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Original Article

Oxidative stress markers, alpha-fetoprotein and alpha-fetoprotein-L3 levels of alcohol dependent subjects

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Abstract
As one of the most important social problems around the world, alcohol addiction damages the body by causing fatty liver and can lead to hepatitis, cirrhosis, and even hepatocellular carcinoma. In the present study we investigated the relationship between oxidative stress and liver cancer risk in alcohol dependent patients using some noninvasive pro- and antioxidant markers. We also examined the relative value of individual markers and their relationship with liver tumor markers including alpha-fetoprotein (AFP) and AFP-L3. Serum samples were collected from age-matched males who are in either healthy control or alcohol dependent groups. The effect of alcohol dependence on the serum proteins including ceruloplasmin and prealbumin, oxidative stress markers, AFP and AFP-L3 levels were evaluated. In the alcohol dependent group, the levels of lipid peroxidation end-product malondialdehyde and protein oxidation marker protein carbonyl were significantly higher than the control group. Similarly, serum ceruloplasmin, prealbumin, AFP and AFP-L3 levels were found to be slightly higher in the alcohol dependent group. However, superoxide dismutase enzyme activities were significantly lower in the alcohol dependent group than in the control group. These results showed that AFP-L3 tends to correlate positively with alcohol consumption. Further studies including higher number of study subjects will be needed to demonstrate a potential statistical significance of increased AFP-L3 levels in alcohol dependent subjects and may contribute to determining the relations between alcohol dependance and liver cancer risk.

INTRODUCTION

Chronic alcohol consumption is a major health issue worldwide, leads to addiction and damage in some consumers, and can cause damage to nearly every organ of the human. In most Western societies, at least 90% of people consume alcohol at some time during their lives, and 30% or more of chronic drinkers develop alcohol-related medical issues [1]. Alcoholic hepatitis, hepatic fibrosis, cirrhosis and liver carcinogenesis are among the serious diseases resulting from alcohol addiction. Biochemical markers for detection of alcohol abuse are important for screening treatment, and prevention of alcohol dependency and other alcohol-related medical diseases [2]. Additionally, the use of alcohol is also known to be associated with an increased risk of cancer. Hepatocellular carcinoma (HCC) is the most common type of liver cancer caused by alcoholism. Unfortunately the diagnosis of HCC mostly occurs in advanced stages of cancer, so that curative treatments are typically not possible [3].

In recent years, studies have focused on the effects of alcohol-induced oxidative stress on the pathological changes of the diseases associated with alcohol. There is persuasive evidence that the potential of alcohol to induce oxidative stress may be an important pathogenic mechanism for the increased occurrence of HCC [3, 4]. The diagnosis of HCC may be considered

for a patient with underlying liver disease that develops a rising serum alpha-fetoprotein (AFP) level [5]. The lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) has been used as a diagnostic marker for HCC and is reported to be highly specific in predicting the prognosis of the disease [6]. Therefore, some oxidative stress markers, AFP and AFP-L3 levels have been investigated in this study; it was also aimed to evaluate possible changes in several serum proteins in alcohol dependent patients.

MATERIALS AND METHODS

Subjects and study design

The study was conducted at the Fatih University Faculty of Medicine (Istanbul, Turkey), and Treatment and Training Center for Alcohol and Substance Dependence (AMATEM; Ankara, Turkey). Forty-five patients (male; mean age of 35.5 ± 1.01 years) diagnosed as abusing alcohol were selected from AMATEM and compared to thirty-one healthy age-matched control subjects (male; mean age of 35.9 ± 1.24 years). Subjects were excluded if they did not agree to participate, had a history of diabetes mellitus, cardiovascular disease, hepatic or renal disease, and if they were being treated with antihypertensive, antihyperlipidemic or antioxidant medications. There was no considerable difference between patients and controls with regard to diet and smoking habits.

All patients and control subjects were admitted for study after being assessed by a psychiatrist at the Psychiatry Clinic of Numune Training and Research Hospital, Ankara, Turkey. Based on DSM-IV dependence criteria of the American Psychiatric Association (APA) [7], all patients had been diagnosed with alcohol dependence, except two. On the morning of the procedure, after an overnight fasting period (10-12 h), blood samples were collected, stored and analyzed by laboratory.

Blood samples were centrifuged within 30 min of collection at 3000 rpm for 10 min at 4°C. Each serum sample was separated into two portions: one for alanine- (ALT), and aspartate transaminase (AST), gamma glutamyl transpeptidase (GGT), prealbumin, ceruloplasmin, and AFP tests carried out on the same day; and the other serum sample was stored at –40°C to study malondialdehyde (MDA), protein carbonyl content (PCC), superoxide dismutase (SOD) and AFP-L3 at a later date. This study was approved by the Ethics Committee of Fatih University School of Medicine (December 08, 2011/01). All patients confirmed agreement for participation in the study by written consent.

Measurement of biochemical markers

Serum ALT, AST, GGT, prealbumin, and ceruloplasmin levels were assayed using a Cobas Integra 800 analyzer (Roche Diagnostics, Geneve, Switzerland). Serum AFP levels were determined by chemiluminometric assay using "ADVIA Centaur XP" immunassay analyzer (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The analytical performance of these methods was within the specifications of the analyzers.

Measurement of oxidative stress markers

The lipid peroxidation end-product MDA, was estimated using the thiobarbituric acid (TBA) test, as described previously [8]. Briefly, the formation of MDA was quantitated using 1,1,3,3-tetraethoxy-propane as a standard, and the absorbance of MDA was read at 532 nm using a Shimadzu UV 1601 spectrophotometer (Tokyo, Japan).

The serum PCC were determined spectrophotometrically by a method based on the reaction of the carbonyl group with 2,4-dinitrophenylhydrazine (DNPH) to form 2,4-dinitrophenylhydrazone [9]. The PCC were calculated from the peak absorbance at 370 nm using an absorption coefficient of 22,000 for the aliphatic hydrazone. The results were given in nanomoles of carbonyl per miligram of total serum protein.

Total SOD activity was determined according to the method of Sun *et al* [10]. The principle of the method is based on the inhibition of nitroblue tetrazolium reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was expressed as U/ml.

Measurement of alpha-fetoprotein-L3

Serum AFP-L3 levels were analyzed by ELISA kits (USCN E91117, Life Science Inc.; Wuhan, Hubei, PR China) using an ELISA microplate strip washer (ELX 50; BioTek Instruments, Winooski, VT, USA), and ELISA microplate reader (ELX 808; BioTek). All processes were performed automatically according to the spesific manufacturer's protocols.

Statistical analysis

The Statistical Package for Social Sciences (SPSS, version 13.0; Chicago, IL, USA) for Windows was used to analyze the data. Continuous variables were checked for the normal distribution assumption using the Kolmogorov-Smirnov statistics and those that did not satisfy the criteria were log-transformed to obtain a normal distribution. For continuous variables, an independent t-test and Mann-Whitney U-test were used to analyze the variance among groups, as appropriate. Results were expressed as mean values and standard deviation. P values below 0.05 were considered to be statistically significant.

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RESULTS

All results based on serum mesurements are presented in Table 1. The age distributions of the patients and control groups were homogeneous and there was no statistical difference between the groups ($P > 0.05$). Serum AST and GGT levels were significantly higher in the patient group compared to the control group ($P < 0.05$ and $P < 0.001$, respectively). There was no significant difference in the serum ALT and prealbumin levels between the two groups.

In the alcohol dependence group there was a significant increase in serum ceruloplasmin levels compared with the control group ($P < 0.05$). There was also a remarkable increase in AFP levels in patients with alcohol dependence compared with healthy subjects ($P < 0.05$), but despite the high levels of AFP compared with the control group, the differences in AFP-L3 levels were not statistically significant.

Of the oxidative stress markers, both MDA and PCC levels of patients were significantly higher than the controls ($P < 0.001$ for both). On the other hand, the SOD activity of the alcohol dependent patients was significantly lower than in the control group ($P < 0.001$).

DISCUSSION

Chronic alcohol consumption is a well-known risk factor for liver disease such as cirrhosis and HCC. Progression of alcohol-induced liver disease is a multifactorial process that involves a number of genetic, nutritional and environmental factors [11]. Experimental and clinical studies increasingly show that oxidative damage induced by alcohol contributes in many ways to the pathogenesis of hepatoxicity, and especially to development of alcoholic cirrhosis and HCC [11-13]. Soffritti *et al* [14] reported that prolonged exposure to alcohol result in carcinogenicity in animals. A meta-analysis in humans also showed that alcohol consumption was a strong risk factor for HCC [15].

More recent data demonstrated that chronic alcohol consumption is associated with hepatic neoplasia, increased cytochrome P450 2E1 (CYP2E1) activity and intrahepatic oxidative stress in rats [13]. There are many causative factors that contribute to oxidative stress in the process of cancer development, such as acetaldehyde-derived DNA adduct formation, CYP2E1-mediated reactive oxygen species (ROS) generation, iron-associated oxidative stress, lipid peroxidation, glutathione (GSH) depletion, inflammation, p53 mutation and induction of fibrosis in the liver [16, 17]. Following induction, CYP2E1 becomes a primary pathway for alcohol metabolism, and in conjunction with ADH-dependent alcohol metabolism, contributes to increased hepatic acetaldehyde production. Owing to an effective acetaldehyde metabolizing system, acetaldehyde concentrations are significantly lower in the liver; so, in the pathogenesis of HCC, oxidative stress may be one of the most important factors [4].

Although ROS can form DNA and protein adducts directly, they can also react with lipid molecules in the cell membrane. Hepatocellular lipid peroxidation leads to the formation of reactive lipid aldehydes in particular, 4-hydroxynonenal and MDA [18]. Furthermore, alcohol promotes the generation of ROS and/or interferes with the body's normal defense mechanisms against these compounds through numerous processes, particularly in the liver. It seems highly probable that alcohol-induced lipid peroxidation and ROS consume antioxidants, such as SOD and ceruloplasmin. Consequently, decreases in CuZn-SOD activity may increase hepatocyte susceptibility to injury against the oxidative stress in alcoholic liver diseases including cancer. The present study also resulted in higher MDA and protein carbonyl levels, as well as lower SOD activities in chronic alcoholic patients compared to healthy subjects.

Table 1. Comparison of measured study parameters between alcohol dependent patients and healthy controls

	Alcoholic patients (n = 45)	Control (n = 31)	P value
Age	35.9 ± 1.24	35.55 ± 1.01	> 0.05
Alanine transaminase (U/l)	26.8 ± 2.89	26.71 ± 3.3	> 0.05
Aspartate transaminase (U/l)	34.11 ± 4.08	23.19 ± 1.61	**< 0.05**
Gamma glutamyl transpeptidase (U/l)	113.53 ± 23.41	31.54 ± 3,29	**< 0.001**
Prealbumin (mg/dl)	29.6 ± 1.11	26.6 ± 1.08	> 0.05
Ceruloplasmin (mg/dl)	33.44 ± 1.24	29.57 ± 0.92	**< 0.05**
Alpha-fetoprotein (ng/ml)	6.9 ± 0.34	5.95 ± 0.34	**< 0.05**
Alpha-fetoprotein -L3 (ng/ml)	2.01 ± 0.18	1.95 ± 0.23	> 0.05
Malondialdehyde (nmol/ml)	11.09 ± 0.96	5.47 ± 0.26	**< 0.001**
Carbonylated protein (nmol/mg)	408.4 ± 86	124.49 ± 8.01	**< 0.001**
Superoxide dismutase (U/ml)	426.59 ± 16.92	528.9± 13.38	**< 0.001**

Hirano [12] suggested that a high concentration of alcohol and an irregular diet increased liver cancer risk. The most important risk factors for the development of HCC are chronic liver diseases caused by viral hepatitis B and C, and alcohol consumption, while the significance of each of the individual factor differs from country to country [3, 4].

The correlation between AFP levels and the incidence of HCC has been discussed over a long period [19, 20]. AFP is used as a serological marker of HCC and employed in combination with ultrasonography for HCC screening [21]. Numerous studies have demonstrated an elevated AFP level to be a risk factor for the development of HCC in hepatitis C virus infected patients [22, 23]. However, the biological and pathophysiological roles of the association of AFP with an increased risk of HCC development remain unclear. One of the first study results showed a significant AFP elevation in the serum of 23 chronic alcoholics [24]. A relatively new test, called AFP-L3, might therefore be more specific for HCC than total AFP measurements and may help differentiating between malignant and nonmalignant liver disease [3, 6]. Numerous reported studies from Japan and other Asian countries have demonstrated that an increase in the AFP-L3 fraction of serum AFP correlates more strongly than conventional serum AFP with adverse histological characteristics of HCC and predicts unfavorable outcome [25, 26]. AFP-L3 is routinely used in Japan when AFP exceeds cut off level, and it is significantly related to portal vein invasion and patient outcome, suggesting it could be a useful prognostic marker for HCC [27]. According to some Japanese investigators [28], any circulating AFP value higher than 10 μg/l in patients with chronic liver disease should be regarded as suspicious of HCC and prompt further investigation using AFP-L3.

Ceruloplasmin, a copper-containing glycoprotein, is a famous serum inflammation marker. It has several characteristics such as ferroxidase, copper transport, antioxidant and proinflammatory activity. Ferroxidase activity is associated with anti-oxidative or anti-inflammatory activity by catalyzing the oxidation of iron. The finding of decreased enzymatic activity in alcoholic subjects is interesting because ceruloplasmin is one of the plasma factors with antioxidant activity and it is generally accepted that oxidative stress and ROS participate in the pathogenesis of alcoholic liver diseases [29]. In addition, disturbed ceruloplasmin functional activity could participate in increased oxidative stress in these patients as well as in the development of steatohepatitis and fibrosis [29, 30].

In conclusion, the findings of this study suggest that alcohol consumption is associated with early liver injury responses, including oxidative damage in the patients with alcohol dependence. ROS production and oxidative stress in liver cells play a central role in the development of alcoholic liver disease and carsinogenesis. It can be proposed that some biochemical markers of risk assesment for liver cancer, taken together with the findings of oxidative stress markers will provide important contributions to the early diagnosis and treatment options in the patients with alcohol dependence. It is possible to achieve particularly accurate results for HCC screening with the use of AFP and AFP-L3 in combination with oxidative stress markers. It could be important to confirm these findings in a larger patient group with liver cancer, and further studies should be conducted at the molecular level in order to explain these findings more clearly.

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COMPETING INTERESTS

There are no conflicts to declare.

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