Carbohydrate deficient transferrin and alcoholism

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Abstract

Alcohol abuse is an important public health problem, with major implications in patients with a pre-existing liver pathology of viral origin. Hepatitis C, for example, is one of the diseases in which alcohol consumption can lead to the transition from a fairly benign outline to a potentially life-threatening liver disease.

Alcohol abuse is usually identified on the basis of clinical judgment, alcoholism related questionnaires, laboratory tests and, more recently, biomarkers. Also on this list of tests, carbohydrate deficient transferrin (CDT) is widely available and useful for determining recent alcohol consumption, particularly when corroborated with elevation of other liver-associated enzymes. Clinicians should be aware of the indications and limitations of this test in order to better evaluate alcohol consumption in their patients.

Keywords Carbohydrate deficient transferrin, hepatitis, alcohol consumption

Alcohol abuse is an important public health problem. This condition is usually identified on the basis of clinical judgment, alcoholism related questionnaires and laboratory tests, i.e. gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST) or mean cell volume (MCV). However, the relatively low sensitivity and specificity of these tests (50% sensitivity and 96.7% specificity for GGT, 70% and 93.3% for AST and MCV)¹ led to a search for a specific marker. Several tests have recently been proposed, such as mitochondrial acetaldehyde adducts, beta-hexosaminidase or phosphatidylethanol (PEth), with promising results.

Carbohydrate deficient transferrin (CDT) has been shown to be more useful than other widely available biochemical tests for alcohol abuse. Structurally, transferrin is a polypeptide with two N-linked polysaccharide chains, branched with sialic acid residues. According to the level of

sialylation, there are various forms of transferrin, tetrasialotransferrin being predominant. The proportion of transferrin with zero, one or two sialic acid chains increases with alcohol consumption but also in the carbohydrate deficient glycoprotein syndrome, leading to the so-called carbohydrate deficient transferrin.

Elevated levels of CDT are suggestive of recent alcohol abuse, particularly when corroborated with elevation of other liver-associated enzymes. Some of the uses of CDT include long term monitoring for early detection of relapse drinking during medical treatment, permitting early intervention.² It may also aid in the assessment for reinstating driver licenses or in identifying chronic alcoholics among traumatized patients.³

Methods of determination include: ion-exchange chromatography on minicolumns, radioimmunoassay, immunoblotting, densitometry and high performance liquid chromatography (HPLC), which has been recommended as reference method until a standardized mass spectrometric method is established.⁴ Through HPLC, five transferrin isoforms are separated at 460 nm: asialo-, disialo-, trisialo-, tetrasialo- and pentasialo-transferrin isoforms.⁵ The clinical significance of CDT is determined by looking specifically at the disialo and asialo forms. Slight differences between

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testing methods may be related to the tests' ability to separate and detect asialo, monosialo, and disialo isoforms.⁶ The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Working Group on CDT Standardization recommended disialotransferrin as the primary target molecule for CDT measurement and the single analyte for CDT standardization.⁴

CDT has been used as a test for excessive alcohol consumption in research, clinical, and medico-legal settings, but there still is conflicting data on its accuracy, with sensitivities ranging from under 20% up to 100%.⁷ In field literature, the results of paired studies obtained with commercially available CDT assays did not prove to be significantly better than with GGT. Scouller et al., after performing a meta-analysis of the data published so far, concluded that further high-quality studies comparing CDText (modified) and other CDT assays with GGT in the same subjects are needed.⁷

A more recent study¹ compared CDT, MCV, AST, ALT, GGT and sialic acid (SA) in subjects which had declared themselves recently abstinent from alcohol consumption. Their results showed that CDT appeared to have higher sensitivity (95% compared with 70% for MCV and AST, 50% for ALT and GGT and only 10% for SA) but the sensitivity decreased for all studied alcohol markers when the period of abstinence was longer than one week.¹ In this context, it might be useful to also discuss the use of markers which yield a longer time horizon for judging drinking levels. For example, measurement of ethyl glucuronide (EtG) from hair is increasingly used for determining chronically increased alcohol consumption.^{8,9} A study comparing hair EtG and CDT in subjects with a two-week and/or three-months history of 60 g/day ethanol intake showed superior sensitivity of EtG (100% vs. 44% for CDT) with a similar specificity of 93% at two weeks, measured by immunonephelometry for cut-off values of 27 pg/mg for EtG and 2.5% for CDT. The same study reported different results when measurements were performed through HPLC: 96% sensitivity for EtG vs. 50% for CDT and an equal specificity of 70%.¹⁰

One other remarkably good direct marker of alcohol intake is phosphatidylethanol (PEth)¹¹ which can be detected in blood for up to two weeks of sobriety.¹² Other studies focused on the sensitivity and specificity of ethanol and methanol concentration in plasma, and the 5-hydroxy-tryptophol (5-HTOL) to 5-hydroxy-indoleacetic acid (5-HIAA) ratio in urine as laboratory tests to identify acute alcohol consumption. Comparison was made with self reported drinking levels. The study included different drinking categories (nondrinkers, light/moderate drinkers, heavy drinkers, patients receiving treatment for alcohol dependence) from five study populations (Australia, Brazil, Canada, Finland and Japan) and it showed that 5-HTOL/5-HIAA ratio was the most sensitive and ethanol the least sensitive indicator of recent alcohol consumption.¹³

Given the fact that transferrin is a steroid-responsive protein, CDT is known to vary with sex and possibly with age.¹⁴ Therefore, the IFCC Working Group proposed percent carbohydrate-deficient transferrin (%CDT) as preferred method of reporting relative to total CDT.⁴ A widely used clinical cutoff value for heavy drinking is over 2.6%.¹⁵

A limitation for the use of CDT resides in the fact that abnormal serum transferrin glycoforms (with different numbers of sialic acid residues) can be found in carbohydrate deficient glycoprotein syndromes. Iourin et al.¹⁶ confirm the presence of hexa-, penta- and tetra-sialoforms of human serum transferrin in both normal samples and in type I carbohydrate deficient glycoprotein syndrome, concluding that capillary zone electrophoresis can be used as rapid diagnostic test for carbohydrate deficient glycoprotein syndromes,¹⁶ data also confirmed through the research of Wuys et al.¹⁷

Alcohol consumption can be a major issue in patients with a pre-existing liver pathology of viral origin. Hepatitis C, for example, is one of the diseases in which alcohol consumption can lead to the transition from a fairly benign outline to a potentially life-threatening liver disease.^{18,19} Wu et al. hypothesized that nuclear receptor signaling is

altered in patients with chronic hepatitis C, displaying a pattern specific to alcohol drinking history.²⁰ Their study showed that, in chronic HCV infection of the liver, alcohol intake was correlated with an increased expression of genes involved in fatty acid uptake, trafficking and oxidation, and a decreased expression of gluconeogenesis genes.²⁰

Osna et al. reported that alcohol-consuming patients with hepatitis C virus (HCV) frequently have a longer course of infection and more severe manifestations of chronic hepatitis, excessive ethanol consumption being known to synergize with HCV to exacerbate liver injury (through modulation of HCV replication by ethanol metabolism).²¹ In clinical practice, questionnaires are used for estimating the alcohol intake of patients.²² Given the fact that questionnaires are highly subjective, CDT has also been considered in ascertaining alcohol consumption in patients with viral hepatitis. However, since transferrin is synthesized, glycosylated, and secreted by the liver, the use of CDT in patients with liver disease can raise certain problems. For example, elevated CDT values may not accurately correlate with alcohol consumption in advanced liver disease while patients with hepatitis C have been shown to have a higher chance of having a clinically positive CDT compared with patients with other types of liver diseases.²³

Perhaps we are reaching a stage where the CDT value as a percentage may even be a predictor of who would make a good candidate for liver transplant? A value greater than 6.5 for example. Not assuming that it will obviate liver function tests or histology of liver biopsy, the CDT value may however give one a good indication of impending liver failure and the need for a transplant.

In conclusion, CDT is a marker of chronic alcohol abuse and current assays can be used in detecting an alcohol consumption of 40–60 g of ethanol per day, consumed for 2–3 weeks or longer.²⁴ It has been proven to be superior to GGT or MCV as an indicator of chronic alcohol or ethanol abuse.¹ It is a sensitive (95%) and specific (93.3%) marker of chronic imbibing¹ and

alcoholic hepatitis, if not liver cirrhosis (micronodular and macronodular cirrhosis). It may also be a marker of liver or hepatic failure. Liver function tests (e.g., transaminases) may well be elevated and liver biopsies will show steatosis, Mallory bodies and evidence of fatty infiltration while carbohydrate deficient or depleted transferrin is indeed of value in the detection of chronic alcohol abuse.

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