Carbohydrate-Deficient Transferrin is a Sensitive Marker of Alcohol Consumption in Fatty Liver Disease

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Abstract

Background

The prevalence of nonalcoholic fatty liver disease (NAFLD) and alcohol-associated/related liver disease (ALD) with metabolic syndrome is globally increasing. Metabolic syndrome and excessive alcohol consumption synergically exacerbate liver pathologies; therefore, drinking-specific serum markers unaffected by liver injury or metabolic syndrome are essential to assess alcohol consumption. We evaluated the ratio of carbohydrate-deficient transferrin to total transferrin (%CDT) in patients with fatty liver disease, particularly focusing on its correlation with metabolic factors (UMIN000033550).

Methods

A total of 120 patients with fatty liver disease, including ALD and NAFLD, were screened for alcohol misuse using the Alcohol Use Disorders Identification Test. Associations of metabolic syndrome-related factors and hepatic steatosis/liver stiffness with drinking markers such as %CDT, gamma-glutamyl transferase (GGT), and mean corpuscular volume (MCV) were assessed using multiple linear regression analyses.

Results

%CDT significantly increased with 3–4 drinks/day. The optimal cut-off value for identifying non- to light drinkers was 1.78% (sensitivity, 71.8%; specificity, 83.7%; area under the receiver operating characteristic curve [AUROC], 0.851), which was significantly higher than that for GGT. The cut-off value for identifying heavy drinkers was 2.08% (sensitivity, 65.5%; specificity, 86.8%; AUROC, 0.815). Multiple regression analysis revealed that this proportion was negatively correlated with body mass index, whereas GGT and MCV were influenced by multiple factors involved in liver injury and dyslipidemia.

Conclusions

%CDT showed a strong correlation with alcohol consumption, independent of liver damage, steatosis/stiffness, or metabolic syndrome-related factors, indicating that it is a useful drinking marker for the accurate diagnosis of NAFLD and ALD.

Introduction

From 1990 to 2017, the annual alcohol consumption increased by approximately 70% globally, from 20,999 to 35,676 million liters, with the proportion of alcohol drinkers increasing from 45–47% [1]. Alcohol-associated/related liver disease (ALD) is a major contributor to the worldwide burden of cirrhosis, and alcohol-associated/related cirrhosis had the highest average annual increase in cirrhosis-related mortality from 1999 to 2016 in the USA [2]. Furthermore, in Japan, the non-viral/viral hepatocellular carcinoma ratio has increased remarkably in recent years [3]. Unfortunately, there are no established pharmacological treatments for ALD other than alcohol avoidance and abstinence.

Secondary to the growing global population of patients with diabetes and metabolic syndrome (MetS), the prevalence of non-alcoholic fatty liver disease (NAFLD) is also increasing worldwide [4]. Although the pathological features of ALD and NAFLD are very similar that it can be extremely difficult to distinguish them, lifestyle-related treatment guidelines are very different for the two, with alcohol abstinence recommended for ALD and dietary exercise therapy recommended for NAFLD. It is important to note that alcohol can easily cause liver injury in obese animals and humans [5], and even light drinking within the definition of NAFLD can exacerbate liver damage in patients with fatty liver disease [6]. By contrast, type 2 diabetes, MetS, and total alcohol consumption are independent risk factors for mortality in ALD patients [7]. These findings indicate that MetS-related factors and alcohol intake can exacerbate liver damage while interacting with one another [8]. Excessive alcohol consumption is often seen in MetS patients, and alcoholic hepatitis exacerbation due to MetS comorbidity is an emerging clinical problem [9]. Therefore, biomarkers for estimating alcohol consumption independent of MetS-related factors are essential for ALD and NAFLD management.

Transferrin, a protein that transports iron, contains several glycoforms that differ in the number and/or structure of up to two N-linked oligosaccharide chains (N-glycans). The major glycoform under normal conditions, tetrasialotransferrin, contains two disialylated biantennary glycans (i.e., four terminal sialic acids) [10]. Tetrasialotransferrin typically accounts for less than 75–80% of serum transferrin [11]. Chronic alcohol consumption, via ethanol or its metabolite, acetaldehyde, has been reported to inhibit glycosylation/sialylation in the Golgi apparatus of hepatocytes, though the specific enzymes involved in this process remain debatable. This inhibition results in a decrease in the sialylation of transferrin and an increase in the relative amounts of disialo- and asialotransferrin, called carbohydrate-deficient transferrin (CDT) isoforms, despite a normal total transferrin level [12]. Measuring the proportion of carbohydrate-deficient transferrin to total transferrin (%CDT) has been mainly performed in Europe, with only a few reports on the utility of %CDT measurement in Asia, including a pilot study evaluating the %CDT in 13 patients with non-alcoholic steatohepatitis (NASH) and 26 with alcoholic hepatitis [13]. Instead, gamma-glutamyl transpeptidase (GGT) and mean corpuscular volume (MCV) are more widely used as drinking markers in Asia [14, 15].

Therefore, this study aimed to evaluate the utility of %CDT measurement in an Asian population with NAFLD or ALD and comorbid MetS-related factors. We identified drinking habits and/or alcohol dependence using the Alcohol Use Disorders Identification Test (AUDIT) and determined the effects of alcohol consumption on %CDT values in order to determine whether this variable may have more utility as an alcohol-intake marker than GGT or MCV. Finally, the associations of liver injury/steatosis/stiffness/MetS-related factors with %CDT values and other drinking markers were assessed using single and multivariate analyses.

Materials And Methods

Participants

This study was approved by the Institutional Review Board of Juntendo University Hospital (no. 18–025) and was conducted in accordance with the Declaration of Helsinki and current ethical guidelines. A cross-sectional study was conducted on 120 patients aged 23–87 years who were admitted to our hospital between September 2018 and October 2020 and were diagnosed with fatty liver, including ALD and NAFLD. This study was registered with the University Hospital Medical Information Network (UMIN000033550). After providing an adequate explanation, written informed consent for participation in this study was obtained from all subjects before measuring %CDT. All participant information, including names, was replaced with numbers, and personal privacy was not compromised in any way. The exclusion criteria were a history of chronic liver diseases, such as chronic hepatitis B, hepatitis C, autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, hemochromatosis, Wilson's disease, or hepatocellular carcinoma.

Serum Collection And Laboratory Evaluations

Participants answered the AUDIT questionnaire at the time of assignment to the study, and serum for %CDT measurement was collected within 3 months after the AUDIT and stored at – 80°C until measurement. Clinical data, including age and history of treatment, as well as laboratory results, including the concentrations of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), GGT, alkaline phosphatase (ALP), total bilirubin, MCV, platelets, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides (TG), and hemoglobin A1c (HbA1c) were collected.

Quantification Of Hepatic Steatosis And Liver Stiffness

Hepatic steatosis and liver stiffness were assessed using vibration-controlled transient elastography (Echosens, Paris), which records controlled attenuation parameter (CAP) values and liver stiffness measurement (LSM).

N-latex Cdt And Nephelometry System

The serum %CDT was assayed using an N-Latex CDT assay. This assay is conducted using a monoclonal antibody (mAb) that specifically recognizes transferrin glycoforms that lack one or both of the complete N-glycans (i.e., disialo-, monosialo-, and asialotransferrins [CDT glycoforms]) in combination with a simultaneous assay that determines the total transferrin (N antiserum to human transferrin; Dade Behring). Polystyrene particles coated with CDT mAb are agglutinated by CDT-coated polystyrene particles, with CDT inhibiting this reaction in a dose-dependent manner, allowing nephelometric CDT quantification [16].

Statistical Analyses

Two groups of variables were compared using the Mann-Whitney test. Multiple groups of quantitative data were compared using the Kruskal-Wallis analysis of variance on ranks, followed by all pairwise multiple comparisons using Dunn's method. Multiple groups of qualitative data were compared using the chi-squared test and post-hoc residual analysis. For the estimation of multivariate models, all parameters that showed significance in a simple linear regression analysis ($p < 0.05$) were fitted to a multiple linear regression model. The residual analysis was performed using Microsoft Excel 2019, while all other analyses were performed using SigmaPlot 14.0 (Systat Software Inc., San Jose, CA). Values are presented as mean $\pm$ standard error of mean (SEM). Differences were considered statistically significant at p -values of < 0.05 .

Results

Relationships between alcohol consumption and %CDT

Participants were divided into four groups according to their alcohol consumption per week, including non-drinkers, light drinkers (< 210 g pure ethanol for males and < 140 g for females), moderate drinkers (≥ 210 g for males, ≥ 140 g for females, and < 420 g for both), or heavy drinkers (≥ 420 g). Detailed characteristics of each group are summarized in Table 1. The %CDT, GGT, and MCV values were significantly higher in the heavy-drinker group than in the non- or light-drinker groups. The percentage of males was significantly higher in the light- to heavy-drinker groups than in the non-drinker group. BMI, HbA1c, and CAP values were significantly lower and the HDL value was significantly higher in the heavy-drinker group than in the non-drinker group.

A receiver operating characteristic curve (ROC) analysis indicated that the area under the ROC curve (AUROC) for the use of %CDT to distinguish non- or light drinkers from all others was 0.851 (95% CI, 0.781–0.922). For this evaluation, the cut-off value with the largest Youden's index (sensitivity + specificity – 1) and a specificity of over 80% was 1.78% (sensitivity, 71.8%; specificity, 83.7%). This %CDT AUROC (i.e., 0.851) was significantly higher than the AUROC of 0.711 for GGT (95% CI, 0.611–0.810) ($p = 0.025$) and tended to be higher than the AUROC of 0.766 for MCV (95% CI, 0.678–0.855) ($p = 0.118$) (Fig. 1a). The AUROC of %CDT for identifying heavy drinkers was 0.815 (95% CI, 0.725–0.905), with a sensitivity and specificity of 65.5% and 86.8%, respectively, which also tended to be higher than the AUROC of 0.723 for GGT (95% CI, 0.607–0.840) ($p = 0.191$) and the AUROC of 0.764 for MCV (95% CI, 0.666–0.862) ($p = 0.353$) (Fig. 1b).

For the correlation between %CDT values and the frequency/amount of alcohol intake based on AUDIT scores (Table 2), the %CDT was significantly higher for “drinks 4 times or more per week” than for other groups with less frequent alcohol use (Q1). In addition, the %CDT was significantly lower for “more than 0–2 drinks/day” than for others with more alcohol intake (Q2). The group with a “daily or almost daily” frequency of heavy drinking of ≥ 6 drinks on one occasion showed a significantly higher %CDT value

than for non-drinkers (Q3). By contrast, no significant %CDT increase was observed in the correlation analysis of alcohol dependence and experience of harm-related questions (Q4–8) on the AUDIT (Online Resource 1), whereas significant differences were found between alcohol-related injuries (Q9) responses of “No” or “Yes, within the year,” and others concerned about drinking (Q10) responses of “No” or “Yes, within the year” (Online Resource 2).

Effect of liver injury and MetS-related factors on drinking markers

To clarify the effects of liver injury, steatosis/stiffness, and MetS-related factors on drinking markers, two-group comparisons were performed using binary data for the presence or absence of each factor (Table 3). All drinking markers were significantly higher in the moderate- or heavy-drinker groups than in the non- or light-drinker groups. Increases in serum AST and ALT levels over the normal range were significantly correlated with elevated GGT levels; however, neither the CAP nor the LSM affected any of the drinking markers. For MetS-related factors, the %CDT was significantly lower in the high body mass index ($\text{BMI} \geq 25 \text{ kg/m}^2$) group, and the MCV was significantly higher in the low-LDL group ($\text{LDL} < 140 \text{ mg/dL}$). Other MetS-related factors were not correlated with drinking markers.

Moreover, simple and multiple linear regression analyses were performed to determine the quantitative effect of each factor on drinking markers (Table 4). Simple linear regression analysis showed that the drinking volume affected all drinking markers and that the %CDT was negatively correlated with CAP, BMI, LDL, and HbA1c. GGT was positively correlated with AST, LSM, and TG and negatively correlated with LDL and HbA1c. MCV was positively correlated with AST, HDL, and BMI and negatively correlated with LDL and HbA1c. All significant parameters identified in the simple linear regression analysis were also evaluated using a multiple linear regression analysis (Table 5), which showed that the %CDT value was significantly positively correlated with alcohol consumption and negatively correlated with BMI. GGT was also positively correlated with the alcohol amount consumed, strongly positively correlated with AST, and positively correlated with LSM and TG. MCV was weakly positively correlated with alcohol intake, strongly positively correlated with AST and HDL levels, and strongly negatively correlated with LDL.

Discussion

In this study, we evaluated the utility of %CDT measurement in an Asian population with NAFLD or ALD and comorbid MetS-related factors. The male:female ratio was approximately equal in the non-drinker group; in contrast, the majority of patients were men in the other groups. The heavy-drinker group had higher HDL and lower BMI values, which are consistent with the general characteristics of patients with ALD (Table 1). Because even a relatively small amount of alcohol can cause liver injury in patients with obesity and/or MetS [8], Obtaining detailed quantitative information on alcohol intake is vital in the medical care of patients with fatty liver disease. Our cohort included more than half of participants with light or moderate drinking levels, in contrast to previous studies on %CDT involving patients with alcohol addiction [17–19]; thereby enabling the investigation on the usefulness of %CDT in patients with light-to-moderate alcohol intake.

This study showed that %CDT had higher AUROCs than either GGT or MCV, indicating good diagnostic accuracy for distinguishing the absence of significant drinking (< 210 g/week for men, < 140 g/week for women), which is an important diagnostic criterion for NAFLD, from the presence of heavy drinking (> 420 g/week), which is a diagnostic criterion for ALD. Particularly, the %CDT showed a significantly higher AUROC than GGT for identifying a lack of significant drinking (Fig. 1).

To our knowledge, our study, which was conducted on a cohort in which the majority of patients exhibited light-to-moderate drinking levels, was the first to show that %CDT is more useful than GGT or MCV for determining the presence or absence of drinking using the diagnostic criteria for NAFLD and ALD. A %CDT cut-off value of 1.78% was used to identify non- or light drinkers, and a cut-off value of 2.08% was used to identify heavy drinkers. These values showed high sensitivities of 71.8% and 65.5%, respectively, and excellent specificities of 83.7% and 86.8%, respectively. Indeed, in the moderate- to heavy- drinker groups, 10 of 14 patients had low GGT levels that were less than the upper limit of normal (ULN, 75 U/L). These patients were considered GGT non-responders, with excessive drinking identified by a %CDT value exceeding the $\geq 1.78\%$ cut-off. By contrast, 20 of the 29 non-to-light drinkers had GGT values more than the ULN but a %CDT of < 1.78%. Similarly, 25 out of 33 moderate- to heavy-drinkers with MCVs less than the ULN (99.0 fl) showed a %CDT of $\geq 1.78\%$, whereas 1 out of 3 non- to light-drinkers with MCVs higher than the ULN showed a %CDT of < 1.78% (data not shown). These findings indicate that %CDT can be used to correctly identify non-light drinkers, even in patients with misleading GGT or MCV values.

Although several studies have shown that the %CDT is an excellent alcohol marker for detecting heavy drinkers, most of these studies were conducted in extreme cohorts of heavy drinkers or those practicing abstinence. A clinical study in Germany showed that the %CDT was more specific than either GGT or MCV for distinguishing ALD due to current alcohol dependence from liver damage due to other causes (mostly viral hepatitis) [18]. A Finnish study reported that the %CDT was more sensitive than GGT or MCV for distinguishing alcohol abusers from healthy volunteers who were either abstainers or moderate drinkers [20]. In Asia, Liang et al. reported that the %CDT was a better indicator than either GGT or MCV for distinguishing an alcoholic group from healthy controls or a non-alcoholic liver disease group in China [21], while Suzuki T et al. showed that the %CDT was a more useful biomarker of chronic alcohol abuse than GGT during treatment of patients with alcoholism in Japan [19].

Serum markers that can identify insignificant alcohol intake for the diagnosis of NAFLD have not yet been established. In this study of 120 Japanese patients, the AUROC of the %CDT was greater for detecting non- or light drinkers than heavy drinkers (Fig. 1), and the %CDT value was significantly elevated with only minimal increases in daily drinking from 0–2 drinks to 3–4, indicating a sharp increase with a small EtOH increase of only 20 g/day (Table 2). Therefore, the %CDT level appears to be very helpful for identifying heavy drinkers and distinguishing non- or light drinkers from moderate drinkers. A threshold effect on manufacturer-recommended CDT value of 2.6% in healthy subjects with an alcohol intake of over 40 g per day was reported in France [22]. Thus, racial differences in %CDT values may need to be considered in the discrimination of insignificant drinkers. Regarding drinking frequency, the %CDT did not significantly increase until the frequency was as high as four or more times per week. Similarly,

even with heavy drinking of six drinks or more at a time, the %CDT did not significantly increase unless the frequency was as high as “almost daily.” Thus, using an elevated %CDT to evaluate alcohol consumption did not require clinically significant daily doses but only frequent drinking.

Candidate factors that could potentially influence drinking markers, including serum AST and ALT levels; hepatic steatosis by CAP values; hepatic elasticity by LSM; BMI; and other MetS-related factors such as HDL, LDL, TG, hypertension, and HbA1c were evaluated in this study. To evaluate these factors, the study cohort was divided into two groups depending on whether each factor was within its respective threshold, and two-group comparisons of drinking markers were performed (Table 3). The GGT value fluctuated depending on whether the AST and ALT values were less than the ULN, suggesting that liver damage can affect GGT. The MCV was significantly lower with high LDL levels, indicating it may be affected by lipid metabolism. The %CDT was low in the high-BMI group, consistent with previous studies reporting that high-BMI groups are less likely to have an increased %CDT than low-BMI groups [23, 24]; however, the detailed mechanisms underlying this phenomenon remain unknown [23].

A simple linear regression analysis (Table 4), followed by a multiple linear regression analysis (Table 5), demonstrated that a low BMI independently affected the increase in %CDT, while many other factors did not affect it. By contrast, GGT was independently influenced by various factors, including the AST, liver stiffness, and TG. MCV increased the *p*-value associated with alcohol intake to the limit of significance, suggesting that MCV is also influenced by many background factors. MCV was independently affected by several factors, including the AST, HDL, LDL, and HbA1c. Taken together, the %CDT value was found to be an almost-pure drinking marker, whereas GGT and MCV values reflected not only the amount of drinking, but also liver damage and disorders of lipid metabolism.

In 2020, the European Association for the Study of the Liver proposed metabolic dysfunction-associated fatty disease (MAFLD) as a new pathological concept, indicating that fatty liver disease can be associated with metabolic disorders.[25] This major shift in the field of liver disease suggests that fatty liver disease will be regarded as a series of liver diseases centered on dyslipidemia, with drinking being a major factor. Therefore, it is expected that using %CDT, a drinking marker not affected by liver damage or dyslipidemia, to objectively analyze a predominant factor underlying the pathogenesis of these disorders and determine a treatment policy for patients with MAFLD will become vitally important.

In conclusion, this study showed that the %CDT is a highly useful drinking marker for diagnosing NAFLD and ALD. While a low BMI could affect %CDT, the %CDT was not influenced by liver damage or lipid metabolism disorders, unlike the MCV or GGT, indicating that this parameter can be used to identify fatty liver disease associated with NAFLD and ALD comorbid with MetS, which is currently the predominant form of liver disease and is expected to account for a higher proportion of patients in the future.

Declarations

Compliance with Ethical Requirements

Conflict of interest statement: Maki Morinaga, Kazuyoshi Kon, Akira Uchiyama, Hiroo Fukada, Kyoko Fukuhara, Reiko Yaginuma, Eisuke Nakadera, Shunhei Yamashina, and Kenichi Ikejima declare that they have no competing interests.

Informed consent in studies with human subjects: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study.

Animal studies: This article does not contain any studies with human or animal subjects.

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None

Competing interests

Maki Morinaga, Kazuyoshi Kon, Akira Uchiyama, Hiroo Fukada, Kyoko Fukuhara, Reiko Yaginuma, Eisuke Nakadera, Shunhei Yamashina, and Kenichi Ikejima declare that they have no competing interests.

Ethics approval

This study was approved by the Institutional Review Board of Juntendo University Hospital (no. 18-025) and was conducted in accordance with the Declaration of Helsinki and current ethical guidelines.

Consent to participate

After providing an adequate explanation, written informed consent for participation in this study was obtained from all subjects before measuring %CDT.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Authors' contributions

Material preparation: MM, KK, AU, HF, KF, RY, EN, SY; data collection and analysis: MM and KK; manuscript drafting: KK; experiment supervision and paper review: KI.

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Tables

Due to technical limitations, table 1 to 5 are only available as a download in the Supplemental Files section.

Figures

Figure 1

The ROC curve of %CDT, GGT and MCV. a) The ROC curves for detecting non- or light-drinkers. The area under the ROC curve was 0.847 (95% CI, 0.776-0.918), 0.706 (95% CI, 0.608 To 0.805), and 0.751 (95% CI, 0.660-0.843) for %CDT, GGT and MCV, respectively. b) The ROC curves for detecting heavy-drinkers. The area under the ROC curve was 0.815 (95% CI, 0.725-0.905), 0.723 (95% CI, 0.607 To 0.840), and 0.764 (95% CI, 0.666-0.862) for %CDT, GGT and MCV, respectively.

Supplementary Files

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