SEMI-AUTOMATED CARBOHYDRATE-DEFICIENT TRANSFERRIN IN PRIMARY BILIARY CIRRHOSIS: A PILOT STUDY

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Abstract — Primary biliary cirrhosis (PBC) is one of the few non-alcohol induced liver pathologies which causes false positive results in the evaluation of carbohydrate-deficient transferrin (CDT) for the diagnosis of alcohol misuse. This phenomenon has only been observed when using the CDTect assay (Pharmacia & Upjohn, Uppsala, Sweden). In this study, we evaluated CDT in female PBC patients (n = 14) by a new CDT procedure, the %CDT turbidimetric immunoassay (TIA, Axis Biochemicals, Oslo, Norway) using the isoelectric focusing/immunoblotting/laser densitometry (IEF/IB/LD, Specialty Laboratories, Santa Monica, CA, USA) procedure as the gold standard. One of the PBC patients tested CDT+ by IEF/IB/LD (cut-off >9 densitometry units, DU) and %CDT TIA (cut off >6%); one patient tested at the cut-off point of the IEF/IB/LD and another one tested at the cut-off point of the %CDT TIA. Thus, unlike CDTect, the %CDT TIA is a procedure that produces few false positives in PBC.

INTRODUCTION

Excessive alcohol consumption, defined as >60 g of alcohol/day for 7-10 consecutive days, causes increased concentrations of carbohydrate-deficient transferrin (CDT) in serum (Stibler, 1991; Allen et al., 1994; Huseby et al., 1997). CDT is a heterogeneous group of molecules bearing a decreased content of oligosaccharides due to the detrimental effects of alcohol in the liver (Xin et al., 1995). Isoelectric focusing/immunoblotting/laser densitometry (IEF/IB/LD, Specialty Laboratories, Santa Monica, CA, USA), designed in the early 1990s, measures molecules of transferrin-bearing glycans with two or no sialic acids (Anton and Bean, 1994; Bean and Peter, 1994). The %CDT turbidimetric immunoassay (TIA, Axis Biochemicals, Oslo, Norway), recently introduced to the market (Heggli et al., 1996; Bean et al., 1997), measures 50% of the transferrin molecules containing three sialic acids and all of the transferrin molecules containing two, one, and no sialic acids (Bean et al., 1997). The classical method for diagnosis of alcohol misuse is abnormal γ-glutamyltransferase (GGT), an enzyme elevated in several other disease states (Meregalli et al., 1995). In contrast to GGT, elevated CDT concentrations are almost always indicative of alcohol misuse in well-defined populations of heavy drinkers (Stibler, 1991; Allen et al., 1994; Anton and Bean, 1994). Indeed, PBC is one of the few non-alcohol-induced liver diseases that can cause false positive results in the CDT test for diagnosis of alcohol abuse (Bell et al., 1993; Bean et al., 1995; Niemela et al., 1995; Stauber et al., 1995; Perret et al., 1997). Previous studies comparing the specificity of IEF/IB/LD and CDTect (Pharmacia & Upjohn, Uppsala, Sweden) showed that, for the same PBC patients analysed in this study, the rate of false positive results for CDTect was 36% (Bean et al., 1995). The purpose of the present study was to determine the false positive rate of the new %CDT TIA and compare the results to those obtained by the IEF/IB/LD, used as a gold standard.

MATERIALS AND METHODS

Patients and controls

Sera from PBC patients (<15 g of alcohol/day for >2 weeks before sample collection) were...
Fig. 1. Quantification of carbohydrate-deficient transferrin (CDT) by two methods. Quantification of CDT was performed in primary biliary cirrhotic (PBC) patients, controls and alcohol misusers by using either (A) IEF/IB/LD or (B) %CDT TIA.
CDT IN PRIMARY BILIARY CIRRHOSIS

20 40 60 80 100

SPECIFICITY

100

Fig. 2. Receiver-operating characteristic (ROC) plot of two methods for CDT quantification in primary biliary cirrhotic patients.

The ROC plot was constructed by determining sensitivity and specificity rates at several cut-off points derived from the corresponding dot plots for the alcohol misusers and the primary biliary cirrhotic groups.

obtained from middle-aged (35-65-year-old) women attending the Hepatology Unit of Los Angeles County–University of Southern California Hospital (n = 14). Diagnosis of PBC was made from a combination of increased serum alkaline phosphatase in the absence of biliary obstruction, pruritus, a positive mitochondrial autoantibody test (13/14), and liver biopsy showing active destructive bile duct lesions.

Sera from female alcohol misusers (n = 21) were obtained from individuals whose histories of alcohol consumption and laboratory test results were used in previous studies (Bean et al., 1995, 1997). Sera from non-misuser females (n = 31) were obtained from employees of Specialty Laboratories drinking in the range of 0-15 g of alcohol daily for years. All sera were kept frozen at -70°C until the time of this study. CDT testing was performed at Specialty Laboratories and Axis Biochemicals.

IEF/IB/LD

Sera were partially saturated with iron and analysed in acrylamide gels containing a gradient of ampholytes as previously described (Bean and Peter, 1994). A cut-off of 9 densitometry units (DU) was used, intra-assay and inter-assay variation are 11 and 21%, respectively (Anton and Bean, 1994).

%CDT TIA

The Axis %CDT TIA is a procedure based on microcolumn separation followed by a turbidimetric immunoassay as described previously (Bean et al., 1997). The cut-off value is >6% CDT; intra- and inter-assay variations are 4 and 5%, respectively. This method is now available in the USA under the name %CDT UltraQuant™ from Specialty Laboratories.

Statistical analysis

Diagnostic test performance was evaluated by receiver-operating characteristic (ROC) analysis and the area under the ROC curve was calculated by the method of Hanley and McNeil (1983).

RESULTS AND DISCUSSION

PBC, chronic viral hepatitis, and hepatic tumours are among the few liver disorders that can cause false positive CDT results in the absence of prolonged and heavy alcohol consumption (Bell et al., 1993; Niemela et al., 1995; Stauber et al., 1995; Perret et al., 1997). However, in this study, we show that new CDT procedures, such as %CDT TIA, exhibit a diagnostic performance that resembles IEF/IB/LD, rather than CDTect.

CDT quantified by IEF/IB/LD yielded mean ± SD values of 4.3 ± 3.5 and 3.8 ± 2.3 DU for the PBC patients and non-drinkers respectively. For female alcohol abusers (n = 21), CDT quantification averaged 17.7 ± 20.4 DU (Fig. 1A). Using a cut-off of >9 DU the rate of false positive results for CDT in PBC patients and control non-abusers was 7% (1/14) and 3% (1/31), respectively. This cut-off ensures optimal clinical utility of CDT testing in females as it provides >90% specificity for the diagnosis of alcohol abuse. Clinical sensitivity of CDT for the assessment of harmful alcohol consumption in females was 45% (10/22).

Serum CDT quantified by %CDT TIA yielded mean ± SD values of 3.3 ± 0.7 and 3.3 ± 1.9 %CDT for the 31 normal controls and 14 PBC patients respectively (Fig. 1B). CDT quantification by %CDT TIA from female alcohol abusers averaged 7.8 ± 5.0 %CDT. Thus, for a cut-off of
>6% CDT, the rate of false positive results for CDT in PBC patients was 7% (1/14). Specificity rate for control non-abusers was 100% and the sensitivity of the %CDT TIA assay for evaluation of harmful alcohol ingestion in females was 43% (9/21).

The ROC plot shows the diagnostic performance of the %CDT TIA and the IEF/IB/LD methods when comparing alcohol abusers with PBC patients (Fig. 2). The area (0.79) under the %CDT TIA curve was the same as the area under the IEF/IB/LD curve. At 100% specificity, the sensitivity rates for the IEF/IB/LD and %CDT TIA were approximately 40%. There was a good discrimination between alcohol abuse and PBC using either of these two CDT methods.

The difference between CDTect and the two CDT procedures described in this study could be accounted for by the inclusion of transferrin isoforms exhibiting a higher content of sialic acids and the quantification of CDT as a ratio of total transferrin, rather than absolute values of CDT.

REFERENCES


