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Alteration of Biological Markers in Alcohol-dependent Individuals without Liver Disease during the Detoxification Therapy

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Abstract. *Background/Aim: The need for sensitive biological markers to detect and prove recent drinking has been the focus of many research groups. The aim of our study was to investigate the alterations of biological markers in a population of alcohol dependent individuals during the detoxification period. Patients and Methods: Fifty-two alcohol-dependent individuals were admitted for alcohol detoxification on an inpatient basis. Carbohydrate-deficient transferrin (CDT), gamma-glutamyl transpeptidase (γ-GT), interleukin-6 (IL-6), mean corpuscular volume (MCV), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) were obtained at admission and on a 15-day basis. Comparisons between measures were made with t-test. Results: All biochemical parameters associated with alcoholism, with the exception of MCV, were statistically significantly decreased during the detoxification process ($p<0.05$). Conclusion: CDT is an excellent marker of alcohol overconsumption during evaluation, as well as during the detoxification treatment. IL-6 could serve as an additional marker to CDT, a point needing further investigation.*

Alcohol dependence represents a major social and medical issue worldwide, with severe adverse complications and consequences (1). The World Health Organization estimates that about 140 million people throughout the world suffer from alcohol dependence. Thus, it is understandable why so much effort has been made to identify, at an early stage, those patients who are at risk of later developing severe alcohol-induced neuropsychological disorders (2). Excessive drinking can lead to both reversible and permanent impairment of cognitive function and structural brain changes (3). Identification of alcoholism can be difficult. There are tools, such as the CAGE, MAST and AUDIT questionnaires, and some laboratory markers which may reflect both recent and chronic alcohol abuse. For the best results possible, laboratory tests should be combined with a detailed personal record, clinical examination and questionnaire (4, 5). In clinical practice, the need for sensitive biological markers to detect recent drinking has been the focus of many research groups. The conventional laboratory markers used are serum γ-glutamyl transferase (γGT), serum aspartate and alanine aminotransaminases (AST, ALT), and the mean corpuscular volume (MCV). Among the latest biological markers, the most important are serum carbohydrate-deficient transferrin (CDT) and interleukin-6 (IL-6) (6, 7). CDT, which reflects changes in the carbohydrate composition of serum transferrin, is a more specific marker for identifying excessive alcohol consumption and monitoring abstinence during outpatient treatment, as well as for the distinction of hepatic from non-hepatic disease (8-12).

The aim of our study was to investigate the alterations of these biological markers in a population of alcohol-dependent individuals on admission, two weeks later and after the completion of the detoxification period, as well as to assess their association by statistical methods at these time points. All our results were compared with a control group, consisting of 100 healthy blood donors used for the establishment of the normal values.

Patients and Methods

Patient population. The sample of the study comprised 52 alcohol-dependent individuals (37 males and 15 females), enrolled over a one-year period, who had consecutively contacted the Drug and Alcohol Addiction Clinic of the Eginition University Hospital in Athens, Greece. All patients fulfilled the DSM-IV diagnostic criteria for alcohol abuse/dependence-'primary alcoholism' (13) and were

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admitted to this specialized Department for alcohol detoxification on an inpatient basis. The patients had abstained from alcohol for an average of 24.0±12.2 hours prior to their admission to the clinic. Informed consent was obtained from each individual, and participation in the project was on a voluntary basis. Detailed information on the objectives of the study and the research therapeutic protocol was provided to all participants. Ethical permission for the study was obtained by the responsible Special Scientific Committee on human experimentation of the Eginition Hospital and the procedures followed were in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 1983. The study participants had to fulfill the following criteria: (i) age between 22-76 years; (ii) absence of serious physical illness, as assessed through physical examination and routine laboratory screening; (iii) absence of another pre- or coexisting major psychiatric disorder on the DSM-IV axis I; (iv) absence of other substance abuse; the mere presence of affective symptoms was not considered to be an exclusion criterion. Alcohol abusers who fulfilled the DSM-IV diagnosis of depressive disorder were excluded from the study if a major depressive episode had been recorded before the beginning of alcoholism. When the depressive episode was present concurrently with an alcohol-abusing period, these patients were not excluded.

Study design. Upon admission, alcohol detoxification was initiated and completed over one week (approximately 7 to 10 days) in all participants. Detoxification consisted of vitamin replacement (vitamins of B complex, vitamin C, vitamin E) and oral administration of diazepam (30-60 mg daily in divided doses), with gradual tapering off over a week. Thereafter, the participants followed an inpatient standard treatment program with a short-term psychotherapy of cognitive-behavioral orientation. This program lasted for 4-5 weeks. It consisted of individual sessions (twice a week), as well as of family interventions (at least once every 2 weeks). It is self-evident that alcohol consumption was prohibited during hospitalization.

Assessments. The participants were diagnosed by the Schedules for Clinical Assessment in Neuropsychiatry (14) and assessed through the Composite International Diagnostic Interview (15) for their pattern of alcohol abuse, potential major life problems related to alcohol consumption and the occurrence of withdrawal symptoms in the past; a structured questionnaire similar to that proposed by the World Health Organization (16) was also used to assess the pattern of alcohol use. This questionnaire includes items related to lifetime, past year and past month frequency and quantity of alcohol use. Furthermore, sociodemographic data (age, socioeconomic status, marital status, level of education) and previous psychiatric history (pre-existing diagnosis, medications and hospitalizations) were recorded. All data pertaining to alcohol use were self-reported. However, to ascertain the accuracy of the information, a relative was also interviewed to corroborate the current status and psychiatric history. Four appropriately trained psychiatrists, who work in this specialized section, carried out the interviews. The mean inter-rater reliability was 0.90. A different assessor, who was blind to the previous scores, conducted the evaluation each time.

Laboratory examination. Fasting blood from all patients was obtained within 24 h upon admission for detoxification to our Department, two weeks later and after the completion of the detoxification period (4-5 weeks). The levels of hepatic enzymes (AST, ALT, γ-GT) were measured using conventional clinical chemical diagnostic automated photometric methods (Olympus Diagnostic Systems, Hamburg, Germany). MCV and CDT were determined at the same time points. CDT concentration was determined by using a commercial nephelometric N Latex CDT kit (Dade Behring Marburg GmbH, Marburg, Germany). Serum concentration of IL-6 (detection range: 0.1-4.6 pg/ml) was measured by using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) (Quantikine, R&D Systems Inc., Minneapolis, MN, USA). Samples and standards were run in duplicate, and the average of the optical density was considered for the calculation of the concentration. The lower detection threshold for IL-6 was 0.1 pg/ml. Intra-assay and inter-assay precision was given by the manufacturer.

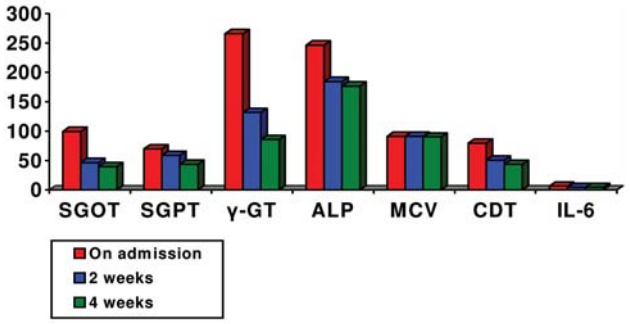

Figure 1. *Alterations in the serum concentrations of biological markers.*

Results

There was a progressive decrease in the means of all parameters, proceeding from the first to the last sample. By using the *t*-test for the study of quantitative features, it was shown that the changes in parameters from the first to the last blood sample are statistically significant ($p<0.05$) in all cases except for the modification of MCV (Figure 1). It seems that all the parameters associated with alcoholism, with the exception of MCV, significantly decrease during the detoxification process.

Discussion

In the current study, we investigated the alterations of several biological markers in alcohol-dependent individuals without liver disease. Serum γGT activity increases in hepatic and biliary tract disease, as well as after severe consumption of alcohol. Its sensitivity as a marker is 60-90% in addicted individuals and 20-50% in nondependent high consumers of alcohol; in addition, specificity is 55-100%. However, a variety of abnormalities or medication can raise γGT and give false-positive results (4, 5, 8, 17-22). AST and ALT activities are in many cases

moderately elevated in alcoholics. AST as a marker has a sensitivity of 25-60% and that of ALT of 15-40%. The use of AST/ALT ratio improves sensitivity, a ratio of >1.5 strongly suggests liver damage, while a ratio of >2.0 is practically indicative of alcohol-induced liver damage. In only one study did AST/ALT appear to be the best marker for the distinction between alcoholic and non alcoholic hepatic disease (4, 5, 10, 17, 19, 20). Increased MCV usually accompanies chronic excessive alcohol consumption and is associated with both the quantity and frequency of consumption. Its main disadvantage is its low sensitivity of 40-50%, but specificity is high 80-90% (8, 18). Lipids do not seem to be either sensitive or specific markers and are therefore not used in diagnosis and monitoring of alcoholism (21). Transferrin exists in normal serum in various forms containing 0-9 sialic acid residues, predominantly tetrasialotransferrin (80%). CDT consists of the asialo, monosialo, and disialo isoforms which are less negatively charged at ph 7.4. Alcoholics consuming 50-80 g alcohol daily for at least one week will show an increase of CDT value in their blood serum. During abstinence, CDT normalizes, with a half-life of 15 days, and this is why it remains elevated for several weeks. A review summarizing 20 studies involving 2500 established alcoholics calculated overall sensitivity at 82% and specificity at 67%. In an attempt to improve the low sensitivity, the CDT/total transferrin ratio has been proposed as a better marker (23). The main advantage of the CDT/total transferrin ratio is its high specificity (80-95%) as reported in most of the studies, which is decreased by several conditions and diseases. False-positive results arise in non-alcoholic hepatic disease such as primary biliary cirrhosis and chronic hepatitis C (8, 24-28). Furthermore, CDT seems to be an excellent marker of alcohol overconsumption, as well as for follow-up during detoxification. IL-6 is a multifunctional protein mainly produced by lymphocytes but also other types of cells such as fibroblasts, endothelial, cardiac and tumor cells. In recent research, it was found that increased IL-6 values characterize alcoholics without hepatic disease. It seems that immunological mechanisms influence a sequence of events which cause hepatic disease in some high alcohol consumers (6, 29). In our study, IL-6 values decreased significantly during the detoxification process. However, its alteration during and after therapy is not yet clearly understood. In our study, there was no significant MCV decrease during detoxification. More studies are needed for the evaluation of this marker.

In conclusion, CDT is an excellent marker of alcohol overconsumption during evaluation of patients, as well as during their detoxification treatment. IL-6 could serve as an additional marker to CDT, a point needing further investigation.

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