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Biochemical Indicators and Cardiac Function Tests in Chronic Alcohol Abusers

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Aim To determine the effect of chronic alcohol abuse on cardiac function, antioxidant system, trace elements, and liver function tests.

Methods Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), superoxide dismutase (SOD), malondialdehyde (MDA), as well as zinc, magnesium, and copper were assayed in 25 chronic alcoholic patients and their 25 healthy relatives matched in age and gender. Echocardiographic parameters were evaluated for subjects.

Results Mean corpuscular volume (96.7 fL vs 92.4 fL) and mean corpuscular hemoglobin levels (31.4 pg vs 30.5 pg) were found to be significantly increased in the patient group ($P=0.002$ and $P=0.048$, respectively). The results of the SOD and MDA assays showed no significant differences between the two groups. AST (38.7 U/L vs 22.1 U/L) and GGT (104.2 U/L vs 34.2 U/L) levels were found to be significantly increased in the patient group compared with controls ($P=0.005$ and $P<0.001$, respectively). Magnesium (1.6 mmol/L vs 1.8 mmol/L) and zinc levels (14.9 μmol/L vs 19.2 μmol/L) were significantly decreased, whereas copper levels (19.3 μmol/L vs 17.9 μmol/L) were increased in alcoholics ($P=0.042$, $P<0.001$ and $P=0.003$, respectively). Echocardiographic examination showed a significant decrease in mitral and tricuspid ratio of peak early and atrial flow velocity (E/A ratio) in alcoholics.

Conclusion Decrease in mitral and tricuspid E/A ratios accompanied with low levels of magnesium and zinc, and increased levels of copper indicate that alcoholics already have heart muscle disease even if they don't have symptoms.

Alcoholism is one of the major causes of non-ischemic heart damage. The association between chronic ethanol abuse and alcoholic heart muscle disease (AHMD) is increasingly recognized (1,2). However the pathogenic mechanisms responsible for the observed myocardial abnormalities are still poorly understood (1,2). Previous studies have demonstrated that long-term alcohol consumption leads to biochemical changes in the myocardium either directly or indirectly by its metabolites (3). The intensity and duration of alcohol use, as well as the patient susceptibility, are responsible for the observed toxic effects of ethanol (4). Indeed, the cardiomyopathic state of the heart due to alcohol consumption may result in compromised ventricular functions. The liver is known to be the major site of ethanol oxidation: it is first oxidized to acetaldehyde by alcohol dehydrogenase, a zinc-dependent enzyme found in the cytosol, and then, in the second oxidation step, rapidly oxidized to acetate by liver acetaldehyde dehydrogenase. These metabolic changes and the generation of toxic free radicals are thought to cause heart muscle damage (5,6). The electron transport

chain within the mitochondria contributes significantly to the production of reactive oxygen species (ROS), and the exposure to ethanol augments the ROS production, which may further be exacerbated by a decrease in mitochondrial antioxidant defenses (7). These toxic free radicals cause a decrease in contractility of the heart muscle (8). Therefore, the activity of superoxide dismutase (SOD), an important antioxidant scavenger enzyme, and the levels of malondialdehyde (MDA), as markers of oxidative stress and lipid peroxidation, could be altered by long-term alcohol abuse (9,10).

Copper and zinc are important trace elements within SOD (11). In addition, muscle zinc deficiency may be important in cardiac dysfunction pathogenesis (12). Similarly, magnesium is another important element involved in muscle contraction (13).

The aim of this study was to investigate the relationship of antioxidant generation and trace element concentrations to cardiac function parameters in alcoholic patients as assessed by echocardiography.

Patients and Methods

Subjects

This study involved 25 men who were diagnosed as "alcoholic" according to the American Psychiatry Association criteria (14) and hospitalized by the Department of Psychiatry (n = 25, mean age ± standard deviation, 39.8 ± 6.4 years) and the control group chosen from the age- and gender-matched healthy relatives of the patients (n = 25, age: 39.9 ± 5.5 years). The average amount of alcohol consumed by alcoholics was 204.6 ± 87.0 g/day (range 124-376) and the duration of consumption was 13.2 ± 5.2 years (range 8-28). Only four patients had alcoholic hallucinosis and none of them had Korsakoff syndrome or *delirium tremens*. None of them had liver biopsy. Chosen volunteers were healthy as defined by the International Federation of Clinical Chemistry (15). All patients were informed about the nature of the clinical trial, and the informed consent was obtained in accordance with the Helsinki Declaration. The study was approved by the institutional Ethics Committee.

Hematological and Biochemical Analysis

Venous blood was drawn into two different tubes (with and without Na-EDTA) during the first morning within 24 hours of hospitalization. The hematological parameters were assayed by Coulter Counter T-890 (Beckman Coulter, Miami, FL, USA). The erythrocyte SOD enzyme activity was measured according to the method of McCord et al (16) and expressed as units per gram of hemoglobin (U/g Hb). The principle of the method depends on xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium-chloride (INT) (Sigma Chemical Co, St. Louis, MO, USA) to form red formazan dye. The optical density of this substance was measured at 505 nm by Schimadzu UV 260 (Schimadzu Co., Kyoto, Japan) (16).

Malondialdehyde, a product of lipid peroxidation, was assayed in plasma in the terms of thiobarbituric acid reactive substance/malondialdehyde (TBARS/MDA) using 1,1,3,3-tetramethoxypropane (Sigma Aldrich Co, Steinheim, Germany) as a standard and expressed as μmol MDA formed per liter plasma. This spectrophotometrical method is based on the concentration of pink chromogen compound that forms when MDA binds to thiobarbituric acid, and the absorbance was read at 532 nm wavelength by Schimadzu UV 260 (Schimadzu Co. Kyoto, Japan) (17).

The serum enzyme activities of AST, ALT, and GGT were measured by enzymatic colorimetric methods (Olympus 5200, Tokyo, Japan).

Serum copper, zinc, and magnesium levels were determined by Atomic Absorption Spectrometry (Perkin Elmer 2380, Boston, MA, USA). The samples were collected into acid washed tubes and stored at -20 °C until the analysis. Specimens were diluted 1:1; 1:5; 1:50 with deionized water for the determination of copper, zinc and magnesium, respectively. Calibration standards and quality control specimens were prepared in the same way and readings for copper, zinc, and magnesium were performed at the wavelength of 324.8, 213.9 and 285.2 nm, respectively (18).

Echocardiographic Examination

A complete M-mode, two-dimensional, and Doppler echocardiographic examinations were performed with Toshiba SSH-160 A equip-

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ment (Toshiba, Tokyo, Japan) and a 2.5- or 3.5-MHz transducer. Echocardiographic images were obtained from the parasternal and apical windows with the patients in the left lateral recumbent position. All recordings were obtained at the end of expiration to get good quality images. M-mode measurements were performed according to the recommendations of the American Society of Echocardiography (19). Left ventricular (LV) mass was calculated using the method of Devereux et al (20) and normalized for body surface area. Transmitral and transtricuspid inflow velocities were recorded by pulse wave Doppler echocardiography from the apical four-chamber view. Peak early flow velocity (E-wave velocity), peak atrial flow velocity (A-wave velocity), the ratio of peak early and atrial flow (E/A) were measured as diastolic function indices for the left and right ventricles.

Statistical Analysis

Statistical analyses were performed using Statistical Package for Social Sciences version 12.0 (SPSS Inc., Chicago, IL, USA). For each continuous variable, normality was checked by Kolmogorov-Smirnov test and histograms. The data were not normally distributed, so an appropriate non-parametric test was chosen. Comparisons were made using the Mann-Whitney *U* test and *P* value of <0.05 was considered to be statistically significant. Results were presented as mean and 95% confidence interval.

Results

Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) levels were significantly higher in alcoholics than in healthy subjects ($P=0.002$ and $P=0.048$, respectively; Table 1). Further, there were no statistically significant differences between the alcoholics and the control group in their SOD and MDA enzyme levels (Table 1). Serum magnesium and zinc levels were significantly lower whereas copper levels were higher in alcoholics ($P=0.042$, $P<0.001$ and $P=0.003$, respectively, Table 1). AST and GGT levels were significantly higher in alcoholics ($P=0.005$ and $P<0.001$, respectively). Except for E/A ratios, all echocardiographic parameters did not differ between groups. E/A ratios for both mitral and tricuspid valves were significantly lower in alcoholics (Table 2).

Table 1. Biochemical findings (mean; 95%CI) of the alcoholics and the control group*

Parameter	Alcoholics (n=25)	Controls (n=25)	*P*†
MCV (fL)	96.7 (94.2-99.2)	92.4 (90.8-94.0)	0.002
MCH (pg)	31.4 (30.6-32.3)	30.5 (29.9-31.1)	0.048
AST (U/L)	38.7 (25.1-52.3)	22.1 (17.4-26.8)	0.005
ALT (U/L)	30.0 (19.9-40.1)	23.2 (16.8-29.6)	0.420
GGT (U/L)	104.2 (76.6-149.4)	34.2 (20.0-48.0)	0.000
SOD (U/g Hb)	1216.0 (1031.0-1401.0)	1024.1 (894.0-1154.0)	0.081
MDA (μmol/L)	2.4 (1.7-3.1)	2.2 (1.6-2.8)	0.854
Zn (μmol/L)	14.9 (13.4-16.4)	19.2 (17.8-20.5)	0.000
Mg (mmol/L)	1.6 (1.4-1.7)	1.8 (1.7-1.8)	0.042
Cu (μmol/L)	19.3 (18.0-20.66)	17.9 (16.7-19.1)	0.003

*Abbreviations: CI – confidence interval; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; AST – aspartate transaminase; ALT – alanine transaminase; GGT – gamma glutamyl transferase; SOD – superoxide dismutase; MDA – malondialdehyde.
†Mann-Whitney *U* test.

Table 2. Echocardiographic parameters (mean; 95% CI) of alcoholics and control group*

Parameters	Alcoholics (n=25)	Controls (n=25)	*P*†
LVDSB (mm)	48.1 (45.9-50.3)	48.3 (46.8-49.8)	0.977
LVSSB (mm)	30.2 (26.7-33.7)	30.4 (27.6-33.2)	0.949
EF (%)	64.6 (58.7-70.5)	66.4 (62.7-70.1)	0.797
FS (%)	35.2 (31.6-38.8)	36.0 (32.7-39.3)	0.779
IVS (mm)	10.1(9.7-10.5)	9.8 (9.4-10.2)	0.157
LVMI (g/m^2)	105.6 (92.2-119.0)	98.9 (89.4-108.4)	0.234
Mitral E/A	1.1 (0.98-1.22)	1.4 (1.28-1.52)	0.004
Tricuspid E/A	1.1 (0.98-1.22)	1.3 (1.18-1.42)	0.014

*Abbreviations: CI – confidence interval; LVDSB – left ventricular diastolic diameter; LVSSB – left ventricular systolic diameter; EF – ejection fraction; FS – fractional shortening; IVS – interventricular septal thickness; LVMI – left ventricular mass index; E/A – ratio of peak early and atrial flow velocity (E/A).
†Mann-Whitney *U* test.

Discussion

Alcoholic heart muscle disease reflects the cardiac dysfunction in an alcoholic in whom no other cause of myocardial disease has been found (21). In our patient group, MCV and MCH were significantly increased, which reflected nutritional folate deficiency usually seen in chronic alcoholics (22,23). DeRitis ratio (AST/ALT) was increased to over one, and GGT levels were higher most likely due to the pyridoxal phosphate deficiency and enzyme induction, respectively. Excessive ROS quantities are produced in oxidative stress conditions (23,24). Increased oxidative stress is one of the possible mechanisms of ethanol toxicity (25). Microsomal ethanol oxidation system (MEOS) aggravates the oxidative stress directly and indirectly by impairing the defense systems (8). Although the activity of SOD, an important part of the free radical scavenging system, is expected to be increased in alcoholics, there are controversial results in the literature (8,25,26). Zinc is essential for the normal activity of SOD (7). In our study, decreased zinc levels in the patient group may explain the inadequate rise in SOD. Malondialdehyde is a non-lipophilic peroxidation

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product of polyunsaturated fatty acids containing three or more methylene interrupted double bonds. Production of MDA reflects lipid peroxidation caused by oxidative damage (28). After acute ethanol poisoning, there is a reduction in MDA content of mouse blood plasma and liver (29). Our patients had insignificant increase in MDA levels which could be due to chronic alcohol exposure.

One of the major limitations of this study is very small sample size. The second limitation is that some other elements such as calcium, chromium, iron, and selenium were not measured.

Ethanol consumption or liver disease may alter liver content of several trace elements. Zinc is a cofactor of alcohol dehydrogenase and the metabolism of zinc is significantly affected by alcohol consumption (30,31). In our patient group, we found significantly decreased zinc and magnesium and increased copper levels. The decrease in zinc and magnesium levels could be related to the impairment of the immune system in alcoholics (8). The observed increase in copper levels may be caused by low levels of zinc, since zinc deficiency enhances copper absorption and increases copper levels in sera by inducing the intestinal metallothionein and hepatic ceruloplasmin (32).

Alcohol-induced cardiac damage is a relatively widespread phenomenon that affects up to one third of those who misuse ethanol (2,21). Alcoholic heart muscle disease starts before overt congestive heart failure occurs (33). The transition from subclinical alcoholic heart muscle disease to its full expression carries a poor prognosis and since the changes in myocardial function are reversible at the beginning, early detection is of crucial importance (34).

Alcoholic heart muscle disease is characterized by cardiomegaly, dilatation of the left ventricle, and systolic dysfunction involving both ventricles; occasionally diastolic dysfunction may be the first abnormality. Echocardiographic features in the subclinical phase of the disease are left ventricular hypertrophy and a decrease in peak early diastolic flow velocity (35). In general, early signs of AHMD appear as increase in end-diastolic and end-systolic dimensions, wall thickening, systolic dysfunction, and increased LV mass (2,34, 36). However, some studies have shown that wall thickening and increased LV mass are early findings rather than LV dilatation in asymptomatic patients (37-39). Some other studies have indicated that ventricular dilatation and systolic dysfunction in alcoholic patients are not consistent findings, especially in asymptomatic AHMD population (34), as it was observed in our patients. We found no differences between controls and alcoholics in the ejection fraction and fractional shortening as markers of systolic function, ventricular dimensions, and LV mass index. We belive that diastolic dysfunction usually starts earlier and may progress to systolic dysfunction. Kupari et al (40) showed the impairment in Doppler indices of diastolic function suggesting abnormal relaxation and impaired early filling of the ventricle, including decreased peak early diastolic E/A velocity ratio. We found a decrease in mitral and tricuspid E/A ratio as a marker of left and right ventricular diastolic dysfunction.

In conclusion, decrease in mitral and tricuspid E/A ratios, along with the low levels of magnesium and zinc, and increased levels of copper, MCV, MCH, AST, and GGT may indicate that alcoholic patients have heart muscle disease even if they are asymptomatic.

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