: Mo3B11). After mixing, incubation was performed for two hours, and the dish was washed with washing buffer (10 mmol/L phosphate buffer (pH 7.4) containing 0.1% BSA, 150 mmol/L NaCl and 0.1% ProClin950). Then, a substrate solution (200μg/mL 3, 3', 5, 5'-teramethylbenzidine with 0.001% H2O2) was added for the coloring reaction. After 20 minutes, the reaction was terminated by adding a stop solution (0.5 mol/L H2SO4), and the absorbance at 450 nm was measured using a microplate reader.

Statistical analysis

The statistical analysis was performed using the IBM SPSS Version 20.0 (IBM SPSS Statistics, Armonk, USA) and Stat Light Yukms ver2.00 (Yukms Co., Ltd., Tokyo, Japan) software programs. The data are expressed as medians, with 25th and 27th percentiles. Statistical comparisons between the different categories of chronic liver disease were made using the Steel-Dwass test, while comparisons between the non-LC and LC groups or the high- and low-OCT subgroups were made using the Mann-Whitney U-test. The Kruskal-Wallis test was used to assess whether there were significant differences between any of the eight groups. The chi-square test was employed for categorical factors. If there were significant differences in the OCT levels or OCT/AST and OCT/ALT ratios between the non-LC and LC groups, a receiver operating characteristics (ROC) analysis was performed to assess the predictive value for LC. The area under the ROC curve (AUROC) was calculated to estimate cut-off values predicting LC with the optimum sensitivity and specificity. Spearman’s rank correlation analysis was performed to assess the associations between the Plt and OCT levels and the OCT/AST and OCT/ALT ratios among the patients with each type of chronic liver disease.

Results

Characteristics of the non-LC group

Fig. 1 shows the method used to group the patients in the present study. The characteristics of the non-LC group (n=344) are listed in Table 1. The median ages of the patients with hepatitis C, AIH, ALD, PBC and PSC were in the 60’s, whereas those of the patients with hepatitis B and NASH were in the 40’s. Men accounted for approximately 50% of the patients with hepatitis B, hepatitis C, NASH and PSC. Although only approximately 10% of the patients with PBC and AIH were men, all of the ALD patients were men.

The median OCT levels were ranked in the following order: ALD, PSC, NASH, PBC, hepatitis C, AIH and hepatitis B. The differences were significant according to the Kruskal-Wallis test (p<0.001), and the median OCT level of the patients with each disease was also significantly higher than that of the control subjects according to the Kruskal-Wallis test (p<0.001).

In the non-LC group, the patients with NASH, ALD, PBC and PSC were classified into the high-OCT subgroup, while those with hepatitis B, hepatitis C and AIH formed the low-OCT subgroup. The differences in the OCT levels between the high-OCT subgroup (54.4 ng/mL) and the low-OCT subgroup (32.2 ng/mL) were statistically significant (p<0.001). The OCT/AST ratios in the high-OCT subgroup were significantly higher than those observed in the low-
OCT subgroup (1.6 vs. 1.1, p<0.001); the same trend was noted for the OCT/ALT ratio (1.3 vs. 1.1, p<0.001).

**Characteristics of the LC group**

The characteristics of the LC group (n=96) are displayed in Table 2. Patients with hepatitis B (n=2) and AIH (n=2) were excluded from the analysis due to their small numbers. The median age of the LC patients was older than that of the non-LC patients, except for those with ALD and PSC.

The LC patients with NASH, ALD, PBC and PSC comprised the high-OCT subgroup, while those with hepatitis C formed the low-OCT subgroup. Interestingly, the OCT level that defined the high-OCT subgroup among the non-LC patients also identified the high-OCT subgroup among the LC patients, while the hepatitis C patients were classified into the low-OCT subgroup in both the LC and non-LC groups.

The differences in the OCT levels between the high-OCT subgroup (101.0 ng/mL) and the low-OCT subgroup (62.4 ng/mL) of the LC group were statistically significant (p=0.019). The OCT/AST ratios were greater in the high-OCT subgroup of the LC group than in the low-OCT subgroup (2.2 vs. 1.3, p=0.005), and the same trend was observed for the OCT/ALT ratio (2.9 vs. 1.7, p=0.034). Among the patients in the high-OCT subgroup, those with PSC had significantly higher OCT levels than those with NASH (p<0.001), ALD (p=0.006) or PBC (p=0.002). The OCT/AST and OCT/ALT ratios were also significantly higher among the patients with PSC than among those with NASH (p<0.001 and p<0.001, respectively), ALD (p=0.002 and p=0.008, respectively) or PBC (p<0.001 and p=0.013, respectively).

**Comparison between the non-LC and LC groups**

Patients with hepatitis B and AIH were also excluded.
from the analysis due to the small number of such patients in the LC group.

Fig. 2 displays the OCT levels, OCT/AST ratios and OCT/ALT ratios for the patients with and without LC who had hepatitis C, NASH, ALD, PBC and PSC. The difference in the OCT levels between the non-LC and LC groups was 20 ng/mL among the patients with hepatitis C, 21 ng/mL among the patients with NASH, 12 ng/mL among the patients with ALD, 29 ng/mL among the patients with PBC and 241 ng/mL among the patients with PSC, with the difference being significant for each disease, except ALD (p<0.001). The OCT/AST ratios were higher in the LC patients than in the non-LC patients with each type of chronic liver disease, except for ALD, with significant differences for NASH (p=0.017) and PSC (p=0.004). The OCT/ALT ratios were also significantly higher among the LC patients than among the non-LC patients with hepatitis C (p=0.011), NASH (p<0.001) and PSC (p=0.003).

Concerning the transaminase levels, the differences in the AST levels between the non-LC and LC groups were significant among the patients with PBC (p=0.001) and PSC (p<0.001), whereas the ALT levels were similar in the non-LC and LC groups, with the exception of the LC patients with PSC (p<0.001). Among the NASH patients, the AST/ALT ratios exhibited a significant difference between those with and without LC (p<0.001), although there were no significant differences among the patients with the other types of chronic liver disease.

**Markers for predicting LC**

The differences in the OCT levels and OCT/AST and OCT/ALT ratios between the non-LC and LC groups were significant among the patients with hepatitis C, NASH, PBC and PSC; therefore, a ROC analysis of the patients with

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Data are expressed as median values, and 25th and 75th percentile (Q25, Q75).

*p values correspond of the eight groups. Kruskal-Wallis test for continuous factors or Pearson’s chi-square for categorical variables were used. NS: not significant.

among these diseases was performed. The predictive value for LC of the OCT level, OCT/AST ratio, OCT/ALT ratio and AST/ALT ratio is shown in Fig. 3 and Table 3. Among these four indexes, the OCT/ALT ratio displayed the largest AUROC for predicting LC (0.75) in the patients with hepatitis C. When the cut-off value was set at 1.26, the OCT/ALT ratio had a sensitivity of 80.0% and a specificity of 60.0% for predicting LC. A similar pattern was observed in the patients with NASH, as the OCT/ALT ratio again had the largest AUROC for predicting LC (0.79). When the cut-off value was set at 1.51, the OCT/ALT ratio showed a sensitivity of 74.4% and a specificity of 75.0% for predicting LC. The OCT level had the largest AUROC (0.64) among the four indexes in the patients with PBC. In the patients with PSC, the OCT level also had the largest AUROC (0.91) for predicting LC among the four indexes. When the cut-off value was set at 96.5 ng/mL, the OCT level showed a sensitivity of 93.3% and a specificity of 68.0% for predicting LC.

Fig. 4 displays the results of the Spearman’s rank correlation analysis of the relationships between the Plt count and the OCT level, OCT/AST ratio and OCT/ALT ratio among all patients with chronic liver disease. There were no significant correlations between these variables. The Plt count and OCT level also showed no significant correlations with LC within each disease category.

Discussion

We investigated the OCT levels in patients with various liver diseases and found that the OCT levels of non-LC patients with steatohepatitis (NASH and ALD) and cholestatic hepatitis (PBC and PSC) were much higher than those of non-LC patients with hepatitis B, hepatitis C or AIH. The non-LC patients with high OCT levels also had higher OCT/AST and OCT/ALT ratios than the patients with low OCT levels. Similar results were obtained in the patients with LC. A comparison of the OCT levels between the patients with hepatitis C, NASH, PBC and PSC showed that those in the LC group had higher levels than those in the non-LC group, and the difference was marked for PSC patients.

Therefore, the OCT levels varied depending on the etiology of liver disease, although they exhibited a similar pattern in the patients with and without LC, and the liver diseases associated with high OCT levels were also associated with high OCT/AST and OCT/ALT ratios. These findings suggest that more severe mitochondrial injury may occur in patients with NASH, ALD, PBC and PSC than in those with hepatitis B/C and AIH throughout the course of disease, as reflected by the pattern of OCT release. Mitochondrial damage due to oxidative stress has been reported to play a very important role in the pathogenesis of ALD and NASH (14). Interestingly, the OCT levels of the ALD patients were extremely high both in the presence and absence of LC; thus, the OCT level may not be a useful biomarker of fibrosis. Among the liver disease groups, the patients in the LC
The OCT/ALT ratio is a well-known biomarker for predicting LC. However, the ROC analysis of the OCT level, OCT/AST ratio, OCT/ALT ratio and AST/ALT ratio in the hepatitis C, NASH, PBC and PSC patients did not identify the AST/ALT ratio to be best predictor of the presence of LC in any of these disease categories. Instead, the OCT/ALT ratio, OCT/ALT ratio and OCT level were found to be the best predictors of the presence of LC in patients with hepatitis C, NASH and PSC, respectively. The comparison between the OCT/AST, OCT/ALT and AST/ALT ratios between the non-LC and LC groups revealed that these three ratios were similar in both the low-OCT subgroups (patients with hepatitis B and hepatitis C). In the high-OCT subgroups (patients with NASH, ALD, PBC and PSC), however, the OCT/AST and OCT/ALT ratios were almost twice as high as the AST/ALT ratio. This finding suggests that the OCT/AST and OCT/ALT ratios were more sensitive markers of fibrosis in the high-OCT subgroup than in the low-OCT subgroup.

There were no significant correlations between the plt count and OCT level in any of the chronic liver disease categories, suggesting that the OCT level is possibly a clinically useful independent serum biomarker of LC. It remains unclear why the OCT levels were higher in the LC patients than in the non-LC patients. One possibility is that even if the degree of hepatocyte necrosis is diminished in LC patients, the hepatocytes in patients with cirrhosis suffer from mitochondrial damage due to remodeling of the hepatic architecture.

The primary limitation of this study is the small number of patients with each type of chronic liver disease, because this was a prospective study and we only assessed new serum samples.
It has been reported that the OCT level is influenced by age (19). However, there were no correlations between the OCT levels and age among any of the patient groups, including all patients with chronic liver disease and the patients in each chronic liver disease category (including the controls), in the present study.

In conclusion, in the current study, the OCT levels were much higher in the patients with NASH, ALD, PBC and PSC than in those with hepatitis B, hepatitis C and AIH. A similar pattern was also observed with respect to the OCT/AST and OCT/ALT ratios. The role of OCT in each type of liver disease should be evaluated further, which may improve our understanding of the pathogenesis of these diseases. Both the serum OCT level and OCT/ALT ratio may be useful surrogate markers of LC. In particular, among PSC patients, the OCT level is a useful biomarker for LC. However, the significant differences observed in the OCT levels between the different disease categories in this study may also be a disadvantage of this marker. The accumulation of more data regarding the OCT levels in various chronic liver diseases would be useful for making the differential diagnosis between these diseases. The OCT level can...
be measured using a simple, reliable and inexpensive test and is a highly liver-specific protein. Accordingly, it may be a useful marker in general practice. However, large-scale studies are needed to confirm the value of the serum OCT level as a marker of LC in patients with various liver diseases.

The authors state that they have no Conflict of Interest (COI).

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