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Determination of serum carbohydrate-deficient transferrin by a nephelometric immunoassay for differential diagnosis of alcoholic and non-alcoholic liver diseases

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ABSTRACT

Background: Carbohydrate-deficient transferrin is a biological marker of excessive drinking. The aim of this study was to evaluate the diagnostic value of a direct nephelometric immunoassay for the differential diagnosis of alcoholic and non-alcoholic liver diseases in comparison with gamma glutamyl transferase.

Methods: Serum samples were obtained from 305 subjects, including 122 patients with alcoholic and 102 cases with non-alcoholic liver diseases. Serum levels of carbohydrate-deficient transferrin were expressed as a percentage of total transferrin.

Results: Serum % carbohydrate-deficient transferrin levels were significantly higher in patients with alcoholic than with non-alcoholic liver diseases. Carbohydrate-deficient transferrin had better specificity than gamma glutamyl transferase to differentiate between alcoholic and non-alcoholic liver diseases.There were 8 alcoholic liver disease patients with normal gamma glutamyl transferase levels, and carbohydrate-deficient transferrin was significantly elevated in 6 of them. On the other hand, there were 25 non-alcoholic liver disease patients with elevated gamma glutamyl transferase levels; their carbohydrate-deficient transferrin levels were within the reference intervals in all cases.

Conclusion: This simple carbohydrate-deficient transferrin immunoassay is useful to detect so-called gamma glutamyl transferase non-responding drinkers and also to exclude the possible role of excessive drinking in apparently non-alcoholic liver diseases. A large-scale prospective study is needed to further confirm the diagnostic utility of carbohydrate-deficient transferrin.

1. Introduction

Excessive alcohol drinking causes not only alcoholic liver disease (ALD) [1] and pancreatitis [2], but it is also a contributory factor to other disorders, such as esophageal cancers [3] and stroke [4]. Although the first line of detecting heavy drinking relies on self-reporting, it is well recognized that self-report surveys result in substantial underestimates of alcohol consumption [5].Therefore, objective markers to identify individuals with alcohol-related problems are needed.

Diagnostic characteristics and applications of alcohol biomarkers have been extensively studied [6, 7]. Gamma-glutamyl transferase (GGT), a membrane-glycoprotein enzyme, has traditionally been used as a marker of excessive drinking. Although sensitive, GGT is not specific enough, and it is elevated in a wide spectrum of non-alcoholic disorders. Indeed, GGT has recently been regarded as a biomarker of oxidative stress independent of alcohol abuse [8]. Measurement of urinary ethyl glucuronide, a nonoxidative metabolite of ethanol, is increasingly used as an indicator of recent alcohol consumption [9]. More recently, ethyl glucuronide in hair and fingernails has been suggested as a potential indicator of long-term alcohol abuse [10].

Since the first report by Stibler and Kjellin in 1976 [11], carbohydrate-deficient transferrin (CDT) has been well studied as a biomarker of alcohol abuse [12]. Various methodologies have been used to measure CDT, as reviewed elsewhere [13]. The traditional methods are

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labor intensive, which has hampered wide use of CDT measurement. The direct immunoassay for CDT [14] has recently been approved as in vitro diagnostics in many countries and is going to be widely used in clinical practice.

GGT levels are elevated in patients with a variety of liver diseases, including not only alcoholic liver diseases, but also non-alcoholic liver diseases. It is necessary to have laboratory tests to specifically exclude an excessive drinking history in patients with apparently non-alcoholic liver diseases.

In the present study, the roles of serum carbohydrate-deficient transferrin (CDT) measurement by the simple nephelometric immunoassay for differential diagnosis of alcoholic and non-alcoholic liver diseases (non-ALD) were tested in comparison with GGT.

2. Methods

2.1. Subjects studied

Serum samples were obtained from 305 subjects, including 122 patients with alcoholic liver diseases who visited the National Hospital Organization Kurihama Medical and Addiction Center seeking treatment for alcohol dependence. Sixteen of these 122 patients had liver cirrhosis based on their clinical and radiological findings. Serum samples were obtained on the day of hospitalization. For comparison, 102 cases (50 men and 52 women) of biopsy-proven non-alcoholic liver diseases (non-ALD) encountered in Chiba University Hospital were also included: 16 hepatitis B virus-related chronic liver diseases, 38 hepatitis C virus-related chronic liver diseases, 19 primary biliary cholangitis (PBC), 17 non-alcoholic fatty liver diseases, 7 autoimmune hepatitis (AIH), and 5 others (such as mild constitutional jaundice). Six patients with PBC and one patient with AIH were under treatment at the time of sampling. For the rest of the cases, blood samples were obtained before starting treatment. Serum samples were also obtained from 81 apparently healthy subjects (33 men and 48 women) without any drinking history to calculate the reference intervals for CDT and GGT. All subjects provided their written, informed consent, and the Ethics Committee of each institution approved this study.

2.2. Determination of CDT and GGT

The nephelometric system BNII (Siemens Healthcare Diagnostics, Japan) and the N Latex CDT assay (Siemens Healthcare Diagnostics, Marburg, Germany) were used. N Latex CDT is a monoclonal antibody-based direct immunoassay detecting disialo-, monosialo-, and asialo-transferrins, and it can quantify those CDT glycoforms [14]. Simultaneous measurements of total transferrin with a polyclonal antibody-based assay (Siemens Healthcare Diagnostics, Japan) performed on the same instrument allowed an automatic calculation of the CDT value as a percentage of total transferrin [14]. Serum GGT activities were determined using an enzymatic assay (Serotec Co, Ltd., Sapporo, Japan) with an autoanalyzer (JCA-2250; JEOL, Ltd., Tokyo, Japan). Separated serum samples were stored in aliquots at −80 °C until analyzed.

2.3. Statistical analysis

The numerical data are presented as means ± SD. All data were analyzed by Stat Flex Ver 6.0. Receiver-operating characteristic (ROC) curves were drawn using Stat Flex Ver 6.0. The reference intervals of GGT and CDT were calculated in a non-parametric way with the 25th to 97.5th percentiles. Multiple comparisons were performed by the Steel-Dwass test using software R (Ver3.3.1). P values < 0.05 were considered significant.

3. Results

Clinical characteristic (including the alcohol drinking history) of ALD and non-ALD patient groups and healthy controls are presented in the Table 1. All but one patient with ALD were males.

Table 1
Clinical characteristics of subjects studied.

	Healthy controls	NALD	ALD
	(N = 81)	(N = 102)	(N = 122)
M/F	33/48	50/52	121/1
Drinking history	No drinking history	No drinking history or occasional light drinking (less than once a month and, < 20 g/day)	> 60 g/day, > 10 yrs
AST (U/L)	20 ± 5	34 ± 18[a]	124 ± 170[b]
ALT (U/L)	28 ± 10	36 ± 32	62 ± 55
GGT (U/L)	17 ± 11	50 ± 84[a]	478 ± 503[b]

[a] P < 0.001 vs controls.
[b] P < 0.001 vs controls, non-ALD.

The reference intervals for %CDT and GGT obtained in 81 apparently healthy subjects without any drinking history were 1.24–2.16% and 10–53 U/l, respectively. Serum levels of total transferrin (Fig. 1A), CDT (Fig. 1B), and %CDT (Fig. 1C) in the three subject groups are presented in Fig. 1.

Serum %CDT levels were 1.67% ± 0.27% in healthy controls, 1.66% ± 0.40% in patients with non-alcoholic liver diseases and 3.73 ± 2.28% in patients with alcoholic liver diseases (Fig. 1C).

The % CDT values were significantly higher in patients with ALD than in healthy controls and in patients with non-ALD. To differentiate between alcoholic and non-alcoholic liver diseases, the area under the ROC curve was the greatest for %CDT (Fig. 2). Among ALD patients, CDT values were not significantly different between patients with and without cirrhosis (2.87% ± 1.17% vs 3.85% ± 2.39%).

No sex differences were seen in %CDT levels in healthy controls (1.66% ± 0.28% vs 1.68% ± 0.28%) and in non-ALD patients (1.63% ± 0.23% vs 1.68% ± 0.24%).

Although the area under the ROC curve of %CDT and GGT were comparable (Fig. 3),

%CDT had better specificity than GGT, whereas GGT had higher sensitivity than %CDT.

to differentiate between alcoholic and non-alcoholic liver diseases (Table 2). Setting the cut-off levels for GGT and %CDT at the upper limit of their reference intervals, GGT had 90% sensitivity to identify patients with ALD, but the specificity was 77%. On the other hand, % CDT had 97% specificity and 72% sensitivity.

There were 8 ALD patients in whom GGT activities were within the reference intervals (Table 3). CDT was elevated in 6 of these 8 patients. There were 25 non-ALD patients (13 men and 12 women) with elevated GGT levels, and their CDT levels were within the reference intervals in all cases (Table 4).

4. Discussion

Transferrin is the most important iron transporting protein, synthesized primarily in hepatocytes. Transferrin has two N-glycan chains terminating with sialic acid molecules. Tetrasialo-transferrin is the most abundant form in serum under nonpathological conditions [15]. Transferrin glycoforms with the isoelectric points (PIs) ≥5.7, namely asialo-, monosialo-, and disialo-transferrins, are collectively defined as CDT [16].

The role of CDT in the clinical diagnosis of ALD is well-known and has been described in clinical practical guidelines [17] as a representative laboratory marker. Wide use of this marker, however, was hampered mainly by a lack of simple and easy methods for its determination until direct immunoassay for quantifying CDT in serum was developed [14]. This assay, called N Latex CDT, uses a monoclonal