CARBOHYDRATE-DEFICIENT TRANSFERRIN AND GAMMA-GLUTAMYL TRANSPEPTIDASE-MARKERS OF EXCESSIVE ALCOHOL CONSUMPTION

DANIELA IONESCU, B.BUMBĂCILĂ**, CARMEN CRISTESCU***, RALUCA DUMACHE**, ELENA GÂLCĂ****, CORINA ŞERBAN*****, GERMAINE SĂVOIU*****, MARIA PUIU*******

*Department of Toxicology, **Department of Biochemistry, ***Department of Clinical Pharmacy, ****Department of Psychiatry Lugoj, ****Department of Medical Genetics, ****Department of Pathophysiology, "Victor Babeş" University of Medicine and Pharmaceutics, Timişoara

Abstract. Carbohydrate-deficient transferrin (CDT) represents an important instrument for the evaluation process of alcohol consumption. But there is not a consensus on its use in the routine practice. The objective of this study was to compare carbohydrate-deficient transferrin and gamma-glutamyl transpeptidase (GGT) assays for the evaluation of alcohol consumption. 165 patients (100 males and 65 females) were included in this study. Patients were divided into five categories according to alcohol consumption: category 1 included non-weaned patients drinking more than 30 g/day for women and more than 50 g/day for men, category 2 included relapse patients, category 3 included moderate drinkers, category 4 included patients weaned less than one month, and category 5 included patients weaned more than one month. A specifically standardized questionnaire was used. Blood samples were drawn at the alcohol-cure outpatient centre. Samples were drawn at the first consultation and later as prescribed depending on the clinical situation. Sensitivity of CDT varied, depending on patient category, from 36% to 96% *versus* 45% to 70% for GGT. Specificity of CDT varied from 72% to 90% *versus* 21% to 61% for GGT. This study shows that carbohydrate-deficient transferrin is more accurate in predicting alcohol consumption compared with gamma-glutamyl transpeptidase in alcoholic patients evaluation.

Key words: Carbohydrate-deficient transferrin, gamma-glutamyl transpeptidase, alcohol consumption, sensitivity, specificity.

INTRODUCTION

Alcoholism is one of the most important and serious addictions with regard to social, economic and medical consequences. Excessive alcohol consumption is a major risk factor for serious social and pathological problems [17].

Declared alcohol consumption, standardized questionnaires and biological markers are three methods used to evaluate alcohol consumption. Mean corpuscular volume (MCV) and serum gamma-glutamyl transpeptidase (GGT)

Received: September 2008.

ROMANIAN J. BIOPHYS., Vol. 18, No. 4, P. 301–307, BUCHAREST, 2008

were the most widely used biological markers [18, 21, 22]. However, GGT is also influenced by a variety of conditions other than alcohol misuse, certain medications and several disorders of non-alcoholic origin, and normal levels vary with sex, age and body weight. Gamma-glutamyl transpeptidase has therefore limited utility as a screening test in the general population [10, 11]. To improve early detection of heavy drinking and monitoring of sobriety during treatment as outpatients, the development and application of markers with higher sensitivity and specificity for alcohol has been demanded [1, 10, 11].

Stibler *et al.* observed a transferrin glycosylation abnormality in the cerebrospinal fluid of alcoholic patients presenting neurological disorders [24]. This abnormality was found in the serum of alcoholic subjects without neuropathy and disappeared after 10 to 14 days of abstinence [25]. At present, measurement of the abnormal micro-heterogeneity of serum transferrin, the so-called carbohydrate-deficient transferrin (CDT), seems to be the most accurate method for detection of recent regular excessive alcohol consumption. Carbohydrate-deficient transferrin is a more specific indicator of heavy drinking than any other laboratory test currently in routine use, and the sensitivity is also relatively high. The exact mechanism by which alcohol causes an elevation of CDT levels is, however, poorly understood. False positive CDT values may be found in non-alcoholic subjects with primary biliary cirrhosis, the transferrin D variant, and a rare neurological disease [3, 5]. Recent studies have suggested the possibility of using marker combinations, which could improve assay sensitivities without sacrificing specificity [2].

The aim of this study was to assess the diagnostic value of serum CDT level in comparison with serum GGT in the evaluation of alcohol intake in patients followed in an outpatient care centre.

MATERIALS AND METHODS

Since February 2005 till February 2006, 165 patients followed at the Psychiatry Department of Lugoj Hospital were included in this study. There were 100 males (mean age 48 ± 9.5 years) and 65 females (mean age 46 ± 10.1 years).

The medical team established a diagnosis of alcohol dependence according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, by administering the alcohol dependence element of the short form composite international diagnostic interview. Declared alcohol consumption was evaluated by one physician specialized in alcohology during a face-to-face interview. Five categories of patients were defined:

1. Non-weaned, alcohol intake > 30 g/d for women and > 50 g/d for men;
2. Relapse, alcohol intake > 30 g/d for women and > 50 g/d for men;
3. Moderate drinkers, alcohol intake < 30 g/d for women and < 50 g/d for men;
4. Weaned for less than one month;
5. Weaned for more than one month

Blood samples were then taken from each patient by venepuncture. Analysis of glutamyltransferase and per cent carbohydrate deficient transferrin was carried out at the Department of Clinical Biochemistry. Blood samples were drawn at the first consultation and later as prescribed depending on the clinical situation with change in drinking behaviour.

The concentration of CDT was measured by a turbidimetric immunoassay (TIA) after ion exchange chromatography. The assay detects primarily a-, mono- and disialotransferrin, although there may be some reactivity towards the trisialofraction of CDT [11]. The measurements were carried out on Behring Nephelometer II (Dade Behring, Behring Diagnostics GmbH, Marburg, Germany). The within-run precision was 4.7%, day-to-day variation was 6.0%, and accuracy 12.7%. Serum GGT was measured using enzymatic colorimetric assay, as standardized against IFCC (International Federation of Clinical Chemistry and Laboratory Medicine). The imprecision within run was 0.85% and the day-to-day variation was 0.54%. The accuracy of the GGT method was found to be 5.0%.

The cut-offs in the above assays were as follows: GGT, men 80 U/L, women 50 U/L; CDT, 2.6%.

We calculated the sensitivity of serum CDT and GGT assays in patients in category 1 (non-weaned), category 2 (relapse), and category 3 (moderate drinkers) and the specificity of the assays in patients in category 4 (weaned < 1 month) and category 5 (weaned > 1 month). Sensitivity and specificity values were determined for the entire cohort and by 10-year age group. The threshold level to calculate serum CDT assay sensitivity and specificity was set at 60 mg/L.

Statistics instruments were EPI Info 6 and Excel.

The study was performed in compliance with the guidelines of the local Ethics Committee of the Hospital and with the HelsinkiDeclaration of 1975, as revised in 2000.

RESULTS

Serum CDT and GGT levels are presented in Table 1 as a function of alcohol intake. Serum CDT level was higher in patients in category 1 (non-weaned) than in patients in the other categories ($p < 0.001$). However, serum GGT levels were lower in patients in category 1 compared with patients in category 2 (relapse) and category 4 (weaned < 1 month) ($p < 0.001$). The sensitivity of the assays for the two markers are presented in Table 2 for patients in categories 1 (non-weaned), 2 (relapse), and 3 (moderate drinkers). Specificity values for the two markers are presented in Table 3 for patients in categories 4 (weaned < 1 month) and 5 (weaned > 1 month). Considering all samples, the positive predictive value of CDT was 92% and the positive predictive value of GGT was 68%.

Table 1

Serum CDT and serum GGT levels according to patient's category

Parameter	n*	CDT (mg / L)	GGT (IU /L)
Category 1 (non-weaned)	32	105 ± 65.5	147 ± 103.6
Category 2 (relapse)	25	55 ± 15.3	51 ± 16.3
Category 3 (moderate drinkers)	44	57 ± 25.4	154 ± 229.1
Category 4 (weaned < 1 month)	51	119 ± 62.2	132 ± 210.1
Category 5 (weaned > 1 month)	13	43 ± 11.7	44 ± 73.2

n* – number of samples
$p < 0.001$

Table 2

Sensitivity of CDT and GGT in patients in category 1 (non-weaned), category 2 (relapse) and category 3 (moderate drinkers) according to age group

Age group (years)	Category 1		Category 2		Category 3	
	CDT	GGT	CDT	GGT	CDT	GGT
< 29	100%	70%	71%	57%	33%	28%
30–39	95%	63%	100%	45%	24%	32%
40–49	90%	73%	88%	53%	38%	42%
50–59	87%	81%	79%	63%	20%	53%
> 60	83%	75%	53%	58%	55%	36%
Total	92%	68%	77%	54%	32%	41%

Table 3

Specificity of CDT and GGT in patients in category 4 (weaned < 1 month) and category 5 (weaned > 1 month) according to age group

Age group (years)	Category 4		Category 5	
	CDT	GGT	CDT	GGT
< 29	50%	67%	100%	64%
30–39	72%	22%	97%	71%
40–49	70%	18%	96%	65%
50–59	65%	35%	95%	59%
> 60	92%	8%	92%	35%
Total	71%	23%	96%	62%

These findings demonstrate the good positive predictive values of serum CDT for the evaluation of alcohol intake. Earlier studies on CDT have been generally conducted in inpatients (hospitals or weaning centres) [4, 7, 9, 15, 21]. Outpatient care centres offer supportive counselling, prevention, screening and care

for alcohol-dependent or alcohol-abuse patients. Studies evaluating serum CDT levels have used alcohol consumption levels varying from 40–80 g/dL [12, 16, 27]. The thresholds retained for this study (30 g/dL for women and 50 g/d for men) were intermediate levels. Throughout the study, the patients' level of alcohol consumption was determined with the face-to-face interview method. A physician highly experienced with this method performed all estimations. With these questionnaires, a positive result orients towards alcohol abuse, without providing a quantitative estimation. Direct interview in a setting of confidence probably provides a better estimation [23].

DISCUSSION

In our study, the sensitivity of serum CDT level varied from 32% to 92% depending on the patient category. The sensitivity of CDT was better than that of GGT for patients in category 1 (92% *versus* 72%) and category 2 (77% *versus* 54%). The sensitivity of CDT ranged from 52% to 100% depending on the study and assay method, with a mean of 82%.

This diversity in sensitivity values is related to the heterogeneous nature of the study populations and severity of their liver disease, as well as the level of alcohol consumption, its duration, and the threshold level for serum CDT retained for the study. Conversely, we found that the sensitivity of serum GGT (41%) was better than that of CDT (32%) in patients in category 3 (moderate drinkers), especially in the subgroup of older subjects (50–59 years). Studies in the literature have reported GGT sensitivity ranging from 34% to 85%, depending on the severity of the liver disease, its association or not with other non-hepatobiliary disease, and patient age and gender. In a study including 1202 patients, the sensitivity of serum CDT exhibited superior sensitivity over serum GGT in men aged less than 40 years [8, 28].

In this study, the specificity of serum CDT varied from 71% to 96%, depending on the patient category. Specificity was however better than that of serum GGT (23–62%). In the review of the literature performed by Stibler [25], specificity of CDT ranged from 80% to 100%, with a mean of 97%. False positive results were noted for certain genetic variants of transferrin (form D) or for carbohydrate-deficient glycoprotein syndrome, or more rarely for patients with primary biliary cirrhosis and chronic hepatitis C [26]. The specificity of GGT reported in the literature has been to the order of 50%, ranging from 11% to 85%. This level of specificity results from elevated GGT observed in patients with non-alcoholic liver disease, obesity, or dyslipidemia, or taking medications [13].

Our findings confirmed the usefulness of serum CDT in following patients during the weaning process. Consequently, a single assay is insufficient for early diagnosis of weaning and repeated tests must be performed. We also observed a

significant decline in the serum GGT levels, but with less specificity. Serum assay of GGT provides a specific and rapid assessment of abstinence [20]. In the event of relapse, we observed a significant increase in both markers. In the literature, serum CDT has been found to exhibit very good specificity (91.7%) in relapse patients as well as better responsiveness than serum GGT in terms of rapid increase [14]. Elevated CDT levels are observed as early as two weeks [20, 26]. During follow-up, intra-individual variations can identify periods of low-level resumed consumption [6, 19].

CONCLUSION

This study confirms the better diagnostic value of carbohydrate-deficient transferrin in comparison with gamma-glutamyl transpeptidase and suggests that carbohydrate-deficient transferrin could be used to follow outpatients, particularly to confirm weaning or screen for relapse.

REFERENCES

1. ALDEN, A., S. OHLSON, P. PAHLSSON, *et al*., HPLC analysis of carbohydrate deficient transferrin isoforms isolated by the Axis-Shield%CDT method, *Clinica Chimica Acta,* 2005 **356**, 143–146.
2. ANTTILA, P., K. JARVI, J. LATVALA, *et al.*, A new modified gamma-%CDT method improves the detection of problem drinking: studies in alcoholics with or without liver disease, *Clinica Chimica Acta*, 2003, **338**, 45–51.
3. BEAN, P. J.B. PETER, Allelic D variants of transferrin in evaluation of alcohol abuse: differential diagnosis by isoelectric focusing-immunoblotting-laser densitometry, *Clinical Chemistry,* 1994, **40***,* 2078–2083.
4. BEHRENS, U.J., T.M. WORNER, L.F. BRALY F. SCHAFFNER, C.S. LIEBER, Carbohydrate-deficient transferrin, a marker for chronic alcohol consumption in different ethnic populations, *Alcohol Clin. Exp. Res.,* 1988*,* **12**, 427–432.
5. BELL, H., C.M.E. TALLAKSEN, K.TRY, E, HAUG, Carbohydrate-deficient transferrin and other markers of high alcohol consumption: a study on 502 patients, *Alcoholism: Clinical and Experimental Research,* 1994, **18***,* 1103–1108.
6. BORG, S., A. HELANDER, A. VOLTAIRE CARLSSON HOGSTROM A.M. BRANDT, Detection of relapses in alcohol-dependent patients using carbohydrate deficient transferrin: improvement with individualized reference levels during long-term monitoring, *Alcohol Clin. Exp. Res.,* 1995, **19**, 961–963.
7. HELANDER, A., A.V. CARLSSON, S. BORG, Longitudinal comparison of carbohydrate-deficient transferrin and gamma-glutamyl transferase: Complementary markers of excessive alcohol consumption, *Alcohol,* 1996, **31**, 101–107.
8. HELANDER, A., E. VABO, K. LEVIN, S. BORG, Intra- and interindividual variability of carbohydrate-deficient transferrin, gamma-glutamyltransferase, and mean corpuscular volume in teetotallers, *Clin. Chem.*, 1998, **44**, 2120–2125.
9. HENRIKSEN, J.H., M. GRONBAEK, S. MOLLER, F. BENDTSEN, U. BECKER, 1997, Carbohydrate deficient transferrin (CDT) in alcoholic cirrhosis: a kinetic study, *J. Hepatol*., 1997, **26,** 287–292.

10. HIETALA, J., K. PUUKKA, H. KOIVISTO, *et al*., Serum gammaglutamyl transferase in alcoholics, moderate drinkers and abstainers: effect on gt reference intervals at population level". *Alcohol and Alcoholism,* 2005, **40**, 511–514.
11. HOCK, B., M. SCHWARZ, I. DOMKE, *et al*., Validity of carbohydrate-deficient transferrin (%CDT), gammaglutamyltransferase (gamma-GT) and mean corpuscular erythrocyte volume (MCV) as biomarkers for chronic alcohol abuse: a study in patients with alcohol dependence and liver disorders of non-alcoholic and alcoholic origin, *Addiction,* 2005, **100**, 1477–1486.
12. HUSEBY, N.E., E. BJORDAL, O. NILSSEN, T. BARTH, Utility of biological markers during outpatient treatment of alcohol-dependent subjects: carbohydrate-deficient transferrin responds to moderate changes in alcohol consumption, *Alcohol Clin. Exp. Res.,* 1997*,* **21**, 1343–1346.
13. LITTEN, R.Z., J.P. ALLEN, J.B. FERTIG, Gamma-glutamyltranspeptidase and carbohydrate-deficient transferrin: alternative measures of excessive alcohol consumption, *Alcohol Clin. Exp. Res.*, 1995, **19**, 1541–1546.
14. MITCHELL, C., D. SIMPSON, J. CHICK, Carbohydrate deficient transferrin in detecting relapse in alcohol dependence, 1997, *Drug Alcohol Depend.,* **48**, 97–103.
15. OUYAHYA, F., Y. BACQ, F. SCHELLENBERG, E.H. METMAN, J. WEILL, Transferrine déficiente en acide sialique et maladies du foie. Etude de 94 malades, *Gastroenterol. Clin. Biol.*, 1995, **19**, 698–702.
16. REYNAUD, M., F. HOURCADE, F. PLANCHE, E. ALBUISSON, M.N. MEUNIER, R. PLANCHE, Usefulness of carbohydrate-deficient transferrin in alcoholic patients with normal gamma-glutamyltranspeptidase, *Alcohol Clin. Exp. Res.*, 1998, **22**, 615–618.
17. ROOM, R., T. BABOR, J. REHM, *Alcohol and public health. The Lancet,* 2005*,* **365**, 519–530.
18. ROSMAN, A.S., P. BASU, K. GALVIN, C.S. LIEBER, Utility of carbohydrate-deficient transferrin as a marker of relapse in alcoholic patients, *Alcohol Clin. Exp. Res.*, 1995, **19**, 611–616.
19. SALMELA, K.S., K. LAITINEN, M. NYSTROM, M. SALASPURO, Carbohydrate-deficient transferrin during 3 weeks heavy alcohol consumption, *Alcohol Clin. Exp. Res.,* 1994, **18**, 228–230.
20. SCHELLENBERG, F., H. MOURAY, La transferrine déficiente en hydrates de carbone: quoi de neuf 20 ans plus tard?, *Ann Biol. Clin.*, 2000, **58**, 298–309.
21. SILLANAUKEE, P., Laboratory markers of alcohol abuse, 1996, *Alcohol,* **31**, 613–616.
22. SILLANAUKEE, P., U. OLSSON, Improved diagnostic classification of alcohol abusers by combining carbohydrate-deficient transferrin and gamma-glutamyltransferase, *Clinical Chemistry,* 2001*,* **47**, 681–685.
23. Société française d'alcoologie, Recommandations pour la pratique clinique, *Alcool Addictol.*, 2001, **23** (4 Suppl), 18–76.
24. STIBLER, H., K.G. KJELLIN, Isoelectric focusing and electrophoresis of the CSF proteins in tremor of different origins, *J. Neurol. Sci.,* 1976, **30**, 269–285.
25. STIBLER, H., C. ALLGULANDER, S. BORG, K.G. KJELLIN, Abnormal microheterogeneity of transferrin in serum and cerebrospinal fluid in alcoholism, *Acta Med. Scand.,* 1978, **204**, 49–56.
26. STIBLER, H., Carbohydrate-deficient transferrin in Serum: a new marker of potentially harmful alcohol consumption reviewed, *Clin. Chem.*, 1991, **37**, 2029–2037.
27. XIN, Y., J.M. LASKER, C.S. LIEBER, Serum carbohydrate-deficient transferrin: mechanism of increase after chronic alcohol intake, *Hepatology,* 1995, **22**, 1462–1468.
28. YERSIN, B., J.F. NICOLET, H. DERCREY, M. BURNIER, G. VAN MELLE, A. PECOUD, Screening for excessive alcohol drinking. Comparative value of carbohydrate-deficient transferrin, gamma-glutamyltransferase, and mean corpuscular volume, *Arch. Intern. Med.,* 1995, **155**, 1907–1911.