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Carbohydrate Deficient Transferrin in Abstaining Patients with End-Stage Liver Disease

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Abstract

Background—Carbohydrate deficient transferrin (CDT), a biochemical marker of chronic alcohol consumption, is used by researchers and clinicians alike in a variety of populations. Levels of CDT may be affected by certain types of medical illnesses and conditions. Thus the interpretation of CDT results may need to be carefully examined in these populations. Because CDT is synthesized, glycosylated, and secreted by the liver, the use of CDT values in patients with liver disease has been an area of focused interest.

Methods—We evaluated the CDT values of 79 abstaining patients with end-stage liver disease. These patients were recruited from a liver transplant clinic while they were listed and waiting for transplantation. Patients were determined to be abstaining both by interview and by random blood alcohol levels in those with a diagnosis of alcoholic liver disease. The severity of the liver disease was categorized by the Child-Pugh score. Correlations were determined between CDT values and liver enzymes, Child-Pugh scores and liver diagnosis.

Results—Nearly 50% of the patients had a CDT value 2.6% or above indicating a clinically positive value. There were strong correlations between CDT and a number of biochemical and physical variables, most importantly the Child-Pugh score ($r=0.52$, $p=0.000$). Specific liver diseases were not associated with absolute CDT values. However, patients with HCV had a significantly higher chance of having a clinically positive CDT compared to patients with other types of liver diseases.

Conclusions—These results suggest that an elevated CDT value may not accurately represent alcohol consumption in patients with advanced liver disease. In fact, in such patients, the CDT may become a marker for the degree of liver impairment in alcoholic and non-alcoholic liver disease. CDT values should be viewed with caution in any patient with liver disease especially when the degree of cirrhosis reaches a Child-Pugh score of C (total score of 10 or above).

Over two decades ago, the identification of carbohydrate deficient (desialylated) transferrin (CDT) and its association with alcohol consumption (Stibler, 1979) captured the interest of clinicians and researchers alike. As a biochemical marker of chronic alcohol use, an elevated CDT could potentially aid in the detection and monitoring of patients with heavy alcohol consumption. Compared to liver enzyme profiles, CDT can have superior sensitivity in detecting alcohol abuse (Litten 1995, Anton 1994). While the clinical utility of CDT is being

explored in a variety of settings, concerns over the impact of various disease states on CDT values have been raised. In particular, because CDT is synthesized, glycosylated, and secreted by the liver, the use of CDT values in patients with liver disease has been an area of focused interest. The earliest work in this area investigated the correlations between various biochemical liver tests, biopsy-verified liver disease and CDT values in patients with liver disease and found that liver disease without current alcohol use was not associated with elevated CDT (Stibler, 1987). However, while the degree of liver fibrosis did not correlate with elevated CDT levels, in that study few patients had advanced liver disease (cirrhosis) and only 10% were clinically affected by their liver disease (Stibler, 1987). In contrast, investigations of other specific populations have reported a high degree of variability in CDT depending on body iron (DeFeo, 1999), gender and exogenous estrogens (Stauber, 1996), and specific types of liver diseases (Murawaki, 1997; Bell, 1993, Stauber, 1995). For example, Heinemann found an unacceptable specificity rate (as low as 20 - 50%) resulting in a high rate of false positives in patients with severe liver disease awaiting liver transplantation (Heinemann, 1998).

We are interested in using CDT values to follow patients' alcohol consumption before and after liver transplantation, specifically for patients with alcoholic cirrhosis. However, given that many of our patients have serious liver disease, we were concerned about the potential poor specificity of CDT values in such patients. Therefore we investigated the impact of the degree of liver disease on CDT values in the absence of alcohol consumption. Our patients all have end-stage liver disease with the majority of patients having non-alcoholic liver disease. In these abstaining patients, the CDT values are expected to be normal. Nevertheless, we hypothesized that while mild to moderate liver insufficiency may not affect CDT values (as seen in prior studies), in our patients, with advanced liver disease, CDT values may be elevated reflecting the degree of liver failure rather than alcohol consumption.

METHODS

Prior to the initiation of the study, approval was obtained from the University of Pittsburgh Medical Center Institutional Review Board. Patients with advanced liver disease, awaiting liver transplantation, were chosen from the University of Pittsburgh Liver Transplant Clinic. Once the patients were informed about the study, they voluntarily signed consent forms and donated a serum sample. Patients were asked about the frequency and amount of recent and current alcohol use. Because the intent was to study the effect of liver pathology on CDT levels independent of alcohol use, patients that reported current regular alcohol use were excluded. On the same date as the CDT sample, demographic and clinical data were obtained from the medical record including total bilirubin, albumin, international normalized ratio (INR), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGTP), ammonia (NH₃), presence of ascites and encephalopathy, and the Child-Pugh scores. Routine blood alcohol levels on patients with alcoholic liver disease were also collected to possibly capture covert alcohol use. These patients had blood alcohol levels checked every 1 to 6 months and on average had 6 negative blood alcohol levels before enrolling in the study.

The Child-Pugh Score is commonly used clinically to quantify the degree of liver cirrhosis (Albers, 1989). Five factors (bilirubin, albumin, INR, ascites and encephalopathy) contribute to the total Child-Pugh score assessing features of synthetic and systemic liver functioning. Scores of 5-6 indicate mild liver disease (Child-Pugh A), 7-9 moderate liver disease (Child-Pugh B), and 10-15 severe liver disease (Child-Pugh C).

Over a 10 month period, 79 consecutive patients were approached and enrolled in the study. Two patients with alcoholic cirrhosis were excluded from the analysis because they admitted to regular alcohol use and both had positive blood alcohol levels [as well as elevated CDT values (3.4 and 7.8)]. These patients were not on the liver transplant list. Only one other patient without alcoholic cirrhosis admitted to any alcohol use. This patient reported drinking 2 to 3 (12-ounce) beers the week prior to coming to clinic and was not excluded from the study. One other patient was excluded after serendipitously discovering she had a genetic variant of CDT (Trout, 2000), a codominant transferrin deficient allelic (polypeptide) variant that is one of the few definitely known causes of false positive CDT. Though our sample was not screened for such variants, in the Caucasian population these variants account for less than 1% of false-positive values (Stibler, 1987).

For conditions in which the total transferrin level could be affected (i.e. liver disease, iron deficiency, pregnancy) a method of CDT determination which does not correct for total transferrin would be misleading. Any alteration in serum total transferrin concentration would markedly decrease the specificity of such an assay (Sorvajarvi, 1996). Thus we chose to use the %CDT which reports the CDT as a percentage of the total transferrin which we believe would be more likely to be accurate in our patients with liver disease. CDT levels are processed according to the Axis-Shield immunoassay for quantitative measurement of CDT in human serum and are reported as %CDT in proportion to the total transferrin (Axis, 1998). Serum samples were stored at -20 Celsius until assayed. The CDT range for total abstainers has been established as 0-2.5% (Anton, 2000). CDT levels 2.6% and higher are considered to be positive for alcohol use. The CDT level can become positive after two weeks of heavy drinking (defined as 60 grams or more of ethanol per day) and with abstinence the values normalize with a mean half-life of 14-17 days (Pharmacia, 1994). Thus a positive CDT can identify recent heavy drinking even after a person has stopped drinking.

STATISTICS

Continuous variables are presented as the mean $\pm$ standard deviation (SD), and categorical variables are presented as proportions. Groups were tested using independent sample t- tests for two groups or oneway analysis of variance for multiple groups. Differences between group means were tested using Scheffe's multiple comparisons test. For lab values that did not have a normal distribution the Mann-Whitney U test was used to compare groups. Crosstabulations were used to test associations between diagnoses and positive CDT values. Correlations between CDT and other biochemical values and physical findings were performed. In order to perform correlations all lab values and CDT values were log transformed due to positive skewedness. A p-value less than 0.05 was considered

statistically significant. All analyses were performed using Statistical Package for Social Sciences SPSS for Windows software (version 9.0).

RESULTS

Descriptions

Patients were predominantly Caucasian (96%) and male (68%) with a mean age of 54 ± 9.8 years. This demographic profile is similar to other liver transplant programs (DiMartini, 2001). Though alcoholic cirrhosis and hepatitis C (HCV) were the most common diagnoses, the causes of end-stage liver disease were varied and 68% had non-alcoholic liver disease (see Table 1). Laboratory values show a wide range, although the mean values of most tests are in the abnormal range (see Table 2). Twenty eight percent of the patients had Child-Pugh A cirrhosis, 48.7% had Child-Pugh B cirrhosis, and 23.6% had Child-Pugh C cirrhosis.

The CDT values ranged from 1.7% to 6.4%. The distribution of the CDT values was positively skewed (asymmetrically shifted towards positive values). The mean CDT value was $3.0\% \pm 1.0$ with a median value of 2.5%. The 25th quartile was 2.3% and the 75th quartile 3.5%. Therefore 49% of the patients had a CDT 2.6% or above indicating a clinically positive CDT value.

Comparisons

There are strong correlations between CDT and a number of biochemical and physical variables, most importantly the Child-Pugh score ($r=0.52$, $p=0.000$) (see Table 3). Patients with positive CDT values had significantly higher Child-Pugh scores ($p=0.01$) than those without positive CDT values. There are differences between the CDT values of the groups of Child-Pugh scores A, B, and C ($p=0.000$). Child-Pugh A (2.4 ± 0.4) was not found to be significantly different than Child-Pugh B (2.9 ± 1.0); however, the CDT values were significantly higher for Child-Pugh C (3.8 ± 1.1) compared to Child-Pugh A ($p=0.000$) and to Child-Pugh B ($p=0.003$).

All of the components of the Child-Pugh Score are significantly correlated with CDT levels (see Table 3.) Serum albumin ($p=.001$), total bilirubin ($p=.001$), and INR ($p=.001$) are also associated with clinically positive CDT values (defined as a CDT $\geq 2.6\%$). In addition to the lab components, the presence and degree of encephalopathy ($p=.001$) was significantly associated with elevated CDT values, though not associated specifically with a clinically positive CDT value. The presence and degree of ascites was not significantly associated with an elevated CDT value ($p=.052$). Additionally ALT, GGTP, and NH3 were not correlated with CDT values.

There are no differences in absolute CDT values comparing all diagnostic groups ($p=.47$), nor are there differences in Child-Pugh scores between diagnostic groups. However, when examining the differences between diagnoses and clinically positive CDT scores ($\geq 2.6\%$), patients with HCV have significantly more positive CDT values than other patients ($p=.048$). Though prior studies suggest that patients with a diagnosis of Primary Biliary Cirrhosis (PBC) could have falsely positive CDT values (Stauber 1995), this is not the case in our sample. Additionally, patients with alcoholic liver disease did not have significantly

more positive CDT values. Table 4 shows the breakdown of Child-Pugh scores within specific diagnoses as well as the percent positive CDT in each group.

CONCLUSIONS

Several hypotheses have been proposed as to the mechanism for the development of CDT, including interference by ethanol with transferrin glycosylation, CDT secretion, or CDT elimination (Stibler, 1987). Cirrhosis develops in end-stage liver disease with the replacement of normally functioning parenchyma with fibrous bands of tissue. With the loss of functioning hepatocytes, normal cellular activity is diminished potentially affecting one or several of these mechanisms. Because the Child-Pugh score is a measure of overall liver functioning, including synthetic, metabolic, and systemic functions, our finding that it was strongly correlated with elevated CDT would suggest that CDT is affected by the degree of liver impairment.

The method by which the severity of the liver disease was calculated may account for inconsistencies between studies investigating the effect of liver disease on CDT values. For example, one study found CDT levels elevated in early alcoholic liver disease but low levels of CDT in the advanced stages of alcoholic liver disease (Niemela, 1995). In contrast, another study found nearly 30% of patients with severe liver disease, but not drinking alcohol, had positively elevated CDT values compared to only 3% of non-drinking controls without liver disease (Meregalli, 1995). Clinical assessments and definitions of serious liver disease may be subjective. However, the Child-Pugh score provides a readily reproducible and standardized method for assessing the degree of liver failure and is generalizable to patients with liver disease regardless of the etiology. Interestingly, a prior study found no association between absolute CDT values (measured as the ratio of CDT to total transferrin) or clinically positive CDT values and Child-Pugh scores in abstaining alcoholics with liver disease (Caldwell, 1995), although numbers of patients with a Child-Pugh score of C were small.

In addition, the specific method for determining the CDT value must be considered in the context of liver disease. CDT assays which do not correct for the serum total transferrin level could be affected by medical conditions (i.e. liver disease) which increase total transferrin and thus decrease the specificity of the assay (Sorvajavi, 1996). The %CDT, used in this study, reports the CDT as a percentage of the total transferrin which would be preferable and more accurate in these cases as it takes into account the transferrin status. Some of the prior studies of CDT and liver disease did not use the %CDT and had equivocal results (Niemela, 1995, Meregalli, 1995).

While it is possible that those patients with elevated CDT values were actually drinking alcohol, we believe this is unlikely due to their negative responses in interview. Furthermore, it would be rare that a patient with terminal liver disease, awaiting transplantation, would be drinking alcohol heavily enough to create a positive CDT level. Patients are counseled on avoiding alcohol and the majority had made this decision and abstained long before coming to our clinic. In addition, nearly 70% of our participants did not have alcohol induced liver disease and did not have histories of prior excessive alcohol

consumption. Random blood alcohol levels are checked for patients with alcoholic liver disease, as part of our routine clinical follow-up. Those who admitted using alcohol or had a positive alcohol level were excluded from the study (n=2). In addition, even if we were to assume that patients with alcoholic liver disease are more likely to deny alcohol consumption, they did not have CDT values that were different from patients with other types of liver disease.

These results suggest that an elevated CDT value may not accurately represent alcohol consumption in patients with advanced liver disease. In fact, the CDT may become a marker for the degree of liver impairment in alcoholic (Niemela, 1995; Yamauchi, 1993) and non-alcoholic liver disease patients. A prior study suggested caution when interpreting CDT values in patients awaiting liver transplantation (Heinemann, 1998), though the degree of liver decompensation was not specified. We would recommend caution when interpreting CDT values in any patient with liver disease especially when the degree of cirrhosis reaches a Child-Pugh score of C (total score of 10 or above).

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Table 1

Diagnoses of 76 Liver Transplant Candidates

Type of Liver Disease	Percentage
Alcoholic Cirrhosis	31.6
Hepatitis C	31.6
Primary Sclerosing Cholangitis	17.1
Cryptogenic	10.5
Autoimmune	7.9
Primary Biliary Cirrhosis	7.9
Hepatitis B	6.5
Cancer	3.9
Non-alcoholic Steatohepatitis	3.9
Hemachromatosis	2.6
Drug Induced	1.3
Gilberts Syndrome	1.3

Patients may be included in more than one category due to multiple diagnoses.

Table 2

Laboratory Values for 76 Liver Transplant Candidates

Lab Type	Minimum	Maximum	Mean	Normal Range
Albumin	2.3	4.8	3.2	3.5-5.5 g/dl
ALT	20	187	66	0-40 IU/L
AST	18	257	73	0-40 IU/L
GGTP	25	1838	225	0-65 IU/L
INR	.9	2.3	1.2	
NH3	1	94	35	9-33 umol/L
Total Bili	.4	13.9	2.8	0.3-1.5 mg/dl
CDT	1.7	6.4	3	0-2.5%

Table 3

Correlations of Variables with CDT

Variable	Pearson Correlation	Significance
Child-Pugh	.520	.000
INR	.483	.000
Total Bili	.516	.000
Albumin	-.413	.000
AST	.323	.004
GGTP	-.151	.195
ALT	.101	.386
NH3	.066	.578
Alcoholic Cirrhosis	-.109	.349
Hepatitis C	-.165	.154

Table 4

Comparison of CDT Values by Diagnosis and Child-Pugh Scores (A, B or C)

Diagnosis	% CDT (Mean)	% Positive (CDT >2.5%)
All Patients		
A (n=21)	2.4	28.6
B (n=37)	2.9	43.2
C (n=18)	3.8	83.3
Total (n=76)	3.0	48.7
Alcoholic Cirrhosis		
A (n=9)	2.5	33.3
B (n=11)	3.3	45.5
C (n=4)	4.2	100
Total (n=24)	3.1	50
Hepatitis C		
A (n=4)	2.7	50
B (n=14)	2.9	57
C (n=6)	4.1	100
Total (n=24)	3.2	66.6
Primary Sclerosing Cholangitis		
A (n=6)	2.2	16.7
B (n=4)	2.2	0
C (n=3)	3.8	100
Total (n=13)	2.6	30.8
Cryptogenic		
A (n=1)	1.9	0
B (n=5)	2.2	20
C (n=2)	4.4	50
Total (n=8)	2.7	25
All Other Diagnoses *		
A (n=6)	2.5	33.3
B (n=15)	3.2	66.7
C (n=7)	3.5	71.4
Total (n=28)	3.1	60.7

* Includes Autoimmune, Primary Biliary Cirrhosis, Hepatitis B, Cancer, Non-alcoholic Steatohepatitis, Hemachromatosis, and Drug Induced.