CARBOHYDRATE DEFICIENT TRANSFERRIN IN ALCOHOLISM

Rajani Prasad, PhD Robert L. Stout, PhD James Smith, MD

Introduction

Alcohol abuse is prevalent all over the world. The United States is no exception and estimates between 11 to 12.5 million alcoholics have been reported.[1]

Drinking is more prevalent in men than women and among young to middle-aged persons than the elderly. However, recent reports indicate that both these segments (women and elderly) population include numerous addictions and the prevalence may be under reported. Women alcoholics have higher mortality and morbidity from alcohol-related problems.[2]

Interviews and history questionnaires are commonly used to aid in initial diagnosis of alcoholism and identification of individuals with suspected alcohol abuse. Two of the widespread measures used as questionnaires are by National Council on Alcoholism Committee and Michigan Alcoholism Screening Test. These have their limitations e.g., only 13% of 104 admissions in general hospital were identified as alcoholic abuse on a basis of the MASP questionnaire although 27% of the admissions were attributed to alcohol consumption.

If alcoholism or alcohol-related disorders were defined by an unambiguous drinking history, the identification of patient's performance and possible diagnostic tests could be assessed. Unfortunately there are confounding issues in each of these areas. The 1980 Version of the Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association (DSM-II) now distinguishes alcohol dependence and alcohol abuse. The latter is a pathologic pattern of alcohol abuse plus impairment in social or occupational functioning and the former includes alcohol abuse plus physiologic tolerance or withdrawal. The term "alcoholism" was omitted and alcohol abuse and dependence were included under a category of substance abuse disorders. Furthermore, the 1987 edition further subdivides abuse and dependence into mild, moderate and severe categories thus emphasizing the degree of disorders.

The search for a single biochemical marker for alcohol misuse with acceptable diagnostic efficiency has not been successful. Liver enzymes such as GGT[3] and AST[4] which showed promise have been found to be too nonspecific to be useful for alcohol abuse. Other markers such as MCV, uric acid, alkaline phosphatase and LDH are also not specific but elevations are frequently found in alcoholics. Recent reports show increased levels of plasma HDL-cholesterol and some apolipoproteins in alcohol population.[2,5] All of these markers have severe drawbacks including lack of specificity and poor sensitivity preceding pathological complications. The association of these markers in abstinent or treated alcoholics has not been defined.

In the mid 70's, the first report of abnormal transferrin in alcoholic abusers was reported.[6] Quantitative measurements have been developed to monitor CDT[7,8,9,10] and have been proven as an ideal marker for alcohol abuse with high specificity (97-100%) and acceptable sensitivities (81-94%).[6,10,11,12] The appearance of CDT in alcoholics is related to a minimum alcohol consumption for a minimum period (greater than 6 oz/day for at least 4 weeks).[13] The specificity for CDT for chronic alcohol misuse is partly responsible for the false negative results in people whose consumption rates <60g of alcohol per day or alcohol use for less than one week.[14] CDT was originally quantitated by isoelectric focusing.[7] However, there have been several improvements and chromatography followed by RIA is now commercially available.[13] We have developed a capillary electrophoresis method to separate and quantitate CDT.[15] A cut-off value for CDT of 3.0% is used to differentiate chronic alcohol users from social, or occasional, drinkers and teetotalers.

In order to study the potential of a biochemical marker for alcohol abuse, definitive and accurate history of alcohol use must be obtained. This should include detailed history of the length and amount of alcohol consumed. Since there has been published sensitivities for CDT ranging from 81-94% for alcoholism, we have completed a blinded study to address the clinical sensitivities of CDT within a well defined population of alcohol users. The objectives of the study were: a) to determine clinical sensitivity and specificity of CDT for alcoholism; b) relationship of CDT with amount and length of alcohol consumption; and c) relationship of CDT with GGT, AST, ALT and blood alcohol.

R.Prasad, *Director of Clinical Services*, Clinical Reference Laboratory, Lenexa, Kansas. R.L.Stout, *President*, Clinical Reference Laboratory, Lenexa, Kansas. J.Smith, *Chief Medical Officer*, Schick Shadel Hospital, Seattle, Washington.

Methods

Individuals presenting at the Schick Shadel Hospital Rehabilitation Center were enrolled in the study after informed consent. A detailed history including alcohol and drug use was elicited by following a standard questionnaire.

Blood was drawn, serum was processed and received in the laboratory the following day. The patients identification was coded to maintain confidentiality and the histories were not examined until the completion of the study. The samples that were received in the laboratory daily for approximately one year were analyzed for serum biochemistries, alcohol and CDT. Thus, the technicians and other laboratory personnel were blinded to the patterns of subject's alcohol use during the course of the study.

CDT was analyzed by capillary electrophoresis following purification of the transferrin from the serum. Fused silica coated capillary with internal diameter of 25 m x 125 cm was used for electrophoresis. The transferrins were separated on the basis of charge due to varying sialic acid contents.

The enzymes ALT, AST and GGT were analyzed by a coupled spectrophotometric assay with a Hitachi 736 automated chemistry analyzer.

Results

After analyzing approximately 100 samples, the codes were broken and the study was unblinded. *Table 1* shows the results from the control group. This group consisted of males (n=15) and females (n=4) who represented social drinkers. These nineteen individuals had CDT values less than 3% which is the cut-off value we have established for abnormality. The values remained normal when the same individuals were redrawn on two other occasions. The AST (normal range = 0-41 U/L), ALT (0-45 U/L), GGT (0-65 U/L) are also within the normal range and does not change the following day. These results were identical in a group of teetotalers.

The population who consumed alcohol at a greater frequency and larger amount than the "social" drinkers were further subdivided based on amount of alcohol consumed regularly. They fell into three categories: 1) alcohol consumption of greater than 80g/day daily for at least 4 times per week for a period of one month. We will refer to this group as the "alcoholic abusers"; 2) alcohol consumption at irregular intervals and irregular amounts (10g-70g/daily). This population will be referred to as "binge" drinkers; and 3) alcohol consumption between 10-70g/daily for at least a month.

Table 2 shows the data for prolonged excess alcohol consumers or "alcohol abusers." The test that picked up the largest number of the 43 alcoholics was CDT.

The CDT values corresponding to the group consuming between 10g-70g daily, and "binge" drinkers are displayed in Tables 3 and 4 respectively.

Discussion

Although CDT has been shown to have a high specificity, the sensitivities range between 81-94%.[6,10,11,12] The discrepancy in the sensitivities may be related to the alcohol consumption history and the errors associated with inaccurate or incomplete histories. We have attempted to obtain detailed history in alcohol consumption with respect to type and amount of alcohol beverages.

Following completion of CDT and serum biochemistry analysis, the study was unblinded and the population was segregated based on the amount of alcohol consumed. The details of the mechanism of CDT formation is not known but it is speculated that the glycosylation is incomplete under the environment of prolonged exposure to alcohol and/or its metabolite. Evidence indicates that other carbohydrate residues besides sialic acids may be missing in CDT.[14,16,17] The sequential addition of the terminal residues could mean that the different glycosyltransferases may be affected under prolonged influence of alcohol.

In the control group which represented "social" drinkers, all individuals had a CDT less than 3%. Similar data has been obtained from a group of teetotalers. The results shown in *Table 1* are consistent with our cutoff of 3% for abnormal CDT. None of the individuals (n=19) had CDT levels greater than three (3) on any occasion (*Table 1*). The specificity of CDT is 100% and agrees with previous reports.[18]

The situation is different for the group that consumes a minimum of 70 g/day of alcohol for a minimum period of four weeks. Out of the 43 individuals (4 females) in this group, 38 of them had abnormal CDT. This results in a sensitivity of 88%. Within this group of alcoholics the distribution of the GGT and ALT is noteworthy. As shown in the *Figure*, only 20 out of the 43 individuals (46%) had abnormal GGT and amongst them, all except 3 were elevated 2x above normal. Only 18 of the 38 individuals that had abnormal CDT had abnormal GGT. 13 out of 43 (30%) individuals had abnormal ALT.

Compared to ALT, only about half the individuals had AST abnormalities (21%). This is consistent with the findings that CDT is a sensitive indicator of alcohol misuse and precedes liver abnormalities.[13] Blood alcohol measurements have better correlation, since 30 of 43 had detectable levels.

There is overlap between the populations with liver enzyme abnormalities. For example, some of the population that are abnormal for ALT and GGT are also represented in the GGT abnormal group. Hence, the total of the different populations exceeds 100%.

The alcohol levels in these individuals ranged from 0.3-150 mg/dL. Only seven (7) of the individuals who were negative for alcohol were abnormal for CDT. This bears out the high probability of a positive alcohol finding in an alcohol abuser even in a random sampling. Thus, alcohol measurements may have better value as a reflex marker for CDT than GGT, ALT, or AST.

In the group of 30 individuals who used lower amounts regularly (10-70 g/day) for at least one week, the blood alcohol was detectable in 13 out of 30 individuals. In contrast to the former group of alcoholics, there were fewer positive alcohols and the range was much lower (0.1-3.4 mg/dL). The CDT was abnormal in 6 out of the 30 individuals in this group, with a sensitivity of only 20%. This data supports the correlation of CDT with amount of alcohol consumption at frequent intervals. The sensitivity of the GGT was 40% (12 abnormal out of 30) and is similar to the alcoholic population. The number of abnormal ALT was 7% and was lower than the alcoholic population. There was only one (1) individual with an abnormality in AST in this population who consumed less alcohol.

The third population that consumed alcohol in modest amount 10-70g at irregular internals (up to twice weekly) constituted the "binge" drinkers. There were a total of 15 individuals in this population and the sensitivity of CDT was similar to the population of moderate-regular alcohol consumption. Only 3 out of the 15 tested abnormal for CDT (20% sensitivity). Out of the 15 individuals, 11 showed detectable levels of serum alcohol ranging from 0.7-70 mg/dL. The range for serum alcohol in these binge drinkers was greater than regular moderate use of alcohol, but lower than the group of chronic excessive alcohol users. None of these individuals had any elevations in ALT or AST and only 2 out of the 15 had abnormal GGT (13.3%). The three individuals who had abnormal CDT had normal liver function tests, but had detectable levels of serum alcohol.

There were six (6) males who had history of drug abuse and claimed to consume moderate amounts of alcohol (10-50g) twice to three times weekly. Three (3) of these individuals had abnormal CDT. The GGT, ALT and AST were normal in all the subjects except one whose AST was 49, slightly above normal. Interestingly, this individual had an abnormal CDT.

For the insurance industry, our laboratory has been reflexing CDT tests based on independent and combined GGT, ALT, AST elevations. We report the CDT results as normal or abnormal based on a cut-off of 3%.

The results of this study examines the role of CDT in a variety of alcohol users. This represents a definitive study of alcohol frequency and amount consumption on CDT. The control population used in our study relied on "social" drinkers rather than teetotalers because it better reflects the insurance population. Interestingly, the CDT results are similar in the teetotaler population. The "social" drinkers are defined for this study as males and females who consume alcohol at irregular intervals, at social events. The amount of alcohol consumed varied between 5-15g during the social event, and these events occurred 2-4 times a month. We have shown elsewhere[15] that CDT does not change from its normal values, in these individuals soon after and the morning following alcohol consumption. This population represents a majority of the applicants in the insurance industry. The results in this study support the theory that abnormalities in CDT appear only under chronic and excessive use of alcohol.

The conclusions from this study are as follows:

1) The prevalence of abnormality in GGT or ALT or AST is 46%, 30% and 21% respectively in a population of documented alcoholics (n=43).

2) The CDT was the most specific indicator for alcohol misuse at a sensitivity of 88.4%.

3) Only 19 out of 38 individuals who were abnormal for CDT had elevations in either one of the liver function enzymes; ALT, AST or GGT. Thus, there is poor correlation of GGT, ALT or AST with CDT abnormality.

4) Use of liver function tests as reflexed for CDT will result in identification of approximately 53% of the alcoholics.

5) Serum alcohol measurements correlated with the history of amount of alcohol consumed.

6) 30 individuals out of 38 who had abnormality in CDT had detectable levels of alcohol. Therefore 79% of all CDT abnormalities were also positive for alcohol.

7) Serum alcohol measurements may be a better reflex for CDT analysis. Alcohol measurements are rapid, simple, and can be adapted for large sample volume.

8) The specificity for CDT in our study was 100% (n=19) and sensitivity 88.4% for chronic alcoholic abusers. The latter was defined as alcohol consumption of at least 70 g/daily for 1 month.

9) The sensitivity for CDT drops to 20% for low to moderate regular alcohol consumption (10-70 g/daily) and for "binge" drinkers. This agrees with the recent report of poor sensitivity for CDT in a population consuming less than 50 g/day.[18]

In conclusion, the high specificity in teetotalers and social, or occasional drinkers, lends credibility for the current use of CDT as the best available alcohol marker.

References

1. Cotton N. The familial incidence of alcoholism study. *Alcoholism* 1979;40:89.

2. Hill SY. Vulnerability to biomedical consequences of alcohol. *Alcohol Problems in Women*. Wilshack S, Beckman LJ, Eds. New York, Guilford Press, 1984:121.

3. Rollason JG, Pincherle G, Robinson D. Serum GGT in relation to alcohol consumption. *Clin Chem Acta* 1982;39:75.

4. Cohen JA, Kaplan MM. The SGOT/SGPT ratio an indicator of alcoholic liver disease. *Diag Dis Sci* 1979;24:835.

5. Baraona E, Lieber CS. Effects of ethanol on lipid metabolism. *J Lipid Res* 1979;20:289.

6. Stibler H, Allgulander C, Borg S, Kjellin KG. Abnormal microheterogeneity of transferrin in serum and cerebrospinal fluid in alcoholism. *Acta Med Scand* 1978;204:49-56.

7. Stibler H, Sydow O, Borg S. Quantitative estimation of abnormal microheterogeneity of serum transferrin in alcoholics. *Pharmacol Biochem Behav* 1980;13(Suppl 1):47-51.

8. Schellenberg F, Weill J. Serum desialytransferrin in the detection of alcohol abuse. Definition of a Tf index. *Drug Alcohol Depend* 1987;19:181-91.

9. Chen A, Leong F, Schanley D, et al. Transferrin and mitochondrial aspartate aminotransferase in young adult alcoholics. *Drug Alcohol Depend* 1989;23:13-8.

10. Vesterberg O, Petren S, Schmidt D. Increased concentration of a transferrin variant after alcohol abuse. *Clin Chem Acta* 1984;141:33-9.

11. Stibler H, Kjellin KG. Isoelectric focusing and electrophoresis of the CSF proteins in tremor of different origins. *J Neurol Sci* 1976;30:269-85.

12. Valmaki M, Salaspuro M, Harkonen M, Ylikahri R. Liver damage and sex hormones in chronic male alcoholics. *Clin Endocuviol* 1982;17:469.

13. Stibler H. CDT in serum: A new marker of potentially harmful alcohol consumption reviewed. *Clin Chem* 1991;37:2029-2037.

14. Stibler H, Borg S, Toustra M. Micro anion exchange chromatography of carbohydrate deficient transferrin in serum in relative to alcohol consumption. *Alcohol Clin Exp Res* 1986;10:535-44.

15. Stout R, Prasad R. Symposium on Capillary Electrophoresis. Manuscript submitted, 1993.

16. Petren S, Westerberg O. *Biochem Biophys Acte.* 1989;994:161-3.

17. Yamashita K, Ideo H, Ohkura T, Fukushima K, Yuasa I, Ohno K, Takeshita K. *J Biol Chem* 1993;268:5783-5789.

18. Jeppsson JO, Kristensson H, Fimiani, C. Carbohydrate-deficient transferrin quantified by HPLC to determine heavy consumption of alcohol. *Clin Chem.* 1993;39:2115-2119.

Table 1
Distribution of CDT and liver enzymes in social drinkers

Mean, ±(SD)

	CDT (%)	AST (U/L)	ALT (U/L)	GGT (U/L)
Baseline	1.7, (0.8)	15, (9)	14, (9)	22, (11)
Day 1	1.5, (0.6)	17, (8)	12, (8)	21, (10)
Day 2	1.7, (0.8)	18, (5)	13, (6)	19, (10)

n = 19

Table 2
Qualitative and quantitative distribution of CDT and liver function tests in chronic alcohol abusers

Number of Individuals

Results	CDT	GGT	AST	ALT	Alcohol
Normal	5	23	34	30	13
Abnormal	38	20	9	13	30
Mean Values	5%	190 U/L	40 U/L	51 U/L	17 mg/dL
Normal Range	0-3%	0-65 U/L	0-41 U/L	0-45 U/L	0 mg/dL

Table 3
Qualitative and quantitative distribution of CDT and liver function tests in "binge" alcohol consumers

Results	Number of Individuals				
	CDT	(65) GGT	(45) AST	(40) ALT	Alcohol
Normal	12	13	15	15	4
Abnormal	3	2	0	0	11
Mean Values	2%	32 U/L	12 U/L	16 U/L	10 mg/dL
Normal Range	0-3%	0-65 U/L	0-41 U/L	0-45 U/L	0 mg/dL

Table 4
Qualitative and quantitative distribution of CDT and liver function tests in moderate alcohol consumers

Results	Number of Individuals				
	CDT	GGT	AST	ALT	Alcohol
Normal	24	18	29	28	17
Abnormal	6	12	1	2	13
Mean Values	2%	79 U/L	19 U/L	28 U/L	2 mg/dL
Normal Range	0-3%	0-65 U/L	0-41 U/L	0-45 U/L	0 mg/dL

Figure
Distribution Among CDT Positive Subjects