SHOULD TRI-SIALO-TRANSFERRINS BE INCLUDED WHEN CALCULATING CARBOHYDRATE-DEFICIENT TRANSFERRIN FOR DIAGNOSING ELEVATED ALCOHOL INTAKE?

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Abstract — CDT (carbohydrate-deficient transferrin) has been identified as a specific marker for chronically elevated alcohol consumption. We investigated the sensitivity and accuracy of using relative concentrations of different isotransferrins in serum for diagnosis of chronically elevated alcohol consumption. The different transferrin variants (isoforms) were quantified by HPLC. Including the tri-sialo-transferrin fraction into the definition of %CDT resulted in an increased accuracy in the detection of chronically elevated alcohol intake in a study among 17 heavy drinkers, 25 healthy individuals with moderate alcohol consumption and nine total abstainers. The results also suggest that desialylation of transferrin is a gradually continuing process, rather than one leading to a single end-result separating asialo-, mono- and disialo-transferrins from trisialo-, tetrasialo-, pentasialo- and higher sialo-transferrins.

INTRODUCTION

During the last two decades, serum CDT (carbohydrate-deficient transferrin) has been studied as a marker for elevated long-term alcohol intake. The clinical specificity and sensitivity of serum measurements of variants (isoforms) of transferrin with different sialic acid contents for monitoring long-term alcohol intake have been well documented (Stibler, 1991). In both the isoelectric focusing (IEF) and high-performance liquid chromatographic (HPLC) methods, CDT is usually expressed as a percentage of total transferrin (%CDT). In minicolumn methods, both absolute units (units/l) of CDT and relative amounts (%CDT) are in use for CDT measurements.

The biological mechanism resulting in the increase in CDT variants in serum has been debated, but it has been generally concluded that it is the sum of transferrin variants with two or fewer sialic-acid residues (pI ≥ 5.7) that are the variants to be included in the estimation of CDT (Stibler et al., 1986). Very few studies have dealt with the tri-sialo-transferrin variant in relation to ethanol abuse. However, some authors concluded that the tri-sialo-transferrin variant shows no increase with, or correlation to, alcohol intake (Stibler et al., 1979, 1986; Vesterberg et al., 1984).

This study presents the effect of including the tri-sialo-transferrins in the CDT parameter, and raises the question whether the clinical biochemical testing for diagnosis of heavy drinking would benefit from including this fraction into the definition of CDT.

METHODS

Clinical serum samples

Heavy drinkers (HD). Serum was sampled from alcohol-intoxicated patients admitted to the Universitätsklinik für Psychiatrie, University of Vienna, in October 1994. The patients were carefully interviewed according to a questionnaire at the time of the admission. For this study, samples were obtained from 17 patients with a drinking pattern characterized by high intake, ranging from 100 to >400 g of alcohol daily for periods ranging from 2 weeks to >6 months. For all patients, the sobriety period before blood sampling was <14 days, with a relapse minimum 3 days before blood sampling. The patients were males aged 34–64 years.

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Fig. 1. The HPLC elution profile of transferrin from iron-saturated serum samples on the porous HQ10 ion exchange column.

(A) Total abstainer. (B) Social drinker. (C) Heavy drinker. Digits below indicate number of sialic-acid residues per transferrin molecule. Dotted line is Cl⁻ gradient. Lower part shows enlarged part of upper chromatograms. The low sialic-acid-containing transferrin variants (CDT) are eluted first in the chromatogram due to lower negative charge of the molecule. The increasing chloride content in the gradient thereafter elutes the transferrin molecules with higher numbers of sialic acid residues. The elution pattern demonstrates an elevation of the relative concentration of both disialo- and trisialo-transferrin concentrations among a heavy drinker (C), as compared to a total abstainer and a social drinker (A and B respectively).

Social drinkers (SD). Serum was sampled from 25 healthy individuals of both sexes from 18 to 70 years old, with moderate alcohol consumption (<40 g per day on average) according to an interview at the time of the serum sampling.

Total abstainers (TA). Serum was sampled from nine individuals (30–82 years old), who were total abstainers, of both sexes and active in an anti-alcohol-organization’s quire. None was known to be a former alcoholic.

HPLC analysis. The serum samples were analysed by the use of strong anion-exchange chromatography using a Poros HQ10 (Perseptive Inc. USA) column (0.5 cm x 5 cm), and Pharmacia Fast Protein Liquid Chromatography equipment with UV detection at 450 nm. Serum samples (120 µl each) were pretreated with 24 µl Fe(III)-maleic-citrate (9.25 mM) solution for iron saturation (30 min). Thereafter, 1.3 µl solution of 100 mg dextran/ml of water and 6 µl solution of CaCl₂ (147 mg/ml of water) were added per 120 µl serum. Samples were kept in a refrigerator for 1 h before they were centrifuged and 100 µl of supernatant was diluted to 2.15 ml with water before 2.0 ml were applied to the column for chromatography.

Transferrin variants were eluted by using a Cl⁻ gradient in a buffer system consisting of buffer A: 20 mM Bis-Tris pH = 6.1; and buffer B: 20 mM Bis-Tris, 0.3 M NaCl pH = 6.0. Flow 3.0 ml/min. A gradient of buffer B mixed with buffer A started with 0–5% from the first 5–7 ml of eluate, increasing thereafter to 18% for the next 7–25 ml and then kept at 18% during analytical run. The 450 nm absorption signal from the different variants of transferrin was recorded and the relative amount of each variant was calculated by peak integration of chromatogram. The impre-
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Fig. 2. The %CDT values of serum samples from different clinical subgroups.

The left-hand side of the Figure shows the results of using di-sialo and lower transferrin variants, whereas the right-hand side shows the effect of using tri-sialo and lower transferrin variants for calculation of %CDT. TA = total abstainers; SD = social drinkers; HD = heavy drinkers.

Precision of the method was found to give a relative coefficient of variation (%CV) of <5% (inter- and intra-day variation).

Statistical analysis

The clinical information for each sample was analysed in relation to the amounts of α-, mono-, di- and tri-sialo-transferrins relative to total transferrin concentration. The effect of including the relative amount of the tri-sialo-transferrin CDT analysed by HPLC was especially investigated. Dunn’s multiple comparison test was performed when comparing the relative amount of tri-sialo-transferrin in the three groups.

RESULTS

Figure 1 demonstrates the typical elution patterns from the HPLC column at 450 nm. Examples are shown of a non-drinker, a social drinker and a heavy drinker. Due to differences in electric charge, the low sialic-containing transferrin variants (CDT) are eluted at the beginning of the chromatogram. The increasing chloride content in the gradient thereafter eluted the transferrin molecules with higher numbers of sialic acid residues.

Figure 2 demonstrates the effect on the results from the groups of the different drinking patterns when: (i) only the transferrin variants with two or fewer sialic acid moieties are included in the calculation of the %CDT; (ii) also including the tri-sialo-transferrins in the calculation of the %CDT. A clearer separation between the heavy

Fig. 3. The 3-sialo-transferrin content in alcohol-drinking subjects.

The Figure shows the differences in the relative amount of 3-sialo-transferrin in the groups: TA = total abstainers; SD = social drinkers; HD = heavy drinkers. Horizontal lines represent median values in each group.
drinkers on the one hand and the social drinkers and non-drinkers on the other, was achieved when the tri-sialo-transferrin variant was included. At a 100% specificity (including the social drinkers with non-alcoholic group), using only variants with two or fewer sialic acid residues resulted in a sensitivity of 82.4, whereas a sensitivity of 100% was obtained by including the tri-sialo-transferrin.

The use of Dunn's multiple comparisons test when comparing the relative amount of tri-sialo-transferrin in the groups with different drinking patterns (see Fig. 3) showed that the relative concentration of tri-sialo-transferrin was significantly raised in the group of heavy drinkers compared with both the total abstainers and the social drinkers ($P < 0.001$ for both). The relative tri-sialo-transferrin contents of the social drinkers were not significantly higher than in the group of total abstainers.

**DISCUSSION**

A general route for the metabolism of serum glycoproteins (e.g. transferrin) is a desialylation process followed by binding to and removal from circulation by the asialoglycoprotein-receptors of liver cells (Ashwell and Harford, 1982; Morell, 1971). These receptors have been demonstrated to be vulnerable to impairment by alcohol feeding of rats (Casey et al., 1989, 1991). Such an impairment may be the reason for elevation of serum desialylated (carbohydrate-deficient) transferrins in heavy drinkers. An elevation of trisialo-transferrin would be expected among heavy drinkers assuming that the metabolic process from high to low sialic-acid transferrin is a continuous one. The present results (Figs 2 and 3) have demonstrated such an elevation and strongly suggest that determination of the tri-sialo-transferrin fraction should be included when the relative CDT value is calculated for diagnosing elevated alcohol intake.

Why have only asialo-, monosialo-, and disialo-transferrins previously been included into the CDT parameter? It is possible that a lack of good quantitative analytical methods is the main reason. With isoelectric focusing, a quantifiable elevation of the trisialo-transferrin variants may be difficult to measure, since non-drinkers and social drinkers have a considerable trisialo-transferrin content in serum. A good linear quantification of both high and low concentrations of transferrin bands is difficult to obtain simultaneously in an isoelectric focusing gel. However, using a high-resolution HPLC method with a good dynamic quantitative range, a clearer separation or distinction between social drinkers and heavy drinkers was obtained when the tri-sialotransferrin variant was included. Our findings suggest that further investigations to establish the best clinically analytical composition of transferrin variants in clinical testing of long-term alcohol intake may be fruitful.

**REFERENCES**


