**Table 2** Characteristics of the subjects

| | Case | Control | *P*-value |
|---|---|---|---|
| *n* | 223 | 669 | |
| Sex (M/F) | 169/54 | 507/162 | Matched |
| Age | 68.0 ± 9.4 | 68.0 ± 9.4 | Matched |
| BMI (kg/m$^2$) | 23.6 ± 3.7 | 22.9 ± 3.2 | 0.2188 |
| Daily alcohol intake (none/<60 g/60–100 g/>100 g) | 61/82/34/46 | 194/433/35/7 | <0.0001 |
| Brinkman index | 544.5 ± 639.3 | 140.2 ± 276.8 | <0.0001 |
| Comorbidity | | | |
| Fatty liver (yes/no) | 30/193 | 150/519 | 0.0038 |
| Hypertension (yes/no) | 40/183 | 129/540 | 0.6940 |
| DM (yes/no) | 87/136 | 49/620 | <0.0001 |
| Hemoglobin (g/dL) | 12.6 ± 2.1 | 14.4 ± 1.5 | <0.0001 |
| Platelet (× 10$^4$/mm$^3$) | 16.5 ± 9.8 | 23.7 ± 5.3 | <0.0001 |
| AST (IU/L) | 57.0 ± 55.9 | 23.1 ± 11.9 | <0.0001 |
| ALT(IU/L) | 50.8 ± 50.3 | 24.5 ± 19.1 | <0.0001 |
| GGT (IU/L) | 251.1 ± 307.8 | 43.8 ± 57.1 | <0.0001 |
| Albumin (g/dL) | 3.55 ± 0.57 | 4.48 ± 0.25 | <0.0001 |
| Total bilirubin (mg/dL) | 1.64 ± 2.65 | 0.89 ± 0.36 | <0.0001 |
| Total cholesterol (mg/dL) | 169.1 ± 46.1 | 206.3 ± 34.2 | <0.0001 |
| Triglyceride (mg/dL) | 108.2 ± 58.4 | 117.7 ± 89.6 | 0.3179 |
| Fasting blood glucose (mg/dL) | 131.2 ± 60.0 | 101.4 ± 18.9 | <0.0001 |
| HbA1c (%) | 6.1 ± 1.4 | 5.3 ± 0.7 | <0.0001 |
| APRI | 1.405 ± 1.472 | 0.313 ± 0.248 | <0.0001 |
| Use of antidiabetic agents (yes/no) | 68/155 | 17/652 | <0.0001 |

Descriptive statistics are expressed as the mean ± standard deviation or the number of patients. Differences between the two groups were analyzed using the Mann–Whitney *U*-test. $P < 0.05$ was considered statistical significant.
ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; DM, diabetes mellitus; GGT, γ-glutamyltransferase; HbA1c, hemoglobin A1c.

level was less than 4.01 g/dL, 82.5% (184/223) of the subjects had NBNC-HCC (Fig. 2).

Among the subjects with an albumin level of 4.01 g/dL or more, the APRI was selected as the variable for the second division with an optimal cut-off of 0.5. In addition, among the subjects with APRI of 0.5 or more, the Brinkman index was selected as the variable for third division with an optimal cut-off of 400 (Fig. 2). Thus, 2.3% (13/574) of subjects had NBNC-HCC when the subjects met the following criteria: albumin level of 4.01 g/dL or more and APRI of less than 0.5 (group 1 in Fig. 2). In contrast, 85.0% (17/20) of the subjects had

**Table 3** Multivariate stepwise analysis for the incidence of NBNC-HCC

| Variables | Unit | Odds ratio | 95% confidence interval | | | *P* value |
|---|---|---|---|---|---|---|
| APRI | 0.1 | 1.07 | 0.98 | to | 1.16 | 0.1283 |
| HbA1c | 0.1 | 1.03 | 0.99 | to | 1.07 | 0.1270 |
| Platelet | 1 | 0.95 | 0.89 | to | 1.01 | 0.0996 |
| GGT | 10 | 1.15 | 1.08 | to | 1.21 | <0.0001 |
| Brinkman index | 100 | 1.17 | 1.05 | to | 1.30 | 0.0047 |
| Use of antidiabetic agents | 1 | 7.42 | 2.42 | to | 22.76 | 0.0005 |
| Total cholesterol | 10 | 0.88 | 0.79 | to | 0.98 | 0.0155 |
| Hemoglobin | 0.1 | 0.95 | 0.93 | to | 0.97 | <0.0001 |
| Albumin | 0.1 | 0.67 | 0.60 | to | 0.76 | <0.0001 |

APRI, aspartate aminotransferase-to-platelet ratio index; GGT, γ-glutamyltransferase; HbA1c, hemoglobin A1c.

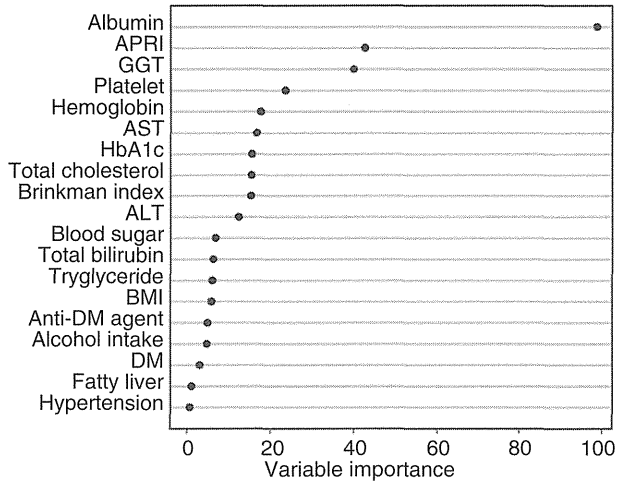


**Figure 1** Random forest analysis for distinguishing between the case and control groups. Variable importance is a general measure of the contribution of each variable in distinguishing the classes. ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; DM, diabetes mellitus; GGT, γ-glutamyltransferase; HbA1c, hemoglobin A1c.

NBNC-HCC when they met the following criteria: albumin level of 4.01 g/dL or more, APRI of 0.5 or more and Brinkman index of 400 or more (group 3 in Fig. 2).

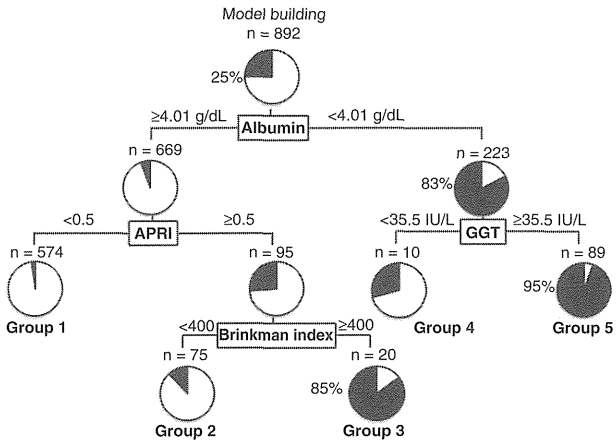


**Figure 2** Decision-tree algorithm of NBNC-HCC predictive factors. The subjects were classified according to the indicated cut-off values of the variables. The pie graphs indicate the percentage of ordinary people (white)/NBNC-HCC patients (black) in each group. APRI, aspartate aminotransferase-to-platelet ratio index; GGT, γ-glutamyltransferase; NBNC-HCC, non-B, non-C hepatitis virus-related hepatocellular carcinoma.

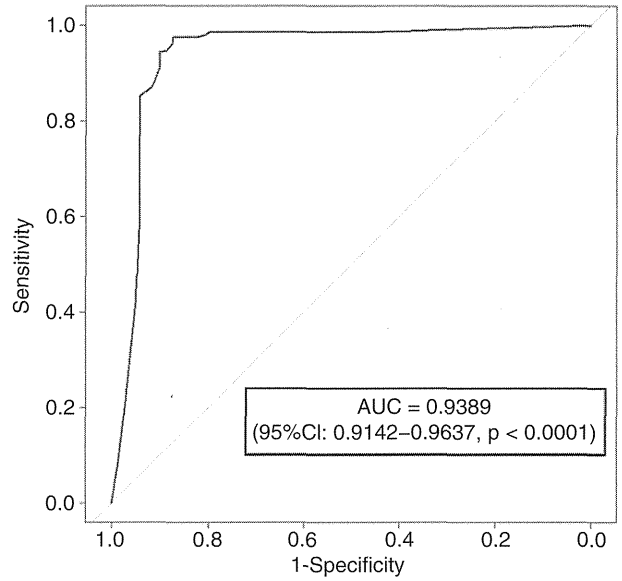


**Figure 3** Predictive accuracy of the decision-tree model by area under the receiver–operator curve (AUC) using a 10-fold cross-validation. The black line indicates the decision-tree model. The gray line indicates the reference. CI, confidence interval.

In subjects with an albumin level less than 4.01 g/dL, however, the GGT level was selected as the variable for the second division with an optimal cut-off of 35.5 IU/L. We observed that 94.5% (172/182) of the subjects had NBNC-HCC when they met the following criteria: albumin level of less than 4.01 g/dL and GGT level of 35.5 IU/L or more (group 5 in Fig. 2). The predictive accuracy of the decision-tree model was validated by AUROC using a 10-fold cross-validation. The AUROC was 0.9389 (95% CI = 0.9142–0.9637, $P < 0.0001$) (Fig. 3).

## DISCUSSION

IN THIS STUDY, we identified independent factors associated with the incidence of NBNC-HCC, including serum levels of albumin and GGT, APRI and the Brinkman index. Our data-mining using random forest analysis and a decision-tree algorithm demonstrated that serum albumin level is the most significant variable associated with the incidence of NBNC-HCC. These findings suggest that profiles consisting of albumin and GGT levels, APRI or the Brinkman index may be useful as screening tools for NBNC-HCC.

Previous studies have shown that obesity, fatty liver and diabetes mellitus are independent factors associated

with NBNC-HCC.[30,31] Our analysis makes the important new observation that serum albumin level is highly predictive of HCC among patients at risk for NBNC-HCC. Although the reason for the high predictivity of albumin for NBNC-HCC is unclear, there are some possible explanations. In the majority of previous studies, control data were obtained from patients with other diseases.[32,33] However, in this study, control data were obtained from a health examination database; thus, the nutritional status of this control group could be better than that of the controls in the other studies, which might have accentuated the difference in serum albumin levels. Another explanation could be the difference in statistical methods used. In this study, we employed a data-mining technique, which is suitable for investigating complex interactions of risk factors with no a priori hypothesis. Although we used three different statistical methods, namely, a random forest analysis, multivariate stepwise analysis and a decision-tree algorithm, the serum albumin level was an independent factor associated with the incidence of NBNC-HCC in all of these analyses. Thus, to our knowledge, our study is the first to show that serum albumin level is predictive of the incidence of NBNC-HCC.

In general, NBNC-HCC is diagnosed at advanced stages. It could be surmised that changes in serum albumin level may be linked to the progression of NBNC-HCC. However, in this study, no significant difference was seen in serum albumin levels among the tumor stages of NBNC-HCC (data not shown). The causal relationship between serum albumin levels and the development of NBNC-HCC remains unclear. However, an increased synthesis of reactive oxygen species is a relevant cause of cancer.[34] It has been established recently that albumin provides the first line of defense against reactive oxygen species in plasma.[35,36] Previous studies have shown that serum albumin levels predict the recurrence of colorectal cancer after elective colorectal resection.[37] Branched-chain amino acids, which increase serum albumin levels, also have been reported to suppress hepatocarcinogenesis.[38,39] Thus, changes in serum albumin level could be involved in the development of HCC.

A variety of factors are involved in hepatocarcinogenesis.[3,32,40]However, interactions between risk factors remain unclear. Therefore, a decision-tree algorithm was created and revealed that the following two profiles are associated with a high incidence of NBNC-HCC: (i) albumin level of less than 4.01 g/dL and GGT level of 35.5 or more; and (ii) albumin level of 4.01 g/dL or more, APRI of 0.5 or more and Brinkman index of 400 or more. The GGT level and APRI have been reported as independent risk factors for hepatocarcinogenesis in NBNC-HCC.[32,41] Using a decision-tree algorithm, we also showed that the GGT level and APRI were the second most significant risk factors after albumin levels. Although smoking is a significant risk factor for the development of several cancers including lung cancer, the impact of smoking on hepatocarcinogenesis remains controversial.[4,42,43] In this study, the decision-tree algorithm identified a Brinkman index of 400 or more as the third most significant factor in patients with an albumin level of 4.01 g/dL or more and APRI of 0.5 or more. Thus, smoking may exert hepatocarcinogenic activity under specific conditions.

Given the role of changes in serum albumin level and relative thrombocytopenia in identifying patients with NBNC-HCC, it may be that the basis of the high predictivity of albumin and APRI for NBNC-HCC is on the basis of consistently identifying patients with cirrhosis, and thus risk for HCC, in this population.

A limitation of this study is that we did not evaluate the impact of occult HBV infection on the incidence of NBNC-HCC because HBV DNA was not tested in the health screening examination. HBV is one of the most transmitted infectious diseases and a cryptogenic cause of HCC;[44] therefore, further studies will be focused on its effect on hepatocarcinogenesis. Another limitation is that we did not evaluate the serum amino acid level, because control data were obtained from a health screening database. Changes in amino acid levels, particularly changes in branched-chain amino acids to tyrosine ratio, are known to precede a reduction in serum albumin level.[45] Thus, the impact of amino acid imbalance on the incidence of NBNC-HCC is an issue which needs to be clarified.

In conclusion, this study has identified independent factors associated with the incidence of NBNC-HCC, such as the Brinkman index, use of antidiabetic agents, hemoglobin level, and serum levels of GGT, total cholesterol and albumin. In addition, random forest analysis showed that serum albumin levels were the most distinguishable factor, and a decision-tree algorithm revealed that it is the initial split variable for the incidence of NBNC-HCC. Thus, data-mining analyses revealed that serum albumin levels were a notable factor associated with the incidence of NBNC-HCC. Furthermore, we obtained a profile associated with the incidence of NBNC-HCC, which consists of albumin and GGT levels, APRI and the Brinkman index. This simple profile could be used in a possible screening strategy for NBNC-HCC.

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VIRAL HEPATITIS

# Independent factors associated with altered plasma active ghrelin levels in HCV-infected patients

Takumi Kawaguchi[1,2], Yumiko Nagao[1] and Michio Sata[1,2]

1 Department of Digestive Disease Information & Research, Kurume University School of Medicine, Kurume, Japan
2 Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Kurume, Japan

**Keywords**
albumin – ghrelin – gut hormone – hepatitis C virus – liver cirrhosis

**Correspondence**
Takumi Kawaguchi, M.D., Ph.D.,
Department of Digestive Disease Information & Research
Kurume University School of Medicine
67 Asahi-machi, Kurume 830-0011, Japan
Tel: +81 942 31 7902
Fax: +81 942 31 7820
e-mail: takumi@med.kurume-u.ac.jp

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## Abstract

*Background & Aims:* Metabolic disorders are frequently seen in hepatitis C virus (HCV)-infected patients. Ghrelin, a gut hormone, regulates hepatic metabolisms, and must be activated to exert its biological effects. The aims of this study were to investigate changes in plasma active ghrelin levels and identify independent factors associated with plasma active ghrelin levels in HCV-infected patients. *Methods:* We enrolled patients with HCV infection ($n$ = 96), hepatitis B virus (HBV) infection ($n$ = 49), non-alcoholic fatty liver disease (NAFLD; $n$ = 20) and healthy subjects (CON; $n$ = 16). Plasma active ghrelin levels were measured using ELISA. Factors associated with plasma active ghrelin levels were assessed by multivariate and Spearman's correlation analyses. *Results:* Plasma active ghrelin levels were significantly lower in relation to the severity of liver disease in both the HBV and HCV groups. Furthermore, HCV infection was identified as an independent factor associated with decreased plasma active ghrelin levels in the multivariate analysis (OR −3.05; 95% CI −0.93 to −19.51; $P$ = 0.0192). Plasma active ghrelin levels were significantly correlated with serum albumin levels in the HCV group ($\rho$ = 0.497, $P < 0.0001$). *Conclusions:* We demonstrated that liver cirrhosis and HCV infection were independent factors associated plasma active ghrelin levels. Moreover, plasma active ghrelin levels were positively correlated with serum albumin levels among HCV-infected patients. Therefore, active ghrelin levels may be regulated by both progression of liver disease and HCV infection and could be involved in the regulation of serum albumin levels in HCV-infected patients.

Various metabolic disorders are more frequently seen in patients with chronic hepatitis C virus (HCV) infection than in those with other hepatobiliary diseases (1, 2). Metabolic disorders including insulin resistance and hypoalbuminemia are risk factors for the development of hepatocellular carcinoma and oesophageal varices, and thus, for increased mortality (3, 4). These metabolic disorders are seen even in early stage chronic liver disease (1, 5) and are partly caused by HCV-induced metabolic dysregulation in multiple organs including the liver, pancreas and intestine (6–8).

Ghrelin, a gut hormone, predominantly secreted from X/A-like stomach cells, was originally identified as a potent growth hormone secretagogue (9). Ghrelin also regulates appetite and its downregulation is involved in the development of malnutrition (10–12). Basic studies have demonstrated that ghrelin plays a significant role in glucose metabolism, fatty acid beta oxidation, hepatic fibrosis and cell proliferation (13–17). Therefore, changes in ghrelin could be associated with various metabolic disorders in HCV-infected patients.

Ghrelin is secreted as a preprohormone; therefore, it must be activated to exert its biological effects, which are tightly regulated by a unique mechanism. Preproghrelin is acylated by ghrelin O-acyltransferase in the serine-3 residue. The acylated peptide is then cleaved by a processing protease prohormone convertase 1/3 to produce active ghrelin (18, 19). Thus, ghrelin is classified into 2 isoforms: des-acyl ghrelin (the inactive form) and acylated ghrelin, so-called "active ghrelin." In healthy adults, approximately 90% of serum ghrelin exists in the inactive form and 10% exists in the active form (20). Although several previous studies have shown inconsistent results regarding serum total ghrelin levels among patients with chronic liver diseases (12, 21–24), changes in plasma active ghrelin level and their clinical association with metabolic disorders have not yet been investigated in HCV-infected patients.

The aims of this study were to investigate changes in plasma active ghrelin levels and identify independent factors associated with plasma active ghrelin levels in HCV-infected patients.

## Subjects and methods

### Ethics statement

The study protocol was approved by The Ethics Committee of Kurume University. All experiments were carried out in accordance with the Declaration of Helsinki. No subjects were institutionalized.

### Materials

All reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan) unless otherwise indicated.

### Patients

We enrolled consecutive patients with HCV-related liver disease (HCV; $n = 96$), hepatitis B virus (HBV)-related liver disease (HBV; $n = 20$), non-alcoholic fatty liver disease (NAFLD; $n = 49$) and healthy control subjects (CON; $n = 16$). All patients and controls were Asian. All diagnoses were based on clinical, serological and/or histological evidence. All data were collected on the same day as collecting blood. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in metres ($kg/m^2$).

### Laboratory tests

Venous blood samples were taken in the morning after a 12-h overnight fast. Plasma glucose, serum aspartate aminotransferase (AST), alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, gamma-glutamyl transpeptidase, total protein, albumin, prothrombin activity, blood glucose, immunoreactive insulin (IRI), haemoglobin A1c, total cholesterol, free fatty acids, total bilirubin, type IV collagen, hyaluronic acid, blood urea nitrogen, creatinine, alpha-foetoprotein and protein induced by vitamin K absence or antagonist-II levels were measured using standard clinical methods (Department of Clinical Laboratory, Kurume University Hospital) as previously described (25). Insulin resistance was calculated on the basis of fasting levels of plasma glucose and IRI using the homoeostasis model assessment for insulin resistance (HOMA-IR) equation: fasting glucose (mg/dl) × fasting IRI (mU/ml)/405 (26).

### Determination of HCV genotype and measurement of HCV viral load

HCV genotyping was performed using Okamoto's method (27), and genotypes were classified according to Simmonds' classification system (28). An Amplicor-HCV-Monitor 1.0 (Roche Diagnostics K.K., Tokyo, Japan) was used to quantify HCV RNA levels.

### Diagnosis of cirrhosis

Cirrhosis was diagnosed by liver histology or AST to platelet ratio index (APRI): serum AST level (U/L)/upper limit of normal AST (33 U/L) × 100/platelet count ($\times 10^4$/ml). APRI is a non-invasive index and can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Patients with APRI values above 1.5 were diagnosed with cirrhosis as previously described (29). In this study, out of 96 patients in the HCV group, 18 patients were diagnosed by liver histology and the remaining 78 patients were diagnosed by APRI.

### Plasma active ghrelin level assay

For measurement of plasma active ghrelin levels, 1 ml of blood was collected in a tube containing 1.25 mg of ethylenediaminetetraacetic acid and 500 KIU of aprotinin, a serine protease inhibitor. Each sample was immediately centrifuged at 1500*g* for 15 min at 4°C and treated with 10% vol/vol of 1 mM hydrochloric acid. Quantification of active ghrelin in plasma samples was accomplished with an Active Ghrelin ELISA Kit (SCETI K.K., Tokyo, Japan) that specifically quantifies active ghrelin. This ELISA kit consists of monoclonal antibodies for both the C-terminal and the acylated N-terminal of ghrelin. The absorbance (450 nm) of each well was then measured with a Bio-Rad Model 550 microplate reader (Bio-Rad, Hercules, CA, USA). Each serum sample was assayed in duplicate and the values were averaged.

### Statistical analysis

All data are expressed as the mean ± standard deviation (SD). Statistical comparisons among multiple groups were performed by analysis of variance (ANOVA) followed by Scheffé's *post-hoc* test. Stepwise forward-selection multivariate linear regression analysis was used to identify any independent variables related to plasma active ghrelin levels, as previously described (30, 31). Spearman's correlation coefficient was calculated to test the relationship between different quantities in a bivariate regression model using JMP version 9.0 (SAS Institute, Cary, NC, USA). $P$ values $< 0.05$ were considered significant.

## Results

### Patient characteristics

The characteristics of enrolled patients are summarized in Table 1. Although serum levels of AST and ALT were significantly higher in the NALFD, HBV and HCV groups than those in the CON group, these levels were not significantly different among the NAFLD, HBV and HCV groups. Serum albumin levels were significantly lower in the HBV and HCV groups than in the CON and NAFLD groups. Serum total cholesterol levels revealed significant depletion in the HCV group compared with the other groups. Significantly elevated

**Table 1.** Patient characteristics

| | Reference value | CON | NAFLD | HBV | HCV |
|---|---|---|---|---|---|
| *n* | 18.5–22 | 16 | 20 | 49 | 96 |
| Age (yr) | N/A | 67.5 ± 5.3 | 62.6 ± 10.8 | 57.3 ± 3.1* | 63.5 ± 12.5 |
| Sex (Female/Male) | N/A | 7/9 | 12/8 | 29/20 | 58/38 |
| BMI ($m^2$/kg) | 18.5–22 | 22.3 ± 0.9 | 26.8 ± 2.2* | 22.3 ± 2.8 | 23.5 ± 3.5 |
| AST (U/L) | 13–33 | 10.6 ± 3.4 | 35.5 ± 22.6† | 31.5 ± 23.8† | 47.9 ± 28.5† |
| ALT (U/L) | 8–42 | 12.7 ± 5.1 | 41.2 ± 24.4† | 35.9 ± 27.8† | 45.2 ± 34.6† |
| LDH (U/L) | 119–229 | 192 ± 51 | 201 ± 35 | 201 ± 52 | 210 ± 42 |
| ALP (U/L) | 115–359 | 242 ± 53 | 262 ± 106 | 261 ± 125 | 281 ± 119 |
| GGT (U/L) | 10–47 | 31.5 ± 17.2 | 51.6 ± 53.4† | 42.2 ± 28.6† | 53.2 ± 68.1† |
| Total protein (g/dl) | 6.70–8.30 | 8.1 ± 0.5 | 7.7 ± 0.3 | 7.3 ± 0.5† | 7.1 ± 0.6† |
| Albumin (g/dl) | 4.00–5.00 | 4.3 ± 0.3 | 4.3 ± 0.5 | 3.8 ± 0.7‡ | 3.7 ± 0.6‡ |
| Prothrombin activity (%) | 60–130 | 105.2 ± 13.3 | 108.7 ± 25.0 | 94.0 ± 8.8‡ | 91.6 ± 19.3* |
| Total bilirubin (mg/dl) | 0.30–1.50 | 0.6 ± 0.2 | 0.9 ± 0.5 | 0.8 ± 0.7 | 1.1 ± 0.5† |
| C-reactive protein (mg/dl) | <0.40 | 0.02 ± 0.02 | 0.03 ± 0.02 | 0.02 ± 0.01 | 0.02 ± 0.01 |
| Total cholesterol (mg/dl) | 128–220 | 197 ± 41 | 251 ± 66* | 199 ± 42 | 163 ± 42* |
| Free fatty acids (μEq/L) | 100–540 | 286 ± 75 | 383 ± 186† | 375 ± 131† | 665 ± 362* |
| Fasting blood glucose (mg/dl) | 80–109 | 96 ± 5 | 121 ± 35† | 103 ± 12† | 109 ± 31† |
| Haemoglobin A1c (%) | 4.3–5.8 | 5.3 ± 0.4 | 6.0 ± 0.7* | 5.6 ± 0.4 | 5.3 ± 0.9 |
| IRI (μIU/ml) | 5.0–20.0 | 6.1 ± 2.3 | 9.5 ± 6.5§ | 7.5 ± 4.4 | 14.3 ± 8.5§ |
| HOMA-IR | <2.5 | 1.3 ± 0.4 | 2.6 ± 1.6§ | 2.0 ± 1.1 | 3.8 ± 1.5§ |
| BUN (mg/dl) | 8.0–22.0 | 14.5 ± 7.1 | 14.2 ± 4.3 | 17.2 ± 2.8 | 15.8 ± 4.3 |
| Creatinine (mg/dl) | 0.4–0.7 | 0.6 ± 0.3 | 0.6 ± 0.2 | 0.7 ± 0.3 | 0.6 ± 0.2 |
| Hyaluronic acid (ng/ml) | < 50 | N/A | 31.6 ± 12.5 | 98.5 ± 72.5‖ | 182.8 ± 155.2* |
| Type IV collagen (ng/ml) | < 140 | N/A | 88 ± 68 | 198 ± 121‖ | 252 ± 146* |
| AFP (ng/ml) | < 8.7 | N/A | 3.4 ± 1.2 | 7.8 ± 2.1‖ | 14.8 ± 36.2* |
| PIVKA-II (mAU/ml) | <40 | N/A | 18.5 ± 12.2 | 21.5 ± 16.2 | 28.5 ± 18.8 |

Data are expressed as mean ± SD or number of patients.

*$P < 0.05$ compared to all of other groups.

†$P < 0.05$ compared to the CON group.

‡$P < 0.05$ compared to the CON and NAFLD groups.

§$P < 0.05$ compared to the CON and HBV groups.

‖$P < 0.05$ compared to the HBV group.

CON, healthy control subjects; NAFLD, non-alcoholic fatty liver disease; HBV, hepatitis B virus; HCV, hepatitis C virus; N/A, not applicable; AST, aspartate transaminase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase; IRI, Immunoreactive insulin; HOMA-IR, homoeostasis model assessment for insulin resistance; BUN, blood urea nitrogen; AFP, α-foetoprotein; PIVKA-II, protein induced by vitamin K absence.

serum insulin and HOMA-IR levels were observed in the HCV group compared with those in the CON and HBV groups. Serum levels of hyaluronic acid and type IV collagen were significantly higher in the HCV group than in the other groups.

### Changes in plasma active ghrelin levels in chronic liver disease

There were no significant differences in plasma active ghrelin levels among the CON, NAFLD and HBV groups. However, plasma active ghrelin levels were significantly lower in the HCV group than in the other groups (Fig. 1).

### Changes in plasma active ghrelin levels in relation to the progression of liver disease

In the HBV groups, plasma active ghrelin levels were significantly decreased in relation to the severity of liver disease (compare with dotted lines in Fig. 2). Similarly, significant decreases in plasma active ghrelin levels in relation to severity of liver disease were seen in the HCV group (compare with dotted lines in Fig. 2).

### The association of plasma active ghrelin levels with virological factors and disease severity in HCV-infected patients

No significant differences in plasma active ghrelin levels were seen among HCV genotypes 1b, 2a and 2b. There was no significant correlation between plasma active ghrelin levels and HCV viral load ($\rho = 0.589$, $P < 0.396$).

### Stage-matched comparisons of plasma active ghrelin levels

In patients with chronic hepatitis, plasma active ghrelin levels were significantly lower in the HCV group than in the HBV or NAFLD group (compare with solid lines in

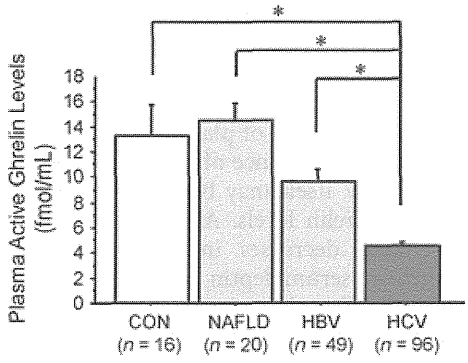


**Fig. 1.** Changes in plasma active ghrelin levels in the CON, NAFLD, HBV and HCV groups. Values are expressed as mean ± SD. Comparisons among groups were made using analysis of variance with Scheffé's *post-hoc* test. *, $P < 0.01$.

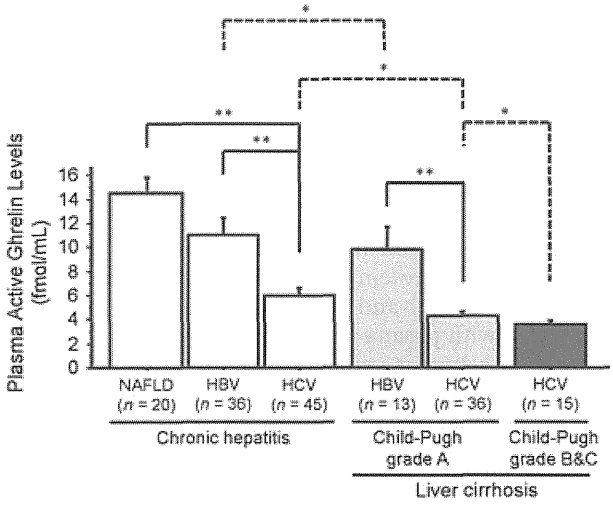


**Fig. 2.** Changes in plasma active ghrelin levels stratified by aetiology and progression of liver disease. Values are expressed as mean ± SD. Comparisons among the groups were made using analysis of variance with Scheffé's *post-hoc* test. *, $P < 0.05$; **, $P < 0.01$.

Fig. 2). In cirrhotic patients with Child-Pugh grade A, a significant decrease in plasma active ghrelin levels was also seen in the HCV group compared with the HBV group (compare with solid lines in Fig. 2).

### Multivariate analysis of plasma active ghrelin levels

In the multivariate analysis, the presence of cirrhosis and low serum albumin levels were independent factors associated with decreased plasma active ghrelin levels (Table 2). In addition, the presence of HCV infection was identified as the most significant independent factor associated with lowering of plasma active ghrelin levels (Table 2).

**Table 2.** Multivariate analysis of plasma active ghrelin levels

| Variable | Odds ratio | 95% confidence interval | *P* value |
|---|---|---|---|
| Liver cirrhosis | −1.82 | −0.37 to −4.33 | 0.0359 |
| Serum albumin levels | 2.39 | 1.32 to 7.95 | 0.0251 |
| HCV infection | −3.05 | −0.93 to −19.51 | 0.0192 |

HCV, hepatitis C virus.

### Correlations between plasma active ghrelin levels and metabolic parameters in HCV-infected patients

A significant negative correlation was observed between plasma active ghrelin levels and BMI ($\rho = -0.137$, $P = 0.0171$) (Table 3). Although plasma active ghrelin levels were not significantly correlated with the parameters indicated in Table 3, plasma active ghrelin levels tend to be correlated with total cholesterol, total bilirubin, type IV collagen and creatinine levels. A significant positive correlation was seen only between plasma active ghrelin levels and serum albumin levels ($\rho = 0.497$, $P < 0.0001$) (Table 3 and Fig. 3).

## Discussion

In this study, we demonstrated that plasma active ghrelin levels were significantly decreased in relation to the progression of liver disease in both HBV- and HCV-infected patients. In addition, HCV infection was identified as the most significant factor associated with decreased plasma active ghrelin levels, independent of the presence of cirrhosis and serum albumin levels. In HCV-infected patients, plasma active ghrelin levels were positively correlated with serum albumin levels. Taken together, these findings suggest that HCV itself, along with cirrhosis, may be involved in decreasing plasma active ghrelin levels, which could be linked to serum albumin levels.

In this study, plasma active ghrelin levels were significantly lower in the HCV group than in the other groups. To assess the effect of advanced liver disease on the plasma ghrelin levels, we performed stratified analyses based on the severity of liver disease. The results indicated that plasma active ghrelin levels significantly decreased in relation to the severity of liver disease in the HBV and HCV groups. The presence of cirrhosis was also identified as an independent factor associated with plasma active ghrelin levels in multivariate analysis. Although changes in total ghrelin levels in cirrhosis remain a controversial subject (12, 21, 24), we have demonstrated that active ghrelin was decreased in patients with cirrhosis. The causal relationship between advanced cirrhosis and changes in active ghrelin levels remains unclear. Plasma active ghrelin levels are regulated by production of preproghrelin or activation of ghrelin (11, 32, 33). Therefore, it is likely that chronic liver injury may change X/A-like stomach cells and

**Table 3.** Correlations between plasma active ghrelin levels and variables

| Variables | ρ | *P* |
|---|---|---|
| Age | 0.048 | 0.6972 |
| BMI | −0.137 | 0.0171 |
| AST | −0.068 | 0.5083 |
| ALT | 0.021 | 0.8371 |
| LDH | 0.093 | 0.3660 |
| ALP | −0.139 | 0.1788 |
| GGT | 0.142 | 0.1674 |
| Total protein | −0.049 | 0.6323 |
| Albumin | 0.497 | <0.0001 |
| Prothrombin activity | 0.163 | 0.1621 |
| Total bilirubin | −0.210 | 0.0533 |
| Total cholesterol | 0.194 | 0.0595 |
| Free fatty acids | −0.190 | 0.4469 |
| Fasting blood glucose | −0.142 | 0.1682 |
| Haemoglobin A1c | 0.162 | 0.1201 |
| IRI | 0.160 | 0.2591 |
| HOMA-IR | 0.120 | 0.3968 |
| BUN | 0.077 | 0.4508 |
| Creatinine | 0.182 | 0.0766 |
| Type IV collagen | 0.378 | 0.0639 |
| Hyaluronic acid | 0.273 | 0.2910 |
| AFP | 0.106 | 0.3022 |
| PIVKA-II | 0.044 | 0.6835 |

BMI, body mass index; AST, aspartate transaminase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase; IRI, Immunoreactive insulin; HOMA-IR, homoeostasis model assessment for insulin resistance; BUN, blood urea nitrogen; AFP, α-foetoprotein; PIVKA-II, protein induced by vitamin K absence.

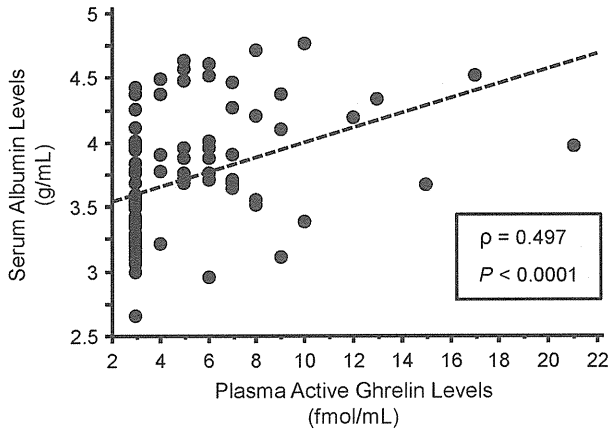


**Fig. 3.** The association between plasma active ghrelin levels and serum albumin levels. This association was analysed using the Spearman's correlation coefficient in a bivariate regression model.

ghrelin-O-acyl transferase and/or prohormone convertase 1/3.

We also examined the effect of HCV infection on plasma ghrelin levels. HCV genotype and HCV viral load were not associated with plasma active ghrelin levels, however; plasma active ghrelin levels were significantly lower in the HCV group than in the NAFLD or HBV group in stage-matched analyses. Moreover, HCV infection was identified as the most significant factor associated with lowering of plasma active ghrelin levels, independent of the presence of cirrhosis. These findings suggest that HCV itself may be involved in decreasing plasma active ghrelin levels. Although the mechanisms of HCV-related decreases in plasma ghrelin levels remain unclear, serum leptin levels are known to be high in HCV-infected patients (34, 35). Given that leptin is a potent inhibitor of ghrelin secretion (36, 37), HCV may suppress ghrelin secretion through up-regulation of leptin.

Various metabolic disorders are frequently seen in cirrhotic patients with HCV infection (38, 39). Therefore, we investigated the correlation between plasma active ghrelin levels and various metabolic parameters in the HCV group. Consistent with the previous reports (40, 41), in this study, plasma active ghrelin levels were found to be negatively correlated with BMI. These changes in plasma active ghrelin levels may represent a physiological adaptation to the positive energy balance associated with obesity. Furthermore, albumin was the only serum parameter, which significantly positively correlated with plasma active ghrelin levels in this study. Serum albumin is a representative marker for both protein metabolism and the severity of liver disease (42); however, other protein metabolism parameters such as total protein level and prothrombin activity were not correlated with plasma active ghrelin levels in this study. Ghrelin is a potent stimulator for secretion of growth hormone (33) and also elicits marked up-regulation of the mammalian target of rapamycin (mTOR) signalling pathway (43). Both growth hormone and activation of mTOR signalling up-regulate albumin synthesis (39, 44). Taken together, these findings suggest that active ghrelin has the novel biological effect on specifically regulating albumin synthesis in hepatocytes.

One limitation of this study is that we did not investigate changes in HCV or HBV carriers with persistently normal ALT levels. As these data may further elucidate the mechanisms underlying the decrease in plasma active ghrelin in patients with liver disease, further study should be conducted while bearing these points in mind.

In conclusion, this study showed that plasma active ghrelin levels were significantly decreased with the progression of liver disease in both HBV- and HCV-infected patients. Moreover, HCV infection was identified as the most significant factor associated with decreased plasma active ghrelin levels, independent of cirrhosis. Furthermore, plasma active ghrelin levels were positively correlated with serum albumin levels in HCV-infected patients. These findings indicated that active ghrelin may be regulated by both the progression of liver disease and HCV infection, and could be related to serum albumin levels.


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***Short Communication***

# Lipid profile is associated with the incidence of cognitive dysfunction in viral cirrhotic patients: A data-mining analysis

Eitaro Taniguchi,[1] Takumi Kawaguchi,[1,2] Masahiro Sakata,[1] Minoru Itou,[1] Tetsuharu Oriishi[1] and Michio Sata[1,2]

[1]*Division of Gastroenterology, Department of Medicine, and* [2]*Department of Digestive Disease Information and Research, Kurume University School of Medicine, Kurume, Japan*

*Aim:* Cognitive dysfunction (CD) is frequently observed in cirrhotic patients. However, the biochemical profiles associated with CD remain unclear. We investigated the biochemical profiles associated with the incidence of CD in cirrhotic patients by using multivariate analyses, including a decision-tree algorithm.

*Methods:* In this study, 27 viral cirrhotic patients were enrolled. All subjects underwent neuropsychiatric tests; two or more abnormal results were defined as CD. A logistic regression model was used for multivariate stepwise analysis. A decision-tree algorithm was constructed, and the categorical differences based on the decision-tree model were analyzed by $\chi^2$-tests.

*Results:* Multivariate stepwise analysis showed the levels of total bilirubin, triglycerides and free fatty acids (FFA) as independent bioparameters associated with the incidence of CD in cirrhotic patients. The decision-tree algorithm showed that among patients with FFA of 514 mEq/L or more, 77.8% had CD. Meanwhile, among patients with FFA of less than 514 mEq/L and triglycerides of 106 mg/dL or more, 20.0% had CD. The sensitivity, specificity and accuracy for the incidence of CD using the lipid profile (FFA >514 mEq/L or triglycerides <106 mg/dL) were 85.7% (12/14), 61.5% (8/13) and 74.1% (20/27), respectively.

*Conclusion:* The levels of total bilirubin, FFA and triglycerides are independently associated with the incidence of CD in cirrhotic patients. In addition, a decision-tree algorithm revealed that FFA of more than 514 mEq/L or triglycerides of less than 106 mg/dL is a profile associated with the incidence of CD. Thus, this lipid profile could be a possible screening bioparameter for CD in cirrhotic patients.

**Key words:** decision-tree algorithm, fatty acid, minimal hepatic encephalopathy, neuropsychiatric test

## INTRODUCTION

CIRRHOSIS IS FREQUENTLY accompanied by various complications, including esophageal varices and hepatocellular carcinoma.[1,2] Cognitive dysfunction (CD) is another frequent complication in patients with chronic liver disease and is known as minimal hepatic encephalopathy or subclinical hepatic encephalopathy.[3,4] CD predicts the development of hepatic encephalopathy and poor prognosis.[5,6] Moreover, CD itself is associated with impaired health-related quality of life[7–9] and serious social issues such as falls and motor vehicle accidents.[10–16] Therefore, CD is one of the critical complications of chronic liver disease.

Because bacterial overgrowth in the intestine and delayed gastrointestinal transit time are associated with the development of CD,[17] ammonia and pro-inflammatory cytokines derived from enteric bacterial flora are thought to be pathogenic factors of CD. In fact, treatment with gut-specific agents such as lactulose and rifaximin can improve CD in cirrhotic patients.[18–22]

*Correspondence: Dr Eitaro Taniguchi, Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan.*
*Email: eitaro@med.kurume-u.ac.jp*
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However, CD is not always correlated with the severity of liver disease, blood levels of ammonia or inflammation.[6] CD can also be caused by malnutrition, cerebrovascular disease secondary to diabetes mellitus and psychoactive agents.[4,12,23,24] These previous reports suggest that complicated interactions between various factors underlie the development of CD in cirrhotic patients.

Data-mining analysis is a set of statistical techniques used to reveal complex interactions within a dataset.[25,26] A decision-tree algorithm is an exploratory data-mining analysis technique that is a series of rules for classification by identifying priorities.[26] This is a quantitative systematic approach that allows clinicians to maximize the net benefit to patients.[27] Decision-tree algorithms are now clinically applied to predict the following issues: response to interferon treatment of hepatitis C virus (HCV);[28] severity of hepatic fibrosis;[29] progression of hepatocellular carcinoma;[29] safety of hepatic resection;[30] outcome of patients with acute liver failure;[31] and dietary factors for normalizing serum alanine aminotransferase levels in patients with HCV infection.[26]

The aim of this study is to investigate the profiles associated with the incidence of CD by using multivariate analyses, including the decision-tree algorithm in cirrhotic patients.

## METHODS

### Subjects

CIRRHOTIC PATIENTS WHO were followed up at Kurume University Hospital were enrolled in this study. The inclusion criteria were viral liver cirrhosis, aged less than 70 year and able to undergo neuropsychiatric (NP) tests. The exclusion criteria were a history of overt hepatic encephalopathy and treatment for transjugular intrahepatic portosystemic shunt or esophagogastric varices. Finally, 27 subjects were enrolled in this study.

Informed consent was obtained from all the subjects. This study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in prior approval by the Ethics Committee of the Kurume University School of Medicine. None of the participants was institutionalized.

### NP tests and definition of CD

The subjects underwent NP tests, including the block design test, digit symbol test, and number connection tests A and B. Patients with two or more abnormal results in these tests were defined as having CD, as described previously.[32]

### Measurement of biochemical parameters

Venous blood samples were collected in the morning after overnight fasting. Biochemical parameters were measured by conventional clinical methods (Department of Clinical Laboratory, Kurume University Hospital), as described previously.[33,34]

### Statistical analysis

Data are expressed as mean ± standard deviation. Nonparametric multiple comparisons were made by the Mann–Whitney *U*-test. Categorical comparisons were made by Fisher's exact test. A logistic regression model was used for multivariate stepwise analysis. A decision-tree algorithm was constructed, and the categorical differences in the decision-tree model were analyzed by $\chi^2$-tests, as described previously.[25,26] The level of statistical significance was set at $P < 0.05$.

## RESULTS

### Analysis of bioparameters associated with CD

THE CHARACTERISTICS OF patients with and without CD are shown in Table 1. Univariate analysis revealed no significant differences between cirrhotic patients with and without CD in age, sex or Child–Pugh grade. No significant differences were observed between patients with and without CD in biochemical parameters such as the ammonia level, branched-chain amino acid/tyrosine ratio, zinc level or Homeostasis Model of Assessment – Insulin Resistance (HOMA-IR) value. In addition, fasting glucose and HOMA-IR were not significantly different between CD and non-CD patients with fasting glucose of less than 140 mg/dL ($P = 0.9096$ in fasting glucose and $P = 0.7055$ in HOMA-IR).

Multivariate stepwise analysis identified the levels of total bilirubin, triglycerides and free fatty acids (FFA) as independent bioparameters associated with the incidence of CD (Table 2).

### Decision-tree algorithm for CD

The decision-tree algorithm showed that all the subjects were classifiable into three groups on the basis of two variables (Fig. 1). FFA was selected as the initial split variable with a cut-off value of 514 mEq/L. Among the nine patients with FFA of 514 mEq/L or more, seven

**Table 1** Characteristics of all subjects

| | CD | No CD | *P* value |
|---|---|---|---|
| *n* | 14 | 13 | |
| Age | 59.8 ± 8.0 | 62.8 ± 6.1 | N.S. |
| Sex (F/M) | 5/9 | 7/6 | N.S. |
| Child-Pugh (A/B/C) | 12/2/0 | 7/5/1 | N.S. |
| Aspartate aminotransferase (U/L) | 68.8 ± 27.4 | 63.4 ± 24.7 | N.S. |
| Alanine aminotransferase (U/L) | 73.1 ± 74.8 | 56.0 ± 29.8 | N.S. |
| Alkaline phosphatase (U/L) | 416.0 ± 400.2 | 366.6 ± 187.6 | N.S. |
| γ-Glutamyltransferase (U/L) | 87.2 ± 128.5 | 56.2 ± 39.6 | N.S. |
| Total bilirubin (mg/dL) | 1.11 ± 0.49 | 1.53 ± 0.83 | N.S. |
| Albumin (g/dL) | 3.46 ± 0.41 | 3.32 ± 0.76 | N.S. |
| Prothrombin time (%) | 86.4 ± 10.2 | 74.5 ± 17.3 | N.S. |
| Ammonia (μg/dL) | 59.2 ± 25.2 | 58.1 ± 31.9 | N.S. |
| Fasting glucose (mg/dL) | 136.4 ± 60.1 | 127.8 ± 56.4 | N.S. |
| Hemoglobin A1c (%) | 5.8 ± 1.9 | 5.6 ± 1.3 | N.S. |
| Fasting immunoreactive insulin (μU/mL) | 16.8 ± 14.0 | 13.9 ± 6.8 | N.S. |
| HOMA-IR | 6.20 ± 7.41 | 4.92 ± 5.07 | N.S. |
| Total cholesterol (mg/dL) | 157.2 ± 31.3 | 152.5 ± 29.7 | N.S. |
| Triglyceride (mg/dL) | 96.6 ± 33.9 | 116.4 ± 47.2 | N.S. |
| Free fatty acids (mEq/L) | 567.9 ± 272.9 | 453.5 ± 229.1 | N.S. |
| Iron (μg/dL) | 274.8 ± 560.0 | 160.4 ± 63.0 | N.S. |
| Ferritin (ng/mL) | 194.6 ± 248.3 | 129.5 ± 115.5 | N.S. |
| Zinc (μg/dL) | 63.6 ± 9.7 | 59.8 ± 22.0 | N.S. |
| Branched-chain amino acids/tyrosine ratio | 3.54 ± 1.26 | 3.54 ± 1.60 | N.S. |

Data are expressed number of mean ± standard deviation.
CD, cognitive dysfunction; HOMA-IR, Homeostasis Model of Assessment – Insulin Resistance; N.S., not significant.

(77.8%) had CD. Meanwhile, among the 18 patients with FFA of less than 514 mEq/L, seven (38.9%) had CD.

Triglycerides were selected as the second split variable with a cut-off value of 106 mg/dL. Among patients with FFA of less than 514 mEq/L, five patients (62.5%) had

**Table 2** Logistic regression analysis for CD

| | OR | 95% CI | *P*-value |
|---|---|---|---|
| Total bilirubin | 0.002 | 5.708e-7–0.154 | <0.05 |
| Triglyceride | 0.889 | 0.748–0.964 | <0.05 |
| Fee fatty acids | 1.015 | 1.004–1.037 | <0.05 |
| Total cholesterol | 1.119 | 1.024–1.323 | N.S. |
| HOMA-IR | 2.053 | 0.889–7.631 | N.S. |
| Zinc | 0.915 | 0.784–1.010 | N.S. |
| Fasting glucose | 1.033 | 0.989–1.102 | N.S. |
| Ammonia | 1.063 | 0.964–1.186 | N.S. |

CD, cognitive dysfunction; CI, confidence interval; HOMA-IR, Homeostasis Model of Assessment – Insulin Resistance; N.S., not significant; OR, odds ratio.

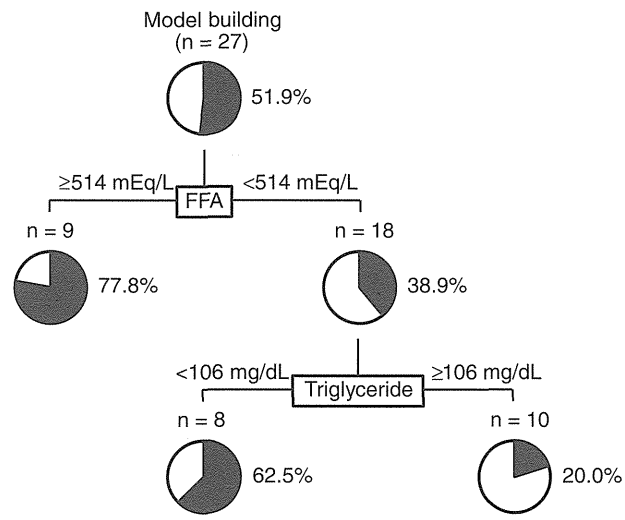


**Figure 1** Decision-tree algorithm for cognitive dysfunction (CD). The subjects were classified according to the indicated cut-off values of the variables. The pie graphs indicate the percentage of CD (black)/no CD (white) in each group. FFA; free fatty acids.

**Table 3** Biochemical profiles and the incidence of CD

| | CD | No CD |
|---|---|---|
| FFA ≥514 mEq/L or triglyceride <106 mg/dL | 12 | 5 |
| FFA <514 mEq/L and triglyceride ≥106 mg/dL | 2 | 8 |

CD, cognitive dysfunction; FFA, free fatty acids.

CD among the eight patients with triglycerides of less than 106 mg/dL, while two patients (20.0%) had CD among the 10 patients with triglycerides of 106 mg/dL or more.

The distribution of CD differed significantly between the groups ($P = 0.0325$).

### Categorical differences according to the decision-tree algorithm for CD

According to the results of the decision-tree algorithm, all subjects were classified into two groups: one group with FFA of 514 mEq/L or more or triglycerides of less than 106 mg/dL ($n = 17$), and another group with FFA of less than 514 mEq/L and triglycerides of 106 mg/dL or more ($n = 10$). The distribution of CD was significantly different between the groups ($P = 0.0183$) (Table 3). The sensitivity, specificity and accuracy using the cut-off values of FFA and triglycerides were 85.7% (12/14), 61.5% (8/13) and 74.1% (20/27), respectively.

## DISCUSSION

THE RESULTS OF this study show that FFA and triglycerides were independent risk factors for CD in cirrhotic patients. Furthermore, data-mining analysis revealed that FFA of more than 514 mEq/L or triglycerides of less than 106 mg/dL is a profile associated with the incidence of CD in cirrhotic patients.

Hyperammonemia and inflammation are known to occur in the pathogenesis of CD in cirrhotic patients.[35–38] However, CD is not always correlated with the severity of liver disease, blood levels of ammonia or inflammation,[6] suggesting the presence of other pathogenic factors. In this study, we demonstrated that FFA and triglycerides are associated with the incidence of CD in cirrhotic patients. Although higher serum FFA levels and lower serum triglyceride levels can be thought to reflect hepatic insufficiency, serum albumin levels and blood ammonia were not identified as risk factors for CD in this study. Moreover, FFA and triglycerides were identified as independent risk factors. Although the precise causal relationship between these factors and CD remains unclear, FFA and triglycerides vary with starvation and meal uptake. Malnutrition is associated with CD,[24] and eating breakfast is known to improve CD in cirrhotic patients.[39] Taken together, changes in lipid metabolism caused by starvation or meal uptake may pleiotropically affect the development of CD in cirrhotic patients.

Data-mining analysis provided FFA of more than 514 mEq/L as the initial classification, suggesting that FFA is the most closely related factor to the incidence of CD in cirrhotic patients. Although the relationship between FFA and CD is unclear, there are some possible explanations. Serum albumin is a carrier protein for various substances, including FFA and tryptophan.[40,41] An increase in FFA–albumin binding results in the dissociation of tryptophan from albumin and a subsequent increase in serum-free tryptophan levels.[41–43] Tryptophan can be transported into the brain across the blood–brain barrier and converted to 5-hydroxytryptamine, which is a neurotransmitter known to be associated with hepatic encephalopathy[44,45] as well as cognitive function.[46]

In patients with FFA of less than 514 mEq/L, triglycerides of less than 106 mg/dL was identified as the secondary classification. Contrary to our results, hypertriglyceridemia is a previously established risk factor for CD.[47,48] Although the reason for this discrepancy remains unclear, it can be speculated that hypertriglyceridemia may cause CD via the micro-impairment of cerebrovascular circulation.[49] Meanwhile, triglycerides, particularly medium-chain triglycerides, are structured lipids and good sources of energy in the brain.[50] In fact, patients with hypotriglyceridemia develop complications such as neurological manifestations with structural changes in the nerves.[51,52] Moreover, treatment with medium-chain triglycerides is reported to improve cognitive functioning in older adults with memory disorders.[53] Thus, lower FFA levels may cause CD via structural or functional nerve impairment.

Cognitive dysfunction occurs in up to 80% of patients at any stage of chronic liver disease[24] and is related not only to poor prognosis[54] but also to social issues, including falls and motor vehicle accidents.[10,13,14,16,55,56] Although NP tests are reliable tools for diagnosing CD in cirrhotic patients,[3,57] they are time-consuming (~30 min) and are affected by the educational status. Both electroencephalogram and critical flicker frequency are rapid tests for diagnosing CD. However, these tests require a trained personnel and

specialized equipment.[6,58] Therefore, a simple screening tool for CD is required for patients with chronic liver disease. In this study, we demonstrated that the lipid profile of FFA of more than 514 mEq/L or triglycerides of less than 106 mg/dL is associated with the incidence of CD with high sensitivity. Because evaluating serum FFA and triglyceride levels are simple objective assessments, further studies will focus on the significance of serum FFA and triglyceride levels as a screening bioparameter set for the presence of CD in patients with chronic liver disease.

The limitation of this study would be related to inclusion criteria. Contrary to expectation, a lower level of total bilirubin was identified as a risk factor. In this study, only the cirrhotic patients with no incidence of overt hepatic encephalopathy were enrolled. Because overt hepatic encephalopathy is generally seen in patients with decompensated cirrhosis or liver failure, the odds ratio of total bilirubin may be influenced by inclusion criteria. CD is also seen in cirrhotic patients with the treatment for overt hepatic encephalopathy; these patients should be included in further study.

In conclusion, the results of this study show that the serum levels of FFA and triglycerides are independently associated with the incidence of CD in cirrhotic patients. In addition, data-mining analysis revealed that FFA of more than 514 mEq/L or triglycerides of less than 106 mg/dL is a profile associated with the incidence of CD in cirrhotic patients. Thus, the lipid profile could be involved in the development of CD and could be considered as a possible screening bioparameter for CD in cirrhotic patients.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

# Decreased expression of insulin and increased expression of pancreatic transcription factor PDX-1 in islets in patients with liver cirrhosis: a comparative investigation using human autopsy specimens

**Masahiro Sakata · Akihiko Kawahara · Takumi Kawaguchi · Jun Akiba · Tomoki Taira · Eitaro Taniguchi · Mitsuhiko Abe · Hironori Koga · Masayoshi Kage · Michio Sata**

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**Abstract**
*Background* Glucose intolerance in patients with liver cirrhosis (LC), known as hepatogenous diabetes, is thought to be distinct from type 2 diabetes (T2DM) in some aspects. Hyperinsulinemia and/or insulin resistance in liver disease is associated with hepatocarcinogenesis, growth of hepatocellular carcinoma, and poor prognosis. However, the pathophysiological processes in islets that are responsible for hyperinsulinemia in LC are still not precisely known. Therefore, we investigated the histopathological differences in islets of Langerhans cells between LC and T2DM.
*Methods* A total of 35 human autopsy pancreatic tissue samples were used in this study (control, $n = 18$; T2DM, $n = 6$; LC, $n = 11$). The expression of insulin, glucagon, somatostatin, pancreatic duodenal homeobox-1 (PDX-1), proliferating cell nuclear antigen (PCNA), and Ki-67 was examined using immunohistochemistry and quantitated by image analysis.
*Results* Islet hypertrophy and a significant increase in PCNA-positive cells in islets were observed in the tissues from LC cases. The insulin-positive areas in islets were significantly decreased in LC cases compared with control and T2DM cases ($P = 0.001$, $P = 0.035$, respectively), whereas the PDX-1-positive area was significantly increased in LC cases ($P = 0.001$) compared with the control. Furthermore, disorganization of pancreatic endocrine cells and nucleocytoplasmic translocation of PDX-1 were both seen in the LC subjects.
*Conclusions* In LC, islets undergo hypertrophy and exhibit paradoxical expression of insulin and PDX-1. In the subjects autopsied, insulin expression was decreased, whereas expression of the pancreatic transcription factor PDX-1 was increased in LC. These results point to important distinctions between LC and T2DM.

**Keywords** Liver cirrhosis · Diabetes · Pancreatic duodenal homeobox-1 · Insulin · Islet

M. Sakata (✉) · T. Kawaguchi · E. Taniguchi · M. Abe · H. Koga · M. Sata
Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan
e-mail: sakata_masahiro@med.kurume-u.ac.jp

A. Kawahara · J. Akiba · T. Taira · M. Kage
Department of Diagnostic Pathology, Kurume University Hospital, Kurume, Japan

T. Kawaguchi · M. Sata
Department of Digestive Disease Information and Research, Kurume University School of Medicine, Kurume, Japan

J. Akiba
Department of Pathology, Kurume University School of Medicine, Kurume, Japan

**Abbreviations**

| | |
|---|---|
| PDX-1 | Pancreatic duodenal homeobox-1 |
| PCNA | Proliferating cell nuclear antigen |
| BMI | Body mass index |
| HbA1c | Hemoglobin A1c |

## Introduction

Insulin, secreted by the pancreas, is one of the most important regulators of plasma glucose. Currently, it is thought that hyperglycemia in type 2 diabetes mellitus (T2DM) is caused by impaired insulin secretion and decreased $\beta$ cell mass together with the development of insulin resistance [1]. On the other hand, glucose intolerance in patients with liver