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# Human Alcohol Dehydrogenase Isoenzyme Activity in the Sera of Non-Alcoholic Liver Cirrhotic Patients

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**Summary:** The activities of class I and II alcohol dehydrogenase isoenzymes were examined in the sera of patients with non-alcoholic liver cirrhosis using a fluorometric method. The analysis of these results shows a statistically significant increase (2,5-times) in the activity of class I alcohol dehydrogenase, and no marked differences in the activity of class II in cirrhotic and control patients. The observed increase in total enzyme activity measured using a photometric method was not very high but confirmed the elevation of class I isoenzyme activity. Activities of both classes of alcohol dehydrogenase isoenzymes have a good correlation with aspartate aminotransferase. Class II isoenzyme activity additionally correlates with alkaline phosphatase. These results suggest that serum activity of class I alcohol dehydrogenase is a better indicator of liver cell destruction during non-alcoholic cirrhosis than total enzyme activity, and is comparable with the value of aspartate aminotransferase.

## Introduction

Human alcohol dehydrogenase[1]) (alcohol : $NAD^+$ oxidoreductase) exhibits multiple forms, which are mainly localized in the cytosol of liver cells. These forms have been grouped into five classes (1, 2).

Isoenzymes of class I are composed of α, β and γ subunits and are encoded by ADH1, ADH2 and ADH3 loci. They were primarily found in the liver (3).

Class II alcohol dehydrogenase contains the π subunits, encoded by the ADH4 locus, and is found only in the liver (4).

Class III is composed of χ subunits, encoded by the ADH5 locus and existed in all tissues examined (5).

Class IV alcohol dehydrogenase is comprised of δ subunits encoded by the ADH7 locus and is detected in the digestive tract organs (6).

A gene of ADH6 was found in human liver and stomach, but has not yet been detected at the protein level (7).

Human alcohol dehydrogenase catalyzes the oxidation/reduction of a wide spectrum of substrates including primary and secondary aliphatic, aromatic alcohols and their corresponding aldehydes and ketones. The activity of the hepatic classes of alcohol dehydrogenase can be measured using two class-specific, fluorogenic substrates – 4-methoxy-1-naphthaldehyde and 6-methoxy-2-naphthaldehyde (8). The first is highly selective for class I isoenzymes, whereas the class II preferentially reduces the second substrate.

Using these substrates, in a previous study, we found a 2-fold increase of class I alcohol dehydrogenase isoenzymes in the sera of alcoholics (9), and a 30-fold increase of class I and a 4-fold increase of class II in the course of viral hepatitis (10). In this situation, it is interesting to investigate the activity of these two classes of alcohol dehydrogenase isoenzymes in the sera of patients with destruction of liver cells in non-alcoholic liver cirrhosis. The total alcohol dehydrogenase activity measured by the photometric method is then compared with the different classes of enzyme tested.

## Materials and Methods

### Patients

The serum samples were taken from 27 non-alcoholic liver cirrhotic patients, 19 male and 8 female, between 27 and 74 years of age. Alcohol dehydrogenase isoenzyme activity was tested in blood samples taken for routine biochemical investigations. All routine tests were performed in the Central Laboratory at the University Hospital. Criteria for the diagnosis of non-alcoholic liver cirrhosis were created on the basis of clinical features of the disease, including portal hypertension, varices, encephalopathy, oesophageal ascites. The diagnosis was further confirmed by ultrasonography, and laboratory investigations (hyperbilirubinaemia, elevation of serum γ-glutamyltransferase, a modest but persistent elevation of aminotransferases with higher elevation of aspartate than alanine aminotransferase, and disturbances of the coagulation system). The diagnosis was further confirmed by liver biopsy (10 from 27). All patients were tested for hepatitis B surface antigen and antibodies against hepatitis C virus. Hepatitis B surface antigen was positive in all patients. Control subjects (20 men and 15 women, between

[1]) Enzymes:
Alcohol dehydrogenase (EC 1.1.1.1)
Alanine aminotransferase (EC 2.6.1.2)
Aspartate aminotransferase (EC 2.6.1.1)
Alkaline phosphatase (EC 3.1.3.2)
γ-Glutamyltransferase (EC 2.3.2.2)
Lactate dehydrogenase (EC 1.1.1.27)

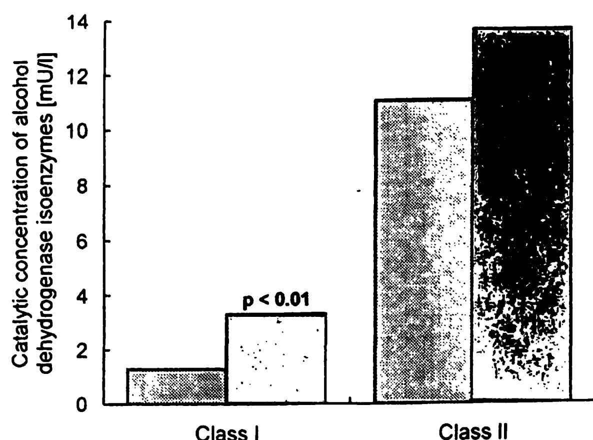


**Fig. 1** Catalytic concentration of alcohol dehydrogenase isoenzymes in the sera of patients with non-alcoholic liver cirrhosis.
□ control
□ tested

20 and 65 years of age) were defined as those with normal liver function tests and normal liver function.

### Alcohol dehydrogenase assays of classes I and II

Serum class-specific alcohol dehydrogenase enzyme activity was measured using a fluorometric method in a reaction mixture (3 ml) containing 60 µl serum, 150 µl of a 300 µmol/l solution of 4-methoxy-1-naphthaldehyde (Aldrich Chemical Company. Inc. Milwaukee, WI) or 6-methoxy-2-naphthaldehyde (synthesized by Dr. *J. Wierzchowski*, Department of Physical Chemistry, Medical School, Warsaw, Poland) (8), 100 µl of 1 mmol/l NADH (Sigma Diagnostics, St. Louis, MO), and 2.69 ml of 0.1 mol/l sodium phosphate buffer, pH 7.6. The reaction was started by the addition of serum. The changes of fluorescence were recorded up to 10 min on an RF-5301 PC Spectrofluorophotometer (Shimadzu) at an excitation wavelength of 316 nm and an emission wavelength of 370 nm for class I and 360 nm for class II. After this, 60 µl of a 200 µmol/l solution of the product (4-methoxy-1-naphthalenemethanol for class I, or 6-methoxy-2-naphthalenemethanol for class II) was added to provide an internal standard. For the evaluation of alcohol dehydrogenase activity, two assays were carried out: one with substrate alone and the second with substrate and 50 µl of a 12 mmol/l solution of 4-methylpyrazole as a specific inhibitor of the enzyme (Aldrich Chemical Company, Inc. Milwaukee, WI). The rate of reaction (initial velocities) was calculated as mU/l (8).

### Total alcohol dehydrogenase assays

The photometric method with *p*-nitrosodimethylaniline (substrate) was used for determination of the total alcohol dehydrogenase activity in the sera of the patients. The reaction mixture (2 ml) contained 1.9 ml of a 26 µmol/l solution of substrate (Aldrich-Chemie D-7924 Steinheim) in 0.1 mol/l Na-phosphate buffer, pH 8.5, and 0.1 ml of mixture containing 0.25 mol/l n-butanol and 5 mmol/l of $NAD^+$ (Sigma Diagnostics). The reaction was started after addition of 0.5 ml serum. The reference solution (2 ml) had the same composition plus 12 mmol/l of 4-methylpyrazole as a specific inhibitor of the enzyme. The solutions were incubated for 20 min at 25 °C, and the reaction was stopped by addition of 50 µl 0.5 mol/l of the inhibitor. The difference of absorbance at 440 nm (Gilford Impact 400E Spectrophotometer) between the samples with and without pyrazole was calculated. Alcohol dehydrogenase activity was determined by means of the graph given by *Skursky* et al. (11).

### Determination of other enzymes and bilirubin concentration

Aspartate and alanine aminotransferase[1]), alkaline phosphatase[1]), γ-glutamyltransferase[1]), lactate dehydrogenase[1]) activities and bilirubin were measured with kits from bioMerieux in an Express Plus biochemical analyzer (Ciba-Corning).

### Statistical analysis

Statistical analysis was performed using *Wilcoxon*'s test and *Pearson*'s correlation coefficients. Differences were considered significant at $p < 0.05$.

## Results

The activity of the two alcohol dehydrogenase classes is shown in figure 1. In comparison with the control level (1.28 ± 0.93 mU/l), the serum activity of class I isoenzyme in non-alcoholic liver cirrhosis increased about 2.5-fold (3.27 ± 3.47 mU/l). In contrast, there was no marked difference in activity of class II alcohol dehydrogenase (control group 11.02 ± 7.76 mU/l, tested group 13.59 ± 17.46 mU/l). Total alcohol dehydroganse activity (table 1) was significantly higher in patients with cirrhosis (1.14 ± 0.86 U/l) than in control patients (0.69 ± 0.21 U/l). The discrepancy between total alcohol dehydrogenase activity, calculated as U/l, and the activity of the two classes, expressed as mU/l, is evidently the result of the different methods and substrates used for these evaluations. The activities of other enzymes, tested as markers of liver cells destruction (aminotransferases), were high, with more evident elevation of aspartate than of alanine aminotransferase. The activities of enzymes commonly accepted as markers of cholestasis (γ-glutamyltransferase and alkaline phosphatase) were also apparently increased. The bilirubin concentration was also elevated in cirrhotic patients.

Serum class I alcohol dehydrogenase activity was highly and positively correlated with the activity of class II

**Tab. 1** Serum activity of alcohol dehydrogenase (total) and other liver injury markers in non-alcoholic liver cirrhosis.

| Group | Alcohol dehydrogenase U/l | Aspartate aminotransferase U/l | Alanine aminotransferase U/l | Lactate dehydrogenase U/l | γ-Glutamyltransferase U/l | Alkaline phosphatase U/l | Total bilirubin µmol/l |
|---|---|---|---|---|---|---|---|
| Control | 0.69 ± 0.21 | 25.34 ± 7.69 | 20.77 ± 9.18 | 340.57 ± 46.71 | 28.05 ± 12.52 | 67.17 ± 14.39 | 11.80 ± 2.91 |
| Tested | 1.14 ± 0.86<br>$p < 0.05$ | 104.67 ± 118.76<br>$p < 0.001$ | 44.59 ± 37.38<br>$p < 0.01$ | 365.52 ± 127.95<br>$p > 0.05$ | 226.29 ± 287.04<br>$p < 0.001$ | 217.59 ± 177.66<br>$p < 0.001$ | 69.59 ± 74.73<br>$p < 0.001$ |

($r = 0.705$, $p < 0.001$). The activities of both classes were correlated with aspartate aminotransferase activity ($r = 0.577$, $p < 0.001$ for class I and $r = 0.622$, $p < 0.001$ for class II). The activity of class II alcohol dehydrogenase was also correlated with the activity of alkaline phosphatase ($r = 0.424$, $p < 0.05$). The total alcohol dehydrogenase activity was positively correlated with bilirubin concentration ($r = 0.425$, $p < 0.05$). In the case of enzymatic markers of cholestasis, only alkaline phosphatase correlated slightly with class II alcohol dehydrogenase.

## Discussion

It is commonly accepted that decrease of enzyme activity in the liver in the course of cell destruction is reflected by increase of the corresponding enzyme activity in the serum. In 1968, *Mezey* et al. (12) first reported total serum alcohol dehydrogenase activity in hepatic cirrhosis. They did not find any differences between cirrhotic and control patients. This was connected with data given by *Panes* et al. (13), who did not observe any significant changes in total hepatic alcohol dehydrogenase activity in non-alcoholic liver cirrhosis, although there was a more than 2-fold decrease of this enzyme activity in the cells of alcoholic, liver cirrhotic patients. Thus, alcohol dehydrogenase activity was significantly lower in liver cells of alcoholics than non-alcoholic cirrhotic patients (13). In addition, the hepatic alcohol dehydrogenase activity was similar in liver cells of patients with chronic hepatitis and non-alcoholic cirrhosis. Other studies, presented by *Vidal* et al. (14), showed no significant differences between alcohol dehydrogenase activity in liver cells of alcoholic and non-alcoholic patients with similar degrees of liver damage. When the mean hepatic alcohol dehydrogenase activity in alcoholic and non-alcoholic cirrhosis was analyzed, a significantly lower activity was found in both groups in comparison to the controls. Patients with cirrhosis unrelated to alcohol had higher alcohol dehydrogenase activity than alcoholic cirrhotic patients. Low liver alcohol dehydrogenase activity in patients with alcoholic cirrhosis has also been suggested by *Coman & Gheorghe* (15). Several other authors, such as *Nuutinen* (16), and *Mezey & Tobon* (17), reported that hepatic alcohol dehydrogenase activity decreases when the liver damage deteriorates into cirrhosis and that this is independent of the aetiology of the liver disease.

Generally, hepatic alcohol dehydrogenase activity was severely reduced in patients with liver disease, and this reduction is a consequence of the liver damage.

In our previous experiments we found an increase of serum total and class I activity of alcohol dehydrogenase in alcoholic patients. Our results are now in agreement with those obtained by *Vidal & Coman*, and follow the rule that serum enzyme activity conversely reflects hepatic enzyme activity. In our present study, we have analysed the changes of serum alcohol dehydrogenase class activity in relation to that of non-alcoholic liver cirrhosis. We found a 2.5-fold increase of class I alcohol dehydrogenase activity in the sera of non-alcoholic liver cirrhotic patients. These changes were confirmed by the increase of total enzyme activity measured using a photometric method. This increase of class I isoenzymes was similar to the changes of aminotransferases (4-times elevation for aspartate and 2-times for alanine aminotransferase) and alkaline phosphatase (3-times above the mean control value), and was positively correlated with aspartate aminotransferase. The analysis indicates non-significant differences between activity of class II alcohol dehydrogenase in the tested and the control groups, but the mean value of activity in cirrhotic patients was higher than that of the control group. The significant increase of class I only may be explained by the presence of many isoenzymes in this class (homo- and heterodimers of $\alpha$, $\beta$ and $\gamma$ subunits), while class II consists only of one isoenzyme ($\pi\pi$ homodimer). The second possible explanation may be selective induction of class I alcohol dehydrogenase in this degree of liver injury. The comparison of total alcohol dehydrogenase activity in the sera of alcoholics (previous study) and non-alcoholic cirrhotic patients (present study) indicates similar changes in both cases, with an evident elevation of

**Tab. 2** Correlation coefficient between activity of two alcohol dehydrogenase classes and other markers of liver cells damage in non-alcoholic liver cirrhosis.

| Isoenzymes | Class II isoenzymes | Total alcohol dehydrogenase | Aspartate aminotransferase | Alanine aminotransferase | Lactate dehydrogenase | γ-Glutamyltransferase | Alkaline phosphatase | Total bilirubin |
|---|---|---|---|---|---|---|---|---|
| Class I | r = 0.705<br>p < 0.001 | r = 0.177<br>p = 0.376 | r = 0.577<br>p < 0.01 | r = 0.059<br>p = 0.768 | r = 0.228<br>p = 0.252 | r = 0.075<br>p = 0.707 | r = 0.253<br>p = 0.202 | r = 0.064<br>p = 0.748 |
| Class II | – | r = 0.065<br>p = 0.747 | r = 0.622<br>p < 0.001 | r = 0.276<br>p = 0.163 | r = 0.182<br>p = 0.362 | r = 0.191<br>p = 0.339 | r = 0.424<br>p = 0.027 | r = 0.139<br>p = 0.487 |
| Total alcohol dehydrogenase | – | – | r = 0.352<br>p = 0.072 | r = 0.273<br>p = 0.167 | r = 0.113<br>p = 0.574 | r = 0.185<br>p = 0.355 | r = 0.232<br>p = 0.244 | r = 0.425<br>p = 0.027 |

class I and total enzyme activity. We suggest that these similarities reflect a similar degree of liver cell damage in these subjects, which supports the results obtained by *Vidal & Coman.*

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