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QUANTIFYING CARBOHYDRATE-DEFICIENT TRANSFERRIN IN SERUM

KVANTIFIKACIJA TRANSFERINA DEFICIJENTNOG UGLJENIM HIDRATIMA U SERUMU

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Summary: Alcohol abuse is a major public health problem with significant consequences for the society and economy. A biomarker commonly used for the analysis of alcohol abuse is serum CDT (carbohydrate-deficient transferrin). Very few conditions other than heavy alcohol consumption over a period of two to three weeks cause serum CDT to rise. Here we report a capillary electrophoresis method that is able to quantify CDT and the high resolution and reproducibility of the method make it possible to identify potential variants while avoiding false results.

Keywords: CDT, alcohol abuse, transferrin

Kratak sadržaj: Zloupotreba alkohola jedan je od glavnih zdravstvenih problema sa značajnim posledicama za društvo i ekonomiju. Biomarkeri koji se obično koristi za analiziranje zloupotrebe alkohola je CDT u serumu. Vrlo malo stanja/poremećaja pored preterane konzumacije alkohola tokom perioda od dve do tri nedelje koje se može kvantifikovati CDT, dok visoka rezolucija i reproducibilnost metode omogućavaju identifikaciju potencijalnih varijanti bez opasnosti od lažno pozitivnih rezultata.

Ključne reči: CDT, zloupotreba alkohola, transferin

Introduction

The abuse of alcohol represents a major public health problem with very important consequences for the society and economy and the European region has the highest alcohol consumption in the world. The total societal costs of alcohol amount to between 1% and 3% of the gross domestic product. The consumption of alcoholic beverages accounts for 10–11% of illnesses and deaths each year (1). A biomarker commonly used for the analysis of alcohol abuse is CDT (carbohydrate-deficient transferrin) (2). The unique advantage of CDT is that very few conditions other than alcohol consumption cause CDT to rise. Because the half-life of transferrin is 14 days, measurement of CDT can reflect the mean blood alcohol concentration (consumption) over the preceding two to three weeks. Capillary electrophoresis has been shown to be a reliable technique for the assay of CDT when compared with traditional immunoassay methods (3).

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Transferrin (Tf) is an iron-transport glycoprotein which consists of a polypeptide chain with two binding sites for iron and two N-linked oligosaccharide chains. The oligosaccharide chains are microheterogeneous and carry sialic acid residues. Transferrin can be separated into several isoforms based on this structure. The isoforms of transferrin have isoelectric points (pI) that range from 5.2 to 5.9, with the predominant isoform (tetrasialo) having a pI of 5.4. This 4-sialo Tf is the most common isoform and represents about 70 to 80% of total Tf content. Other isoforms that can be detected are 6-sialo Tf, 5-sialo Tf, 3-sialo Tf, 2-sialo Tf and sometimes 0-sialo Tf. When analysing serum from an alcohol abuser one can find an increase in 2-sialo-Tf and 0-sialo Tf will gradually appear (4).

Asialo- and disialotransferrin, which result from an impaired glycosylation mechanism, are commonly referred to as carbohydrate-deficient transferrin (CDT), which is an important marker of chronic alcohol abuse. Percentage (%) CDT is generally referred to as the sum of % 0-sialo plus % 2-sialo Tf. Since the half-life of Tf is 10 to 15 days you will see a decrease of this parameter when a patient is entering an alcohol withdrawal treatment.

We have developed a simple and rapid method to quantify the percentage of CDT from serum. With

this method, we are able to show a difference of migration between transferrin carrying two sialic acids after desialylation and the disialotransferrin from serum of alcoholic patients. Conversely, the transferrin carrying three sialic acids after desialylation and the trisialotransferrin co-migrate. This suggests that the disialotransferrin fraction not only carries less sialic acid, but also lacks one of the entire carbohydrate chains. Reproducibility, total imprecision, and the influence of variants were determined.

Materials and Methods

Capillary zone electrophoresis was performed on both a P/ACE™ 5000 system equipped with a UV detector and on a P/ACE MDQ system equipped with a UV detector, sample refrigeration and large buffer reservoir (Beckman Coulter Inc, Fullerton, California). Buffers and diluents were from the CEofix CDT kit (Analis s.a., Namur, Belgium). The borate buffer at pH 8.5 uses a patented double coating of the capillary to improve resolution and reproducibility. The kit also contains an Fe solution for sample preparation. The capillary (Polymicro, Phoenix, AZ, USA) has an internal diameter of 50 µm and a length of 50 cm to the detector. Both the P/ACE 5000 and P/ACE MDQ gave similar results, varying only by migration time. Sample preparation entails mixing volume-by-volume 50 µL serum and the Fe solution in a microvial for the P/ACE 5000 system or 60 µL for the P/ACE MDQ. First the fused-silica capillary is rinsed with the »initiator« solution which coats the capillary wall with a polycation. This is followed by a second rinse with a »buffer« solution, containing a polyanion, which adds a second layer of coating. The »serum solution« is loaded by pressure injection for the P/ACE 5000 system and by vacuum for the P/ACE MDQ. Separation is performed at a voltage of 28 kV and detection is at 200 nm. The corrected peak areas are calculated by the integration software and expressed in area percent of the total transferrin. After separation, the coating is cleared by a rinse with NaOH, and the capillary is ready for the next analysis.

Results and Discusion

Separation Sera were obtained from both men and women with well documented drinking habits. These subjects were classified as heavy drinkers or social drinkers and analyzed with the above method. For social drinkers, the separation and quantification of disialo-, trisialo-, tetrasialo-, pentasialo-, and hexasialotransferrin is shown in *Figure 1*. For heavy drinkers, an increase of disialotransferrin and the presence of asialotransferrin were noted.

Reproducibility and imprecision

Evaluation of the precision performance was conducted in the Department of Clinical Pharmacology, University of Bern, Switzerland, according to »Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline. NCCLS document EP5-A« [ISBN 1-56238-368- X]. This evaluation was carried out on a P/ACE MDQ system. Samples and controls (sera) were run in duplicate, two runs per day, over 20 days. The results are reported in *Table I (*Professor Wolfgang Thormann Ph.D., Professor and Head of the Analytical Laboratory Dept. of Clinical Pharmacology, e-mail: wolfgang.thormann@kp.unibe.ch; and Christian Lanz, University of Bern, Switzerland).

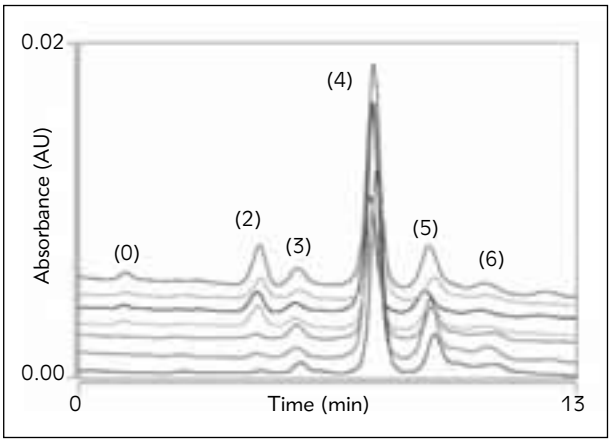

Figure 1 Example of separation of transferrin isoforms obtained for 4 heavy drinkers and 3 social drinkers. Instrument used: P/ACE 5000. Peaks detected: (0) asialotransferrin, (2) disialotransferrin, (3) trisialotransferrin, (4) tetrasialotransferrin, (5) pentasialotransferrin, (6) hexasialotransferrin.

Table I Evaluation of Precision Performance.

Samples and controls (sera) were run in duplicate, 2 runs per day, during 20 days (N = 80). Instrument used: P/ACE™ MDQ.								
	Sample				Control			
	Low		High		Low		High	
	Asialo	Disialo	Asialo	Disialo	Asialo	Disialo	Asialo	Disialo
Mean	–	0.89	0.68	3.20	–	0.94	0.75	3.59
SD	–	0.06	0.12	0.12	–	0.06	0.07	0.13
CV	–	7.2	17.7	3.9	–	6.6	9.0	3.6

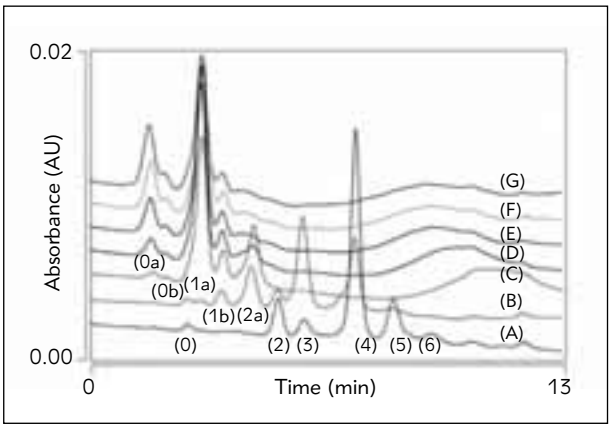

Figure 2 Desialylation obtained by incubation with neuraminidase of serum from a heavy drinker. From electropherogram (A) to (G), each time the Example of separation of transferrin isoforms obtained f-minute longer incubation time. Instrument used: P/ACE. Peaks detected: (0) asialotransferrin, (2) disialotransferrin, (3) trisialotransferrin, (4) tetrasialotransferrin, (5) pentasialotransferrin, (6) hexasialotransferrin. Desialylation of pentasialo-, tetrasialo-, and trisialotransferrin will result in fraction (2a), (1a), and (0a). Desialylation of disialotransferrin will result in fractions (1b) and (0b).

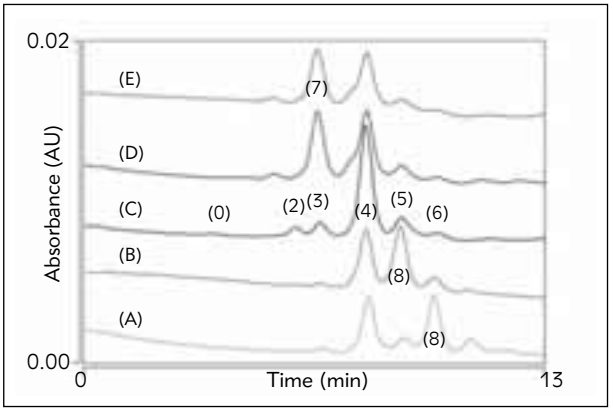

Figure 3 Separation of genetic variants of transferrin. Trace (C) is from heavy drinkers, Traces (A) and (B) are CB phenotypes, while Traces (D) and (E) are CD phenotypes. Instrument used: P/ACE. Peaks detected: (0) asialotransferrin, (2) disialotransferrin, (3) trisialotransferrin, (4) tetrasialotransferrin, (5) pentasialotransferrin, (6) hexasialotransferrin, (7) tetrasialotransferrin of D phenotype, (8) tetrasialotransferrin of B phenotype.

Effect of desialylation on the various isoforms

To demonstrate the ability of the proposed method to resolve all the CDT isoforms, serum of a heavy drinker was incubated with neuraminidase for increasing time to obtain sequential cleavage of the sialic acid residue. These results are shown in *Figure 2*. This electropherogram clearly indicates that after desialylation pentasialo-, tetrasialo-, and trisialotransferrin produce an asialotransferrin called 0a, whereas disialotransferrin produces another asialotransferrin called 0b. Both are different from the asialotransferrin found in sera from heavy drinkers.

Interference of variants

Genetic variants, attributable to substitutions of amino acids in the polypeptide chain of human serum can occur. At least 38 transferrin variants have been described. In addition to the common (C) transferrin type, anodal (B) and cathodal (D) variants have been reported in different human populations. It is important to be able to identify the heterozygous CB and CD phenotypes to avoid false positive or false negative results. The resolution of these variants is highlighted in *Figure 3*.

Conclusion

This capillary electrophoresis method has proven to be effective to quantify CDT which is a valuable marker in alcohol abuse research. This method can be automated easily with the P/ACE capillary electrophoresis system from Beckman Coulter. The high resolution and reproducibility of the method make it possible to identify potential variants while avoiding false results. Commercial kits are available through Analis for research purposes: each kit contains: rinse, conditioner, initiator, buffer and Fe solution. CEofix CDT kit for P/ACE MDQ (100 tests) Analis PN: 10-004740/844111036.

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