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Evaluation of the diagnostic utility of carbohydrate-deficient transferrin in chronic alcoholism

Results from Southwest China

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Abstract

Although recent gathered evidence indicates that obtaining the diagnostic value of serum carbohydrate-deficient transferrin might be more useful for identifying alcohol abuse than other widely available biochemical tests; however, its precise value as an indicator of chronic alcoholism is unclear. The main objective is to investigate the diagnostic significance of carbohydrate-deficient transferrin in chronic alcoholism in the Chinese population.

In this study, we enrolled (1) 52 physically healthy subjects, (2) 20 patients with nonalcoholic liver disease, and (3) 70 alcoholics. Patients with liver injuries and a history of liver surgery were excluded. Serum gamma-glutamyltransferase, aspartate aminotransferase, alanine aminotransferase, and mean corpuscular volume were determined by standard biochemical assays, and serum carbohydrate-deficient transferrin was estimated in each group using capillary electrophoresis. Subsequently, the diagnostic value of carbohydrate-deficient transferrin (CDT) in chronic alcoholism was determined based on differences between each indicator among the three groups.

The CDT level in the alcoholic group was significantly higher than that of the non-alcoholic liver disease and healthy control groups ($P < .05$). The area under the curve for alcoholism diagnosis was the highest for CDT, at 0.922, whereas those for gamma-glutamyltransferase, aspartate aminotransferase, alanine aminotransferase, and mean corpuscular volume were 0.860, 0.744, 0.615, and 0.754, respectively. When the cutoff value of CDT was set at 1.25%, the sensitivity and specificity were 85.5% and 89.6%, respectively. However, the correlation between CDT and daily alcohol consumption was weak ($r = 0.175$; $P = .16$).

Compared with the other parameters evaluated, CDT was a better indicator of alcoholism. It should, therefore, be actively promoted in clinical practice. However, the correlation between CDT and daily alcohol consumption needs further evaluation.

Abbreviations: ALT = alanine aminotransferase, AST = aspartate aminotransferase, CDT = carbohydrate-deficient transferrin, CE = capillary electrophoresis, GGT = gamma-glutamyltransferase, MCV = mean corpuscular volume, NALD = non-alcoholic liver disease.

Keywords: alcoholism, capillary electrophoresis, serum carbohydrate-deficient transferrin (CDT)

1. Introduction

Alcohol consumption is a common social phenomenon.[1] Alcoholism is associated with an increased risk of liver disease and cirrhosis. For occupations requiring persistent and high level of concentration, such as drivers, pilots, and workers in industrial and mining enterprises, alcoholism could significantly increase accidents.[2] According to a report by the World Health Organization (WHO), alcoholism has become 1 of 5 major risk factors for global morbidity, disability, and mortality.[3] Early identification of alcoholism would reduce health-related, social, and economic problems caused by alcohol abuse considerably.

Since alcoholics find it difficult to estimate their alcohol intake and drinking times accurately, it is often difficult to determine their degree of alcohol consumption. The traditional laboratory indicators for the diagnosis of alcoholism include γ- glutamyl-transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and mean red cell volume (MCV). However, these predictors lack specificity for precise identification, and most often cannot distinguish between liver injury caused by different agents. Besides, they have poor sensitivity for the early detection of alcoholism. Moreover, these indicators only change after cumulative liver injury.[4]

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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In recent years, researchers have found that carbohydrate-deficient transferrin (CDT) is closely related to the extent of drinking alcoholic beverages. CDT is derived from transferrin; under normal conditions, transferrin has 2 sugar-binding sites, which can bind to 2, 3, or 4 sugar chains, each of which is a negatively-charged sialic acid molecule. If transferrin is unable to a sugar chain, it is referred to as a sialic acid-deficient transferrin subtype. If the sugar-binding sites bind to 2, 3, or 4 sugar chains respectively, 1 to 8 sialic acid-deficient transferrin subtypes might be presented. When a sugar chain binds to 1 sialic acid molecule, the isoelectric point (pI) decreases by 0.1. CDT refers to the sum of all low sialic acid transferrin, namely, 2 sialic acid transferrin and (or) sialic acid-deficient transferrin.[5,6]

In this study, capillary electrophoresis (CE) was used to detect the serum CDT among alcoholics, patients with NALD, and physically healthy individual controls to investigate the diagnostic value of CDT in chronic alcoholism in the Chinese population.

2. Materials and methods

2.1. Subjects

Male patients of Han ethnicity, aged ≥18 years, who came for treatment at the Department of Substance Abuse, West China Hospital, Sichuan University, China, were included. The diagnoses of alcohol dependence and nonalcoholic liver disease were based on the 10th revised version of the International Classification of Diseases (ICD-10). The patients were divided into 3 groups:

1. case group: 70 alcoholics, with an average age of 47.8 ± 7.6 years,
2. differential diagnostic group: 20 patients with NALD, including patients with a primary biliary cirrhosis, chronic active hepatitis, or hepatocellular carcinoma, with an average age of 46.4 ± 10.1 years, and
3. a healthy control group: 52 patients without any physical organ lesions, history of medical illnesses or alcoholism, or abnormalities in liver function, evidenced by abdominal ultrasonography and liver function tests, which were within normal limits.

The age and gender of the healthy controls were matched with that of the diseased groups, with an average age of 46.2 ± 9.8 years. Patients with liver injuries and a history of liver surgery were excluded. The study was approved by the Institutional Ethics Committee of the West China Hospital in Sichuan University approval No. Clinical Trial [2014 (14)]. Written informed consent was obtained from all subjects.

2.2. Information on drinking habits

The study participants (n = 142) were admitted into the hospital and screened using the Alcohol Use Disorders Identification Test (AUDIT) scale developed by the WHO for alcohol dependence. Low and high-risk drinkers, harmful drinkers, and alcohol addicts were identified. Additionally, patients also responded to a "survey regarding their drinking habits in the last 2 months," which elaborated on the types of drink, frequency, and alcohol intake to obtain more detailed information about recent drinking habits. The 2 aforementioned scales were completed by professional nurses and patients employing a one-to-one approach to ensure the reliability of results.

2.3. Specimen collection and detection

On the morning of the second day after admission, a fasting blood test was performed on all participants. Serum ALT, AST, GGT, and alcohol levels were estimated using an automatic biochemical analyzer (cobas8000, Roche Diagnostics, Germany); MCV was evaluated using an automated blood cell analyzer (Sysmex, Sysmex Corporation, Japan). The serum was frozen at –20 degrees. The serum CDT content was analyzed using an automatic CE instrument (MINICAP, Sebia, Lisses, France) in accordance with the manufacturer's instructions. All analyses were completed within 1 month. MINICAP automatically performs all sequences of operations to obtain a full transferrin map for the quantitative analysis of CDT. The transferrin isolated from the quartz capillary could be determined directly in 200 nm ultraviolet light. The direct determination allowed an accurate quantitative analysis of each CDT component. After extraction of DNA from white blood cells (WBCs), a high resolution melting curve (HRM) was used for transferrin genotyping (Lightcycler 480 Fluorescence quantitative analyzer, Roche Diagnostics, F. Hoffmann-La Roche AG, Basel, Switzerland). Positive specimens were sent to Chengdu Qingkezixi Biological Technology Co., Ltd. for confirmation by sequencing.

2.4. Statistical analysis

Statistical analyses of the data were performed using the SPSS software (Version 19.0, SPSS) package. Data conforming to normal distributions were expressed as the median (25% percentile, 75% percentile). The rank-sum test was used to compare indicators among the 3 groups; receiver operating characteristics (ROC) curves were used to analyze each indicator's diagnostic performance in chronic alcoholism. Pearson correlation test was used to analyze the correlation between CDT and daily alcohol consumption.

3. Results

3.1. Differences in indicators among alcoholics, patients with NALD, and controls

After CE was performed on the 142 specimens in the 3 groups, transferrin components were isolated completely in 136 samples in 136 specimens (as shown in Fig. 1). The peaks of components in the 6 remaining specimens were not well separated, and thus, the content of each component could not be obtained; among them, 2, 3, and 1 specimens were from the healthy, NALD, and control groups, respectively. The comparison between the indicators among the 3 groups after excluding the patients mentioned above is shown in Table 1. No significant difference was noted in the levels of ALT between the alcoholic and control groups. Significant differences were observed in the MCV, ALT, AST, GGT, and CDT levels between the alcoholic and control groups, and between the alcoholic and differential diagnosis groups. Next, the ROC curves were constructed for these indicators, as shown in Figure 2. Among the 5 indicators employed to diagnose alcoholism, the calculated AUC value of CDT was the highest (0.922). In contrast, the AUC values of the other indicators, namely, GGT, AST, ALT, and MCV, were lower (0.860, 0.744, 0.615, and 0.754, respectively). Further analysis showed that when the cutoff value of CDT was set to 1.25%, the sensitivity and specificity were 85.5% and 89.6%,

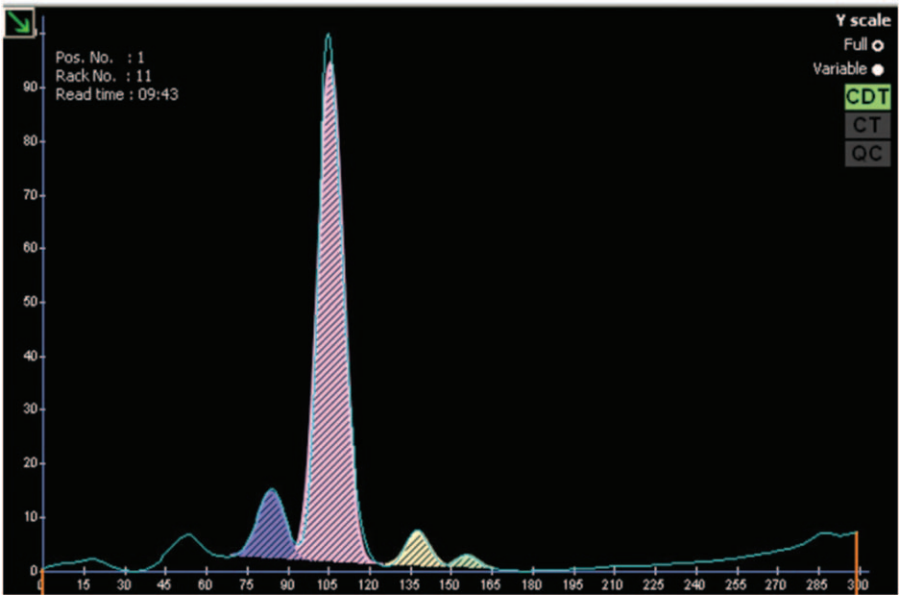

Figure 1. Diagram of carbohydrate transferrin analyzed by capillary electrophoresis.

respectively; when it was set to 1.65%, the corresponding values were 75.4% and 95.5%, respectively.

3.2. Correlation between serum CDT content and average daily alcohol consumption

To further understand whether there was a correlation between serum CDT content and alcohol consumption, patients were required to respond to the "survey on drinking in the last 2 months" during the admission questionnaire. The survey recorded the types of drink (namely, beer, liquor and spirits, red wine, health wine, and home-brewed wine), alcohol strength, quantity, and frequency in detail. The daily consumption of alcohol was obtained after conversion and divided into 5 subgroups according to the extent of intake. The median and interquartile of CDT percentage (%) in each group was calculated. As shown in Table 2, CDT% did not increase significantly with increased alcohol consumption. Pearson correlation analysis showed that the correlation between CDT and daily alcohol consumption was 0.175 ($P > .05$), indicating a minimal relationship between them.

4. Discussion

Although the Food and Drug Administration (FDA) has approved CDT as a marker for evaluating alcohol-drinking conditions in clinical practice, it has not been implemented due to the inconsistencies in detection methods and results between various laboratories. Besides, in China, only a few laboratories have evaluated this marker. Therefore, studies on the reference

Table 1

Comparison of the levels of MCV, ALT, AST, GGT and CDT in patients among 3 groups.

	Reference range	Control group (n = 52)		Non-alcoholic liver disease group (n = 20)		Alcoholism group (n = 70)	
Gender (M/F)		52/0		20/0		70/0	
Ethnicity		Han ethnicity		Han ethnicity		Han ethnicity	
Age		46.2 ± 9.8		46.4 ± 10.1		47.8 ± 7.6	
		Median (IQR)	No. of positive cases (%) [Note1]	Median (IQR)	No. of positive cases (%)	Median (IQR)	No. of positive cases (%)
MCV (fl)	82–100	93.2 (90.1–95.6)	1 (1.9%)[Note2]	92.1 (88.0–98.9)	4 (23.5%)	99.6 (93.1–103)[*,†]	34 (49.3%)
ALT (U/L)	<50	22 (18–31)	0	122 (46–326)[*]	12 (70.6%)	39 (22–67)[†]	25 (36.2%)
AST (U/L)	<40	22 (19–25)	0	135 (63–296)[*]	15 (88.2%)	59 (37–136)[*,†]	51 (73.9%)
GGT (U/L)	<60	20 (13–33)	0	107 (82–219)	15 (88.2%)	209 (63–490) [*,†]	52 (75.4%)
CDT (%)	≤1.6%[Note3]	0.80 (0.60–0.90)	1 (1.9%)	1.00 (0.60–1.30)	2 (11.8%)	2.60 (1.65–5.40)[*,†]	52 (75.4%)

ALT = alanine aminotransferase, AST = aspartate aminotransferase, CDT = carbohydrate-deficient transferrin, GGT = gamma-glutamyltransferase, IQR = interquartile range, MCV = mean corpuscular volume, NALD = non-alcoholic liver disease.

[Note1]: The number in the bracket was the percentage of the number of positive cases in the total number of cases in the group.

[Note2]: Positive MCV referred to the detection value of MCV was greater than 100 fl.

[Note3]: Reference range provided by instructions.

[*] $P < .05$: Comparison with control group.

[†] $P < .05$: Comparison with NALD group.

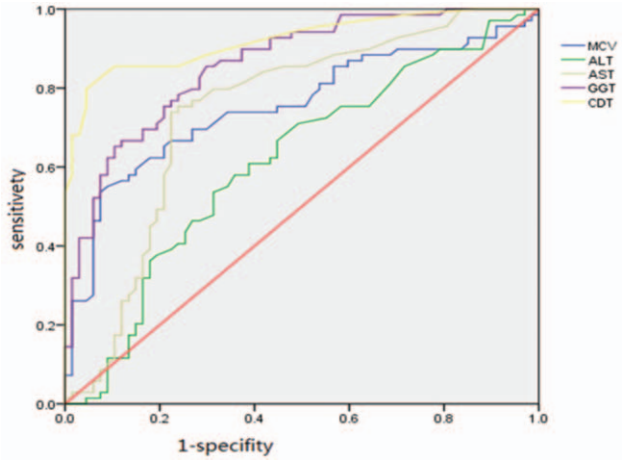

Figure 2. Receiver operating characteristics of mean corpuscular vol, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase, and carbohydrate-deficient transferri for diagnosing alcoholism.

range of CDT in the Chinese population and its application in the diagnosis of alcoholism are scarce.[7]

A recent study suggested that an intake of 60 to 80 g of alcohol per day for at least 2 weeks would increase the CDT level. After abstaining from alcoholic drinks for the same duration, reverted the level to the normal range.[4] The median daily alcohol consumption of the subjects in this study was 133 g, with a minimum and maximum of 24 g and 560 g per day, respectively. As shown in Table 1, the positivity rates for CDT and GGT were both equal to 75.4%, which was consistent with the results reported by Liangpunsakul.[8] However, this value was higher than the positivity rate of 50%, reported by Fagan.[9] In this study, among the 20 of nonalcoholic cirrhosis cases, 15 and 2 cases tested positive for GGT and CDT, respectively. Therefore, the specificity of CDT was significantly higher than that of GGT. The ROC curve showed that the AUC of CDT was the highest (0.922). Therefore, CDT was a better indicator for diagnosing alcoholism.

CDT is influenced by gender, body mass index, and smoking status of individuals.[10] Monika Ridinger et al[11] found that the relative concentration of CDT (%CDT) at study entry was higher in alcohol-dependent males than females (5.67% ± 0.74% vs 3.22% ± 0.37%, $P = .027$), although the daily alcohol consumption was comparable (197.0 ± 17.14 g/d vs 159.4 ± 21.19 g/d). Mundle et al[12] and Niemela[13] had mentioned that the clinical performance of CDT might have gender variation. In fact, it has been suggested that women express higher CDT levels under physiological conditions but might produce less CDT in response to heavy drinking, although the underlying reasons for such findings are still unclear. CDT has limited sensitivity as a biomarker of heavy alcohol consumption, particularly in women, patients with cirrhosis, and those with elevated BMI.[9] Polymorphisms of the transferrin gene also affect the detection of CDT.[14–16] In this study, after 6 interfered specimens were detected by HRM and confirmed by sequencing, 3 cases of transferrin gene linkage [TF-DChi (A > G)] heterozygous mutation were identified; in these cases, the amino acid (aspartate) is replaced by the loss of the sugar chain, resulting in the loss of saliva acid and an increase in the isoelectric point (pI) value, thereby affecting the electrophoretic mobility of each component, resulting in unreliable CDT results.[17]

Table 2

Relationship between CDT and daily alcohol consumption.

Average daily alcohol consumption (g)	No. of cases (case)	CDT (%)
≤60	6	2.45 (1.67, 3.4)
60–100	11	2.4 (0.90, 4.30)
100–160	24	2.45 (1.55, 6.37)
160–280	20	3.40 (2.02, 7.25)
≥280	5	2.10 (1.15, 14.1)

CDT = carbohydrate-deficient transferrin.

An ideal marker should have multiple uses, such as disease diagnosis, evaluation of the degree of illness, and guidance of treatment and prognosis. In this study, the correlation between CDT and average daily alcohol consumption was unsatisfactory. This might have been related to the complex wine culture in China. In the "survey on drinking in the last 2 months," subjects often drank 2 to 3 types of alcohol simultaneously, including beer, liquor and spirits, red wine (purchase or home-brewed), and even health wine, making it difficult for patients to evaluate the strength and quantity of each drink accurately. The estimation of daily alcohol consumption by participants could also have errors. Laatikainen et al found that alcohol consumption was not reliably estimated by self-reporting in a Russian cohort.[18] Bertholet et al reported that CDT and GGT misdiagnosed alcoholism to a certain extent. [19] However, Schellenberg[20] conducted a study on the association between CDT and the self-reported drinking volume in 183 subjects; the results showed a significant correlation between the two. Lee et al reported that advanced liver fibrosis had a possible negative influence on the levels of CDT.[21]

China has a large population with a well-established alcohol culture. However, it also has a large population with hepatitis B. It is often necessary to clinically distinguish between liver injury related to alcohol and other causes; since some patients often conceal their history of alcoholism, it affects the clinical judgment regarding the disease. Therefore, in this study, the NALD group was used as a differential control group to evaluate the clinical applicability of CDT more objectively.

Some new markers have recently been proposed for alcoholism; these include, ethyl glucuronide (EtG), ethyl sulfate (EtS), fatty acid ethyl esters (FAEE), and phosphatidylethanol (PEth), among others.[4,10,21,22] Combining 2 or more biomarkers could improve the diagnostic efficiency of the alcoholics. The combination of GGT and CDT using a mathematically formulated equation GGT-CDT = 0.8 × ln (GGT) + 1.3 × ln (CDT) can improve the detection of excessive alcohol consumption by increasing assay sensitivity without loss in specificity in the diagnosis of excessive alcohol consumption.[23] Helander et al[24] found that the PEth and CDT have a good correlation, which was supported by observing a much better intra-individual agreement between PEth and CDT. The results from Tsanaclis et al[25] showed that a PEth test combined with an EtG test was just as efficient in the diagnosis of alcohol use as a PEth test in combination with EtG and EtPa together in the diagnosis of alcohol use.

5. Conclusion

Our present findings demonstrated that CDT is a better predictor of alcoholism, compared with the other indicators evaluated.

Thus, its use as a biomarker of alcohol abuse must be actively promoted in clinical practice. It is essential to continue with the discovery of more sensitive and specific markers in the future for the early identification of alcoholics to enable the implementation of interventions that would prevent serious consequences such as alcoholic hepatitis, liver fibrosis, and cirrhosis.

Author contributions

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Writing – original draft: Shanshan Liang.
Writing – review & editing: Cheng-yao Jia, Wei Gan.

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