Abstract. Background: Chronic alcohol consumption has been associated with both liver dysregulation and neurotoxic effects in the central nervous system of human beings and experimental animals. Serum levels of S100B protein have been extensively studied in several conditions of neural tissue injury but not in alcohol abuse. The aim of this study was to evaluate the serum levels of S100B in alcohol-dependent individuals and to further investigate the effect of alcohol detoxification on the levels of S100B. Patients and Methods: Our study included 20 alcohol dependent/abusing subjects, diagnosed based on the DSM-IV criteria and treated on an in-patient basis according to a standard detoxification protocol. The serum concentration of hepatic enzymes (ASAT, ALAT, γGT), as well as measurements of anxiety, depression and global functioning were obtained at baseline and at weekly intervals over the period of 4-5 weeks, while S100B levels were measured on admission and discharge. Results: Upon admission, hepatic enzyme levels were found increased compared to normal levels and correlated positively with the degree of alcohol consumption of the last year. Interestingly, the ALAT levels correlated positively with S100B levels upon admission. After completion of alcohol detoxification, the hepatic enzyme levels returned to normal. The S100B levels decreased in 10 patients with a moderate alcohol-consumption over the last year, but increased in 10 patients with high alcohol consumption over the last year. Additionally, a significant correlation was found between the levels of S100B and the global functioning scale at the end of detoxification treatment. Conclusion: S100B protein levels are affected differently in alcohol-dependent individuals with either mild or high alcohol consumption during the period of up to one year before assessment. A good correlation between the release pattern of S100B and global functioning scale was found. Although this is a preliminary study, the present data suggest a possible use of S100B protein measurements in detecting alcohol-dependent individuals with high alcohol consumption and in further monitoring the alcohol detoxification treatment.

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Key Words: Alcohol abuse, S100B, alcohol-detoxification, global functioning.
The sample of our study comprised 20 alcohol subjects. Patients and Methods

Several hypotheses have been proposed regarding the function of S-100 cells: including a role resembling that of adrenocorticotropic hormone (ACTH)-secreting cells (19-22) and their function as stem cells (23), undifferentiated granular cells (24), supporting cells (25), phagocytic cells (26, 27) and nursing cells of the adenohypophysis (28).

Contradictory studies, however, regarding the value of this protein as a marker of alcohol abuse neurotoxicity were reported (29, 30) and there is no evidence on the effect of detoxification treatment in S100B levels.

The aim of the present study was to investigate the effect of chronic alcohol consumption on serum S-100B levels and to further evaluate the influence of detoxification treatment on these levels.

Patients and Methods

Subjects. The sample of our study comprised 20 alcohol dependent/abusing subjects (16 males, 4 females), who had consecutively contacted the Drug and Alcohol Addiction Clinic of the Athens University Psychiatric Clinic at the Eginition Hospital in Athens, Greece. Informed consent was obtained from each participant, 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 subjects. Ethical permission for the study was obtained from the special scientific committee of the Eginition Hospital and the procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

All patients fulfilled the DSM-IV diagnostic criteria for alcohol abuse and dependence (31) presenting for detoxification on an inpatient basis. They were abstinent from alcohol for an average of 28.0±15.2 days before admission to the clinic. Subjects included in the study had to fulfill the following criteria: a) age between 25 and 65 years, b) absence of serious physical illness (as assessed through physical examination and routine laboratory screening), c) absence of another pre- or co-existing major psychiatric disorder on the DSM-IV axis I and d) absence of other drug 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 prior to the onset of alcoholism; subjects were not excluded whenever a depressive episode was present concurrently with an alcohol-abusing period.

The mean age of the subjects was 48.94±9.27 years and the mean alcohol consumption over the last month before admission was 235.17±124.19 g/day.

Study design. Upon admission, the detoxification protocol was initiated and completed over one week (7-10 days). Detoxification comprised vitamin replacement (vitamin C, vitamin E, vitamins of the B complex) and oral administration of diazepam (30-60 mg in divided doses), with gradual taper-off over a week. Thereafter, a standard treatment program with a short-term psychotherapy of cognitive-behavioral orientation was implemented and lasted for 4-5 weeks; it consisted both of individual sessions (twice a week) and family interventions (at least once every two weeks). Alcohol consumption was prohibited during hospitalization.

Assessments. The participants were diagnosed by the Schedules for Clinical Assessment in Neuropsychiatry and assessed through the Composite International Diagnostic Interview (32) 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 the one proposed by the World Health Organization (32) 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-existent diagnosis, medications and hospitalizations) were recorded (32).

The Hamilton Depression Rating Scale (HDRS) (33), the Hamilton Anxiety Rating Scale (HARS) (34) and the Global Assessment Scale (GAS) (35, 36) were used for the assessment of psychopathology. Depressive and anxiety symptoms, as well as the level of functioning, were initially evaluated within 48 h upon entering the program (first assessment at time-point 1) and sequentially assessed 7±2 days apart, over the 4-5 weeks detoxification period (fourth assessment at time-point 4).

The levels of hepatic enzymes (ASAT, ALAT and γGT) were also measured at the beginning as well as at the end of the detoxification program using diagnostic kits from Olympus diagnostic systems, Hamburg, Germany (ASAT, ALAT, γGT: units/ L).

The S100B concentration was measured in all samples by an immunoluminometric assay (Lia-mat Sangtec 100, AB Sangtec Medical, Bromma, Sweden). According to the manufacturer’s instructions, this assay specifically measures the h subunit of the S100 protein, as defined by the three monoclonal antibodies: SMST 12, SMSK 25 and SMSK 28. The h subunit of the S100 protein is known to be predominant (90-96%) in the human brain. Each measurement was performed in duplicate according to the manufacturer’s recommendations and the averages were reported. As indicated by the manufacturer, the limit of detection of the assay (B0±3SD) was 0.02 Ìg/L and the precision (CV) was 5.5% or lower (within-assay) and 10.1% or lower (inter-assay) for concentrations ranging between 0.28 and 4.17 Ìg/L.

For the assessment of the severity of symptoms withdrawal, a modified version of the Addiction Research Foundation Clinical Institute Withdrawal Assessment for Alcohol (CIWA-Ar) was used twice daily during the first week of abstinence from alcohol. This 10-item, 5-point modified scale, which we routinely use for the assessment of alcohol withdrawal symptoms, provides a measure for symptoms of autonomic hyperarousal (nausea, tremor, sweats), anxiety, agitation, perceptual (tactile, auditory, visual) disturbances, and disorientation (rated from 0=absent to 4=severe; maximum possible score=40).

Statistical analysis. Data are expressed as mean±SD and p<0.05 was considered statistically significant. A comparison of medians was performed by Mann-Whitney W-test. The statistical procedures were performed using the STATGRAFICS PLUS version 5.1 for Windows program (Graphic Software System).
Table I. Demographic data of alcohol-dependent individuals (N=20).

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>48.94±9.27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>14</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>73.42±11.43</td>
</tr>
<tr>
<td>Alcohol consumption-last month (g/day)</td>
<td>235.17±124.19</td>
</tr>
<tr>
<td>Alcohol consumption-last year (g/day)</td>
<td>174.72±113.20</td>
</tr>
</tbody>
</table>

Values are expressed as means±SD.

Results

Demographic data. The study included 20 subjects (16 males, 4 females) who completed the 4-week detoxification period (Table I). The mean age was 48.94±9.27 years and there was no statistical age difference between male and female alcohol-dependent individuals. The mean alcohol consumption in g/per day over the last month was 235.17±124.19 and over the last year was 174.72±113.20. The mean age at onset of alcohol abuse was 26.91±10.14 years. The mean weight was 73.42±11.43 Kg. Regarding the socioeconomic status of the participants, all belonged to a middle socioeconomic level.

Biochemical profile. Upon admission, all alcohol abusers presented increased serum levels of ASAT (62.94±59.07 U/L), ALAT (60.61±58.08 U/L) and a marked elevation of γGT (170.83±182.31 U/L). The normal range for ASAT is 7-40 U/L, for ALAT 7-40 U/L and for γGT 7-49 U/L. After the detoxification period, most values were significantly reduced. Specifically, ASAT was 38.28±14.93 (p<0.001), ALAT 34.11±12.63 (p<0.001) and γGT 50.11±30.24 (p<0.001), respectively (Table II).

There was a statistical correlation of all hepatic markers ALAT, ASAT and γGT on admission with alcohol consumption during the last year (ALAT/consumption during the last year: r=0.637, p=0.004; ASAT/consumption during the last year r=0.609, p=0.007; γGT/consumption during the last year r=0.738, p=0.001).

Regarding S100B levels, ten alcohol-dependent individuals presented a decrease in S100B values (0.13±0.04 vs. 0.10±0.02 µg/l, p<0.01) (Figure 1) at the end of treatment and the other ten subjects presented an augmentation of S100B levels (0.13±0.04 µg/l vs. 0.24±0.02 µg/l, p<0.01) (Table III, Figure 2). Interestingly, the levels of S100B correlated positively with ASAT levels (r=0.801, p=0.017) upon admission as well with alcohol consumption over the last year in both cases (Table IV).

Psychopathology data. On admission, the total HDRS mean score±SD was 39.33±2.03, 37.72±1.64 for HARS and 46.11±5.02 for GAS. These scores indicate severe
depressive-like symptoms and high level of anxiety. At the end of detoxification treatment, the alcohol-dependent individuals had an HDRS mean score±SD of 6.22±1.06, HARS of 7.56±1.38 and GAS 79.44±5.39. The differences between the first and the fourth assessment were highly significant \( (p<0.001) \). The values at the end of detoxification treatment were within the normal range and indicated a recovery from the anxiety and depressive-like symptoms. Furthermore, S100B levels showed a positive correlation with the global assessment scale at the end of detoxification period \( (r=0.724, p=0.042) \).

**Discussion**

Several studies have described the effect of alcohol abuse/dependence on various organ systems. However, the multifactorial nature of alcohol dependence has made evaluation of the observed alterations and of the underlying (patho) physiological regulatory mechanisms difficult, with the majority of findings and interpretations remaining controversial. Biochemical markers offer a new strategy in the diagnosis, estimation of clinical prognosis and monitoring of treatment in alcohol-dependent individuals. Although, hepatic enzyme levels are the predominant factors monitoring liver damage, no markers have to date been determined, for the evaluation of alcohol-induced brain damage.

The present study investigated the possibility of alcohol-induced neurotoxic damage to be reflected in the serum concentration of S100B protein, a sensitive marker of neural tissue injury (7-9). Since the levels of S100B protein have been extensively studied in several conditions of neural damage but not in alcohol abuse, the aim of this study was to evaluate the levels of S100B in alcohol-dependent individuals and to further investigate the possible effect of alcohol detoxification on this protein.

S100B is a protein belonging to the multigenic family of calcium-modulated proteins (S-100 proteins) that were first identified as a protein fraction detectable in the central nervous system (CNS), where it exerts neurotropic and gliotropic action (12, 13). In the nervous system, the protein appears to be most abundant in glial cells, although its presence in neuronal subpopulation has also been reported. Since the pioneering observations in CSF, which represents the biological fluid into which brain constituents might most directly be released, the more recent studies were based on the hypothesis that S100B released from the damaged tissue could spread into the systemic circulation (7-9).

The present data provide some preliminary evidence of a different association of S100B levels with alcohol consumption in alcohol-dependent individuals, closely related to the quantity of alcohol consumption over the last year prior to treatment. Specifically, the subjects who
consumed mild quantities of alcohol over the year before entering the detoxification program showed a decrease in their S100B levels at the end of treatment, suggesting a possible improvement of neuronal injury. On the contrary, alcohol-dependent individuals who consumed high concentrations of alcohol over the last year showed a rather constant or a slight elevation in their S100B levels at the end of treatment. A possible prolongation of detoxification, although not tested in the present study, could have succeeded in decreasing S100B levels in heavy alcohol-abusing individuals. Therefore, it can be postulated that S100B levels could represent a marker of alcohol consumption and of the severity of alcoholism in alcohol-dependent patients.

Additionally, since a positive correlation was observed between the levels of S100B and of the hepatic marker ASAT upon admission, it could be suggested that S100B levels may play a role in the evaluation of hepatic function in alcohol-dependent subjects. Furthermore, the positive correlation of S100B protein with the global functioning scale at discharge suggests another property of this protein in influencing the functioning abilities of alcohol-dependent subjects.

The possible mechanism by which alcohol detoxification results in S100B protein level reduction could be through the amelioration of brain inflammation. There is evidence that antibodies to the S-100 protein (propoten-100) could be used in the therapy of patients with alcohol withdrawal syndrome (37).

In conclusion, the present study provides some preliminary data on the possible use of S100B serum levels as a sensitive marker of alcohol consumption over a one-year period. Furthermore, the effect of a four-week alcohol-detoxification treatment seems to depend strongly on the quantity of alcohol consumption over the last year. Future investigation of the possible interrelationships between S100B protein levels, hepatic function and mood status of alcohol-dependent patients is needed in the light of other consequences of long-term heavy alcohol use such as concurrent adaptive changes, organ dysfunction or organ damage. Ensuing studies with larger subject numbers should follow in order to verify these preliminary observations.

References


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