Vitamin B12 and hepatic enzyme serum levels correlate with interleukin-6 in alcohol-dependent individuals without liver disease

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Abstract

Alcohol abuse is a major cause of liver cirrhosis as well as chronic liver disease. The aim of the present study was to investigate the possible correlation, between liver dysfunction biological markers and vitamin B12, with interleukin-6, in the serum of alcohol-dependent individuals without liver disease (AWLD).

In a sample of 43 alcohol abusing/dependent subjects (33 males and 10 females) treated on an inpatient basis according to a standard detoxification protocol, the serum activities of the hepatic enzymes (ASAT, ALAT, γ-GT), as well as the concentration of B12 and IL-6, were determined on admission.

A strong positive correlation has been observed between IL-6 and B12, ASAT, ALAT, and γ-GT at the beginning of the detoxification period.

The results confirmed that in alcohol-dependent individuals, the median serum concentration of IL-6, before the beginning of the treatment, had a significant positive correlation with the liver dysfunction biological markers and B12. In conclusion, IL-6 might be used as an additional diagnostic marker for the degree of liver dysfunction in alcohol dependent individuals.

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Introduction

Alcohol dependence represents a serious health issue with major socio-economic consequences in Western countries. It has been estimated that 20–50% of alcohol-dependent individuals are receiving medical care, whereas the most recent edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) describes alcohol as the most frequently used brain depressant in most cultures and a cause of considerable morbidity and mortality [1,2].

It is well-known that alcohol dependence can potentially affect almost every organ system of the human body, resulting in serious disorders, such as liver disease [3–5], impaired heart function [6], inflammation of the pancreas [7], and alteration in immune regulation, leading to immunodeficiency and autoimmunity. Furthermore, alcoholic liver disease remains one of the most common causes of chronic liver disease in the world, usually accompanied by hepatitis, cirrhosis, and/or hepatocellular cancer [8]. The severity of liver damage related to alcohol abuse varies among different individuals and even within any given individual at different times. It has been estimated that only 30% of alcohol-dependent individuals develop cirrhosis, suggesting that the development of alcohol-induced liver injury requires one or more additional factors [9]. Furthermore, alcohol abuse can exacerbate Hepatitis C viral infection usually
associated with liver disease by causing oxidative stress and promoting fibrosis, thereby accelerating disease progression to cirrhosis [10].

The activities of hepatic enzymes ASAT (aspartate-aminotransferase), ALAT (alanine-aminotransferase), and γ-GT (gamma-glutamyl transpeptidase) are commonly used as clinical markers for recent alcohol abuse [10–12]. The application of these tests improves significantly the information received by single serum determinations, thus allowing a better discrimination between alcoholic and non-alcoholic origin of liver disease [13]. However, their individual concentration can be influenced by additional factors to alcohol abuse.

Liver disease has been frequently associated with elevated vitamin B12 levels in serum [14–18] and with a lowered liver tissue total vitamin B12 concentration [19].

Serum vitamin B12 levels, plasma cobalamin, total corrinoids, and their analogues were found to be elevated in patients suffering from alcoholic cirrhosis [20,21]. Lambert et al. [22] found that the concentration of vitamin B12 bound to transcobalamins I and III was positively correlated with plasma ASAT in alcoholic cirrhotic patients. In patients with alcoholic liver disease, vitamin B12 levels were found to range within normal limits, except for a group of cirrhotic patients where vitamin B12 levels were raised, so biochemical changes in blood vitamin B12 status may precede clinical manifestations of a cirrhotic process and may have prognostic value [21]. Sex differences in the serum vitamin B12 levels have been described in a study by Goldman et al. [23], where high vitamin B12 levels were as accurate an indicator of alcoholism in female patients as elevated ALAT, γ-GT, and mean corpuscular volume (MCV) values. In contrast, for males the vitamin B12 serum levels of alcoholics were not significantly different from non-alcoholics. However, in another study by Himmerich et al. [6], significant positive correlations between vitamin B12 and hepatic enzyme values were found in 80 male alcohol-dependent patients, further indicating a clinical relevance of B12 levels in hepatocellular damage.

Alcohol dependence, complicated by systemic and hepatic manifestations, is thought to reduce natural killer cell responses and to alter cellular immunity by changing the relative balance of Th1 versus Th2 cytokine response profiles [24]. Such alterations may lead to increased susceptibility to infection [25]. Results of early investigations of cytokine abnormalities in patients with alcoholic liver disease (ALD) have demonstrated increased serum concentrations of interleukin (IL)-1, tumor necrosis factor-alpha (TNF-α), IL-6, and IL-8 [26–28].

IL-6 is a multifunctional protein produced by lymphoid and nonlymphoid cells, and by normal and transformed cells [29]. Its production is either positively or negatively regulated, by a variety of signals, including mitogens, antigenic stimulation, lipopolysaccharide, IL-1, tumor necrosis factor (TNF), platelet-derived growth factor, and viruses [29,30]. IL-6 is acting on B cells and T cells in a different way [31,32]. Furthermore, there is evidence that IL-6 helps to maintain the hepatic microcirculation at normal levels [33]. Previous studies have shown that elevation of serum IL-6 levels is always associated with ALD, but the significance of such elevation is not clear. In vivo studies have shown that chronic ethanol consumption induces significant apoptosis in the liver of IL-6 (−/−) mice but not IL-6 (+/+) mice. IL-6 (−/−) hepatocytes are more susceptible to ethanol- and TNFα-induced apoptotic killing, which can be corrected by IL-6. These findings suggest that elevated serum IL-6 levels in ALD may overcome the inhibitory effect of ethanol on IL-6-mediated anti-apoptotic signals and prevent alcohol-induced hepatic apoptosis [34].

Previous data from our group have shown increased serum IL-6 levels in AWLD suggesting that the presence of pro-inflammatory signals in these subjects may be associated with the progression from AWLD to ALD [29]. Thus IL-6 can be an additional useful diagnostic marker for the degree of liver dysfunction [35].

The present study involves investigation of the relationship between the levels of liver dysfunction biological markers (ASAT, ALAT, γ-GT) and vitamin B12 with interleukin-6 in the serum of 43 alcohol-dependent subjects consecutively admitted for detoxification at our department.

Materials and methods

Subjects

The study comprised of 33 males and 10 females, with a mean age of 48.37±9.94 years (age at first use of alcohol 25.79±12.00 years), and mean daily alcohol consumption 272.00±148.83 g/day. The subjects included were randomly enrolled over a 2-year period and fulfilled the DSM-IV diagnostic criteria for alcohol/dependence – “primary alcoholism” – (American Psychiatric Association, 1994). They were admitted to a specialized department of Athens University Psychiatric Clinic for alcohol detoxification on an inpatient basis. The alcohol abusers had been abstinent from alcohol for an average of 24±12.2 h prior to admission to the clinic. Informed consent was obtained from each participant, and their participation in the project was on a voluntary basis. Ethical permission for the study was obtained from the special scientific committee of the hospital and the procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in Hong Kong in 1983. This detoxification program (4–5 weeks) is a specialized abstinence therapeutic program. During this program special care is given to the patient’s physical health and somatic problems. After the completion of the detoxification procedure, the alcohol dependent individuals are referred to an Outpatient Drug Free Drug Addiction Clinic for a follow-up therapeutic program. They are offered individual psychotherapeutic interviews (CBT) at least once a week for two years.

The subjects included in the study had to fulfill the following criteria: (a) age between 18 and 70 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, (d) absence of another 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 before the beginning of alcoholism. There were not excluded, whenever a depressive symptomatology was present, concomitant with an alcohol abusing period. Upon admission, alcohol detoxification was initiated and completed over one week (approximately 7 to 10 days). Detoxification comprised vitamin replacement (vitamins of B complex, vitamin C, vitamin E) and oral administration of diazepam (30–60 mg daily in divided doses), with gradual taper off over a week.

**Assays**

Fasting blood from all patients was obtained within 24 h upon admission for detoxification to our department. The levels of hepatic enzymes (ASAT, ALAT, and γ-GT, U/L) were measured using diagnostics kits from Olympus diagnostic systems, Hamburg, Germany.

Serum B12 levels (180–900 pg/mL) were measured using diagnostic kits from Beckman Access II, USA. Serum concentration of IL-6 (0.1–4.6 pg/mL) was measured by using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) technique (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.

**Statistical analysis**

Due to the skewed distribution of the parameters under study (ALAT, ASAT, vitamin B12, γ-GT, and IL-6), their values were transformed using the natural logarithm. The transformed variables had an approximately normal distribution and the parametric tests assuming a normal distribution were used. On the logarithmic transformed variables, the Pearson’s correlation coefficient and the corresponding coefficients of the linear regression were calculated. Multiple linear regressions having as dependent variable the logarithm of IL-6 and the hepatocellular enzymes ASAT, ALAT, γ-GT as well as vitamin B12. An increased serum concentration of IL-6 and the hepatocellular enzymes ASAT, ALAT, γ-GT as well as vitamin B12 were found to be significantly correlated with smoking habits and alcohol consumption (r=0.7212, p<0.0001; r=0.7212, p<0.0001; r=0.7212, p<0.0001; r=0.7212, p<0.0001).

The present study aimed at identifying a relationship between serum IL-6 levels and vitamin B12 as well as hepatic enzyme levels in alcohol-dependent individuals without liver disease.

Highly significant positive correlations were found between IL-6 and the hepatocellular enzymes ASAT, ALAT, γ-GT as well as vitamin B12. An increased serum concentration of IL-6 is a strong indicator of an imbalance of the proinflammatory mediators, in the direction of inflammatory signals, which thus may participate in the development of future liver disease [29].

IL-6 being a multifunctional protein is produced by a variety of cell types through positive or negative regulation by a variety of signals, including mitogens, antigenic stimulation, lipopolysaccharide, other cytokines, and viruses [5].

Its effects on different cells vary and it has been found to stimulate production of acute-phase proteins by hepatocytes as well as to have colony-stimulating activity on hematopoietic stem cells [4,7].

There is evidence that the hepatoprotective effect of IL-6 is due partly to prevention of sinusoidal endothelial cell injury, which helps to maintain normal hepatic microcirculation. In addition to protection against endothelial cell injury and im-

**Table 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total patients (n=43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>33</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.37±9.27</td>
</tr>
<tr>
<td>Alcohol consumption (g/day)</td>
<td>272±148.83</td>
</tr>
<tr>
<td>Age at first use of alcohol (years)</td>
<td>25.79±12.00</td>
</tr>
</tbody>
</table>

Values are expressed as means±SD.

**Biochemical profile**

The descriptive measures of the parameters upon admission are shown in Table 2. The mean values of the hepatic enzymes were: ASAT 46.27±41.30 U/L (normal values: 7–40 U/L), ALAT 40.67±28.98 U/L (normal values: 7–40 U/L) and γ-GT 153.65±271.92 U/L (normal values: 7–49 U/L). B12 levels were 744.58±412.90 pg/mL (normal values: 240–1100 pg/mL) and IL-6 3.22±4.22 pg/mL. IL-6 showed a positive and significant correlation with all measured parameters: ASAT (r=0.66, p<0.0001), ALAT (r=0.666, p<0.0001), B12 (r=0.4877, p=0.0009), and γ-GT (r=0.96, p<0.0001, Fig. 1). In all cases, an increase in each one of the predictors resulted in increasing values of IL-6 on admission. However, the predictors, were also correlated with each other (ALAT/ASAT, r=0.8751, p<10−4; ALAT/γ-GT, r=0.7315, p<10−4; ASAT/γ-GT, r=0.7212, p<10−4; γ-GT/B12, r=0.4428, p=0.0029). Thus, in a multiple linear regression, the only variable that independently predicted the values of IL-6 was γ-GT (coefficient 0.8538622, p-value <10−4, 95% C.I.: 0.7760483, 0.9316762). The total proportion of the variability in the values of IL-6, explained by this model was 92.29% (R²).

**Discussion**

The study included 43 alcohol-dependent individuals (33 males and 10 females) who entered the 4-week detoxification program (Table 1). There was no statistical age difference between male (45.64±9.94) and female alcohol-dependent individuals (45.42±9.96, min 22, max 76). The mean alcohol consumption in g/per day was 272±148.83. The mean age at onset of alcohol abuse was 25.79±12. The mean weight was 74.42±11.45 kg.
Improvement of hepatic microcirculation, IL-6 prevented hepatocyte death, which may also contribute to its hepatoprotective effect in steatotic isografts [33].

It is well known that both IL-6 secretion by monocytes and plasma concentrations of this cytokine tend to be slightly increased in ALD. IL-6, together with TNF-α, was found to correlate with mortality in alcoholic hepatitis [7,36,37]. Also, in patients with cirrhosis it was observed that the serum concentration of IL-6 was elevated and correlated with increased serum concentrations of immunoglobulin A. Moreover, it was found that the IL-6 overproduction is responsible for the hypergammaglobulinemia observed in cirrhosis [4,5]. Furthermore, IL-6 production in peripheral leukocytes of patients with alcoholic cirrhosis was significantly increased in comparison with findings for control subjects [4].

In the present study, increased IL-6 concentrations were detected in serum samples obtained from patients who were chronically dependent on alcohol but without ALD. Results of several studies have shown a correlation between circulating levels of proinflammatory cytokines and progression of ALD, during chronic ethanol consumption. The exact mechanism of liver injury has not been well defined, but there is evidence that acute alcohol consumption and chronic alcohol dependence can increase gut permeability to endotoxins and impair the reticuloendothelial function of the liver, which may result in increased plasma endotoxin concentrations [38,39].

In our study IL-6 levels correlated positively with serum concentration of vitamin B12. The highly significant correlation was found despite the fact that most vitamin B12 values ranged within normal limits. In addition our results demonstrate a substantial positive relationship between vitamin B12 and hepatic enzyme serum levels. This correlation may be interpreted as meaning that, with increasing hepatocellular damage, as indicated by elevated hepatic enzymes, serum vitamin B12 also tends to be higher. Possible explanations for this phenomenon may be the failure of the damaged liver to take up cobalamin and analogues from the serum [40,41]. Another plausible explanation may lie in the finding that, in hepatic damage, liver tissue vitamin B12 binding and storage of transcobalamin is disrupted and causes vitamin B12 to leak out of the liver into the circulation (42). The latter may have relevant clinical implications: in a malnourished patient with primarily decreased peripheral vitamin B12 levels, the deficiency of vitamin B12 in the periphery may be temporarily masked by vitamin B12 leaking out of hepatic cells affected by acute alcohol consumption. A single measurement of vitamin B12 levels might therefore be misleading in some patients. For a proper interpretation of vitamin B12 serum levels in alcohol-dependent patients, a correlation with ALAT, γ-GT, and B12 concentrations in alcohol-dependent individuals without liver disease.

Fig. 1. Scatter plots showing the correlation of serum IL-6 levels with ASAT, ALAT, γ-GT, and B12 concentrations in alcohol-dependent individuals without liver disease.

Table 2
Descriptive statistics for laboratory parameters of alcohol-dependent patients (n=43)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean±SD</th>
</tr>
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<tbody>
<tr>
<td>ASAT (U/L)</td>
<td>46.27±41.30</td>
</tr>
<tr>
<td>ALAT (U/L)</td>
<td>40.67±28.98</td>
</tr>
<tr>
<td>γ-GT (U/L)</td>
<td>153.65±271.92</td>
</tr>
<tr>
<td>B12 (pg/mL)</td>
<td>744.58±412.90</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>3.22±4.22</td>
</tr>
</tbody>
</table>
dependent patients, it may be clinically relevant to take values of γ-GT, ASAT, and ALAT as markers of hepatocellular damage into account, because, in alcohol-dependent patients, a serum cobalamin test may not reflect the true amount of available vitamin B12 nor the extent of hepatic damage. Baker and colleagues, who investigated vitamin B12 changes in plasma and liver tissue in alcoholics with liver disease, concluded that eventually liver disease could produce enough severe tissue B12 deficits to cause metabolic dysfunction despite elevated plasma total vitamin B12 (42). So if a patient with alcohol dependence shows normal or elevated vitamin B12 levels, the clinician has to pay attention to the hepatic enzyme levels. If those are elevated, the patient could suffer from a deficit of vitamin B12, although he exhibits normal or elevated vitamin B12 levels.

Although the relation between IL-6 serum levels and B12 is not clear it is possible that they are both having an additive effect to hepatocellular damage, since the presence of increased concentrations of proinflammatory signals in alcohol-dependent individuals may be associated with the progression from AWLD to ALD. Furthermore, the present study indicates that determination of serum IL-6 levels may represent a useful diagnostic marker for the degree of liver dysfunction, which, however, needs additional and more extensive investigation.

References


