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Carbohydrate Deficient Transferrin: How reliable is it as a Biomarker for Chronic Alcohol Consumption?

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ABSTRACT: *Alcohol use disorders are a major cause of a number of health, economic and social challenges for individuals, their families and health care systems worldwide. The inadequate and inaccurate assessment of long-term drinking demeanors is a significant and substantial hindrance to its diagnosis and management. Biomarkers for chronic alcohol consumption are now well established as reliable diagnostic aids but their sensitivity and specificity still need to improve. Therefore, there is a definitive need for the development of more sensitive and specific markers of alcohol abuse and addiction. Biological markers of alcoholism are divided into two cohorts: conventional and circumstantial indices. Lineal markers are detected in some biological fluids including blood and urine. The other matrices encompassing hair, saliva and sweat are not yet internationally accepted and approved, despite some studies seems to be promising for some. Among the conventional biomarkers which are tested for alcohol misuse and abuse are ethanol, ethyl glucuronide and ethyl sulfate. The conventional biomarkers, directly detect the alcohol consumption, with variable degrees of sensitivity and reliability. The circumstantial markers including MCV, γ-GT, transaminase enzymes SGOT (AST) (Serum Glutamate Oxaloacetate Transaminase) & SGPT (ALT) (Serum Glutamate Pyruvate Transaminase) and carbohydrate-deficient transferrin. These biological markers are affected by heavy alcohol consumption for long periods.* ***Objective:*** *Our prime objective of this article is to review the available literature on CDT (Carbohydrate Deficient Transferrin) as a biomarker for chronic alcohol consumption and its role in diagnosing and monitoring alcohol use disorders. We also aim to enrich and add to the scientific debate and knowledge about the manifest reliability of this biomarker.*

KEYWORDS: *Alcohol use disorders, Biological markers, Laboratory findings, Alcoholism, Transferrin isoforms, CDT*

INTRODUCTION

Alcohol use is popular and wide spread around the globe. Alcoholism causes a myriad of medical and psychiatric syndromes and complications. For example, alcohol use is a major cause of road traffic accidents. The impact of alcohol abuse is reflected in the biological, psychological, and social domains of our lives. Therefore, there is pressing need for reliable, safe and specific markers for early detection of potential alcohol abuse and follow up of recovering patients. Laboratory testing of alcohol consumption can be of an added value in identifying alcohol ingestion. Yet, the conventional biomarkers, including transaminase enzymes SGOT (AST) (Serum Glutamate Oxaloacetate Transaminase) and SGPT (ALT) (Serum Glutamate Pyruvate Transaminase), Gamma-Glutamyl Transferase (GGT) and (MCV), have mutable and finite sensitivity and specificity.

Discovered in 1976, Carbohydrate-Deficient Transferrin (CDT) was introduced to detect heavy and long-term alcohol abuse. CDT refers to the less sialylated forms of human transferrin: Asialo- and disialo-transferrin (Kent E Vrana et al.-March 25, 2011). It is presumed that alcohol intake of ≥ 50-80 g/day for a period of at least two weeks augments the increase of CDT concentrations. Despite the fact that mechanism of CDT increase still remains poorly understood, a large number of studies suggest and manifest that CDT is a good biomarker for the diagnosis of heavy alcohol consumption, with higher sensitivity and specificity than any of

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the other traditional markers. CDT has become a focal point for alcohol abuse research and clinical studies, besides forensic and judicial applications **(**Peterson, K.-2005, Steven Kipnis-2006).

The novel advances in proteomic technologies have immensely boosted the potential for alcohol abuse biomarker discovery. Quantification of Carbohydrate Deficient Transferrin (CDT) by capillary electrophoresis is used for screening patient serum samples to detect chronic alcohol abuse. Serum transferrin isoforms are separated into five major fractions according to their sialylation level. The human transferrin consists of several isoforms. Each isoform has a different mobility according to its sialic acid content (Isoelectric point between 5.2 and 5.9). The tetrasialo transferrin is the prevalent isoform and represents about 70 to 80% of total transferrin content. Other isoforms that can be revealed are 6-sialo Tf, 5-sialo Tf, 3-sialo Tf, 2-sialo Tf and occasionally 0-sialo Tf. When analysing serum from an alcohol abuser one can find an increase in 2-sialo-Tf and 0-sialo Tf will progressively manifest (Figures 1-4 and Table 1).

METHODOLOGY

Literature search and was conducted on the databases of PubMed, Medline and Embase for all articles published using the key words CDT test & chronic alcohol use. A total, of 907 articles, were found, search date 22/02/2018. For the brief description of the different methodologies generally used for detection and quantification of CDT, several laboratory manuals and standard operation procedures were reviewed.

Carbohydrate Deficient Transferrin

Transferrin is a carbohydrate-protein that relates and carries Fe ions through the blood. In healthy normal individuals, transferrin has specific sialic acid arrangement through its molecule. The sialic acid component of CDT might range from four to six per molecule. The unique structure of transferrin is disrupted as a result of alcohol consumption due to the impediment of sialic acids bonding to Transferrin, which leads to the presence of inadequate amount of sialic acid in blood and consequently carbohydrate deficient transferrin. Transferrin result is reported as % of total transferrin. A result that exceeds 5%, suggests heavy drinking. The FDA ratified this test in 2001. Prolonged alcohol consumption for two weeks or more will increase the rate of formation of CDT in blood, resulting in a higher percentage value raised CDT blood levels return to normal within fourteen to thirty days after the first day of ceasing intense alcohol intake **(**SAMSHA**).**

The levels of CDT in females are always higher than in males, regardless of the any aspect (Allen 2000). Misleading false positive results for CDT testing are seen in many cases and diseases, including, Obstructive liver disease, Post hepatic obstruction, Hepatitis, Liver cirrhosis, Liver Carcinoma, Cardiac insufficiency, Mononucleosis, Renal transplant, Hyperthyroidism, Myotonic dystrophy, Diabetes mellitus, Pancreatitis, Cystic Fibrosis, Low Ferritin, primary biliary cirrhosis, combined kidney and pancreatic transplant, CDG (Carbohydrate Deficient Glycoprotein), congenital glycoprotein metabolism flaw and hereditary variants of transferrin. False negative CDT test result is sometimes seen in females, where further studies are needed to explain the relation of gender to the sensitivity of the test.

The induction or inhibition of sialyl transferase and plasma sialidase may be implicated in the rise of CDT level. Transposition of protein transport during post-translational modification could be a primary mechanism in the decadence of protein metabolism connected with chronic alcohol abuse. Hormonal variations affect sensitivity of CDT. Iron homeostasis and stable equilibrium is

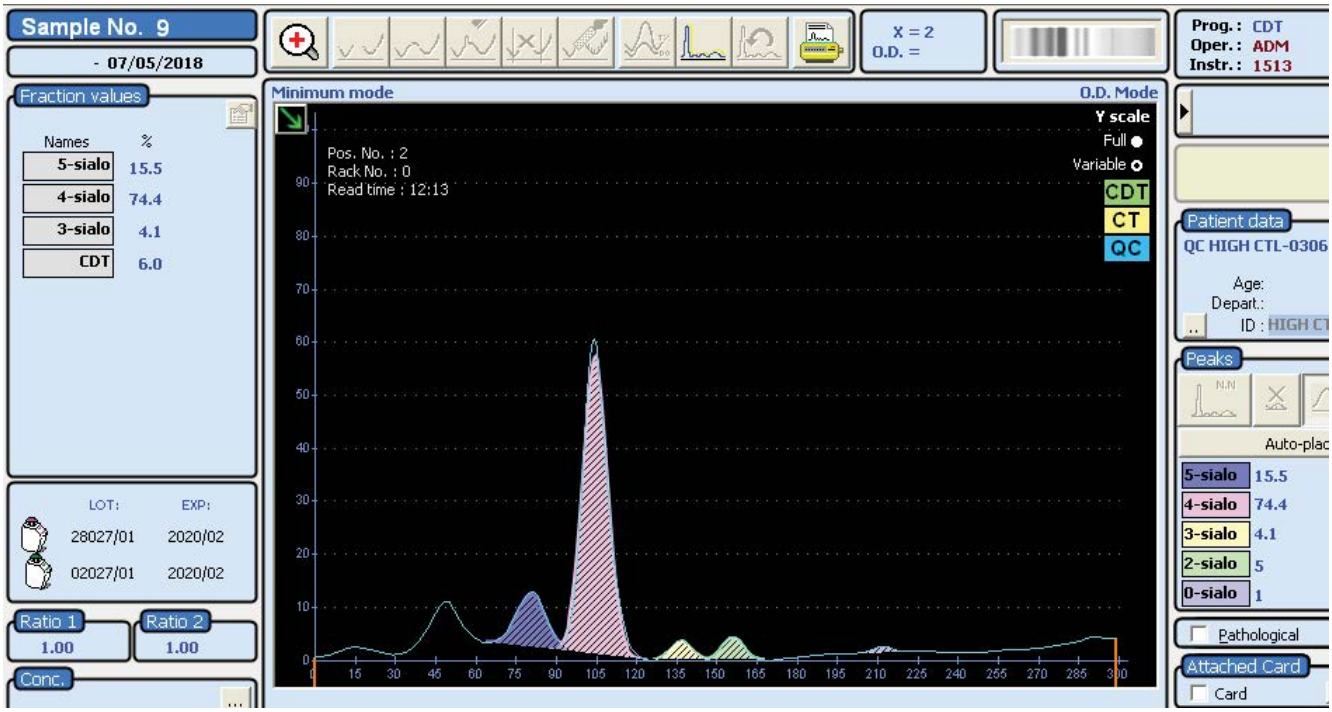
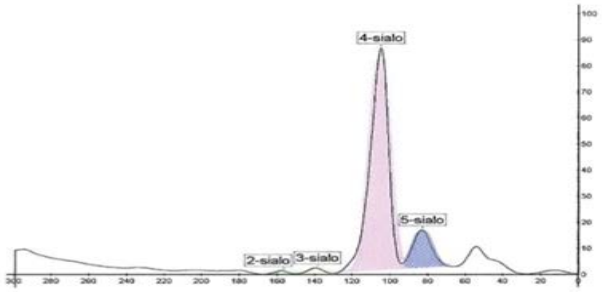


Figure 1: Screen shot for CDT result, from Sebia Capillary 2 equipment in the laboratory at The National Rehabilitation Centre, Abu Dhabi, United Arab Emirates

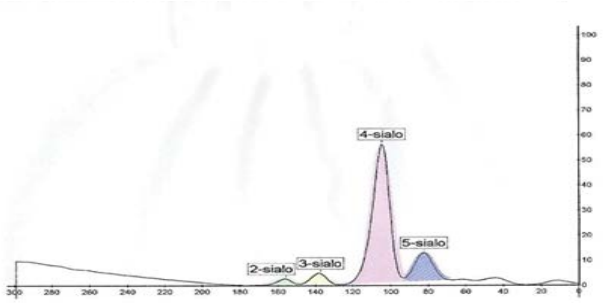


Transferrin isoforms electrophoresis

Fractions	%
5-sialo	14.2
4-sialo	83.4
3-sialo	1.6
CDT (2-sialo = 0.8)	0.8

Normal<=1.3; 1.3< inconclusive <=1.6; pathological>1.6

Figure 2: Normal CDT patient result test done on SEBIA Capillary 2 Equipment, by capillary electrophoresis method. Laboratory at the National Rehabilitation Center, Abu Dhabi, United Arab Emirates, 2018



Transferrin isoforms electrophoresis

Fractions	%
5-sialo	16.1
4-sialo	75.3
3-sialo	5.8
CDT (2-sialo = 2.8)	2.8 >

Normal<=1.3; 1.3< inconclusive <=1.6; pathological>1.6

Figure 3: Pathological CDT patient result test done on SEBIA Capillary 2 Equipment, by capillary electrophoresis method. Laboratory at the National Rehabilitation Center, Abu Dhabi, United Arab Emirates, 2018

AbuseCheck Alcohol Abuse Test Comparison Chart

Products			Detection Window	Markers	Result Details	Benefits	Limitations	
GOLD	SILVER	FAEE Hair Test	1 - 6 months	Fatty Acid Ethyl Esters (FAEEs)	Quantitative; 1ng/mg cut-off	No concerns for environmental 'wash-out' influencers. Non invasive sample collection. Longest detection period available for alcohol abuse. Sample cannot be tampered with.	Some alcohol containing hair products may elevate FAEE levels, and contribute to false-positives. Cannot determine exact dates or amounts of alcohol consumed. Six centimeters length (about 2 1/2 inches) of hair required.	ABUSECHECK PRODUCTS
GOLD	BRONZE	EtG Hair Test	1 - 3 months	Ethyl Glucuronide (EtG)	Quantitative; 30pg/mg cut-off	Provides a lower cost solution for determination of alcohol abuse, although it is recommended that this test is always accompanied with an FAEE test.	Must also take into consideration medical and other contextual information, including FAEE and/or blood alcohol test results. False-negatives are a concern due to wash-out effects from shampooing over time and bleaching. Three centimeters length (about 1 1/4 inches) of hair required.	
		CDT Blood Test	2-4 weeks	Carbohydrate Deficient Transferrin	Qualitative	Can detect prior alcohol abuse 2-4 weeks after donor stopped drinking.	Invasive sample collection; measures only excessive alcohol consumption (average of 60g per day, for at least 2 weeks)	NA
		Breath	Several hours after alcohol consumption	Exhaled alcohol vapor	Quantitative	Non invasive sample collection. Correlates with blood alcohol concentration (BAC). Measures very recent alcohol consumption	High false-positive rate due to alcohol in the air or in the mouth or stomach; also detects molecules similar to alcohol	NA
		Saliva	10-24 hours after alcohol consumption	Alcohol	Quantitative	Non invasive sample collection. Correlates with blood alcohol concentration (BAC). Measures very recent alcohol consumption.	Unclear standards for analysis; chance for false negatives if donor takes measures to "beat" the test.	NA
		Urine	1-3 days after alcohol consumption	Alcohol or Ethyl Glucuronide (EtG)	Positive/ negative	Non invasive sample collection. Detects very recent alcohol consumption.	Cannot determine how much alcohol was consumed; chance of false positive results.	NA
		Standard Blood Alcohol	Several hours after alcohol consumption	Alcohol	Quantitative	Most accurate measure of recent alcohol consumption.	Invasive sample collection.	NA

Figure 4: Abuse check on alcohol abuse test comparison chart

Table 1.
Isoelectric point for human transferrin isomers

Transferrin Isomer	PI (Isoelectric Point)
0-sialo Transferrin	pI=5.9
Hexasialo Transferrin	++++
Pentasialo Transferrin	pI=5.2
Tetrasialo Transferrin	pI=5.4
Trisialo Transferrin	pI=5.6
2-sialo Transferrin	pI=5.7
Monosialo Transferrin	pI=5.8

Table 2.
The assessment of alcohol use utilizing biomarkers

20-99 mg%	Loss of muscular coordination
100-199 mg%	Neurological impairment, ataxia, prolonged reaction time, mental impairment, incoordination
200-299 mg%	Nausea, vomiting, ataxia
300-399 mg%	Hypothermia, dysarthria, amnesia, stupor
400- > mg%	Coma

interrupted in women due to hormonal fluctuation and imbalance in situations of gestation, use of contraceptives, menstruation, menopause, etc. CDT levels are significantly affected by iron homeostasis. Many CDT assay methods appeared to be auspicious, but it is not clearly conspicuous which technique is the most precise and accurate. Furthermore, false-positive results of CDT have been notified in non-alcohol related hepatic failure and in rare conditions. Subsequently, clinical interpretation of CDT result necessitates rigorous assessment in patients with alcohol-related or non-alcohol-related health problems. CDT levels below 1.3% are regarded as normal, but CDT levels above 1.6% are considered to be abnormal and indicative of chronic alcohol abuse. CDT values above 1.3% and beneath 1.6% are inconclusive results (Figures 1-4).

Concentration of Alcohol in Object Blood (BAC)

For neotric and fresh alcohol consumption, it is advisable to determine the blood or breath alcohol concentrations as the most precise and accurate measures with the ease of using simple equipment. Another advantage of BAC is the possibility of correlating the test result with the findings and diagnosis of the treating physician.

The extent of ailment is influenced by degree of indulgence. The metabolism and clearance of alcohol from the body is easy and full. In the majority of people the range is 8-10 hours. As a result of this rapid removal, BAC is regarded as an ineffectual test outside this timeframe, where the window of alcohol metabolism in some people ranges from four to six hours (Table 2).

DISCUSSION

Biomarkers revealing chronic alcohol consumption continue to be a substantial tool for inpatient management and effective outpatient handling. Conventional laboratory investigations, including SGOT (AST), SGPT (ALT), GGT, and MCV, are still used as the standard markers to in defining alcoholism treatment course. CDT, on the other-hand, considered to be a relatively new marker that added an extra value to better interpretation of heavy alcohol consumption with higher sensitivity and specificity. CDT has been extensively studied to help correlate effectiveness in conjunction with other commonly used tests.

CONCLUSION

CDT has bounded sensitivity with regards to being used for confirmation of ponderous alcohol use. Caution should be exercised when ordering and interpreting % CDT results, particularly in women, patients with cirrhosis and those with a high BMI. In deduction, % CDT has limited sensitivity as a thematic biomarker to recognize and characterize subjects consuming mischievous amounts of alcohol. We believe in the very near future, High Resolution Mass Spectrometry, might soon become the method of choice when available for its high degree of accuracy and sensitivity. HPLC and capillary zone electrophoresis are currently widely used for their ease of use and accuracy despite the HPLC methodology being a little bit lengthy.

Definitely, % CDT result would be of higher value in defining heavy alcohol consumption for longer terms when combined with the other conventional markers GGT, SGOT (AST), SGPT (ALT) and MCV for more informative interpretation and hence a clinical decision.

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