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BRIEF ARTICLE

Antioxidative status of patients with alcoholic liver disease in southeastern Taiwan

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Abstract

AIM: To investigate the antioxidative status of patients with alcoholic liver disease (ALD) in southeastern Taiwan.

METHODS: Our study comprised 27 patients with ALD recruited from Taitung Mackay Memorial Hospital, located in southeastern Taiwan. Patients with ALD included 12 non-aborigines (12 men) and 15 aborigines (11 men and 4 women). According to the severity of ALD, patients with ALD included 10 with hepatitis (9 men and 1 woman) and 17 with cirrhosis (14 men and 3 women). Twenty-two age- and gender-matched healthy adults served as the control group in this study. Venous blood (10 mL) of each subject was drawn into EDTA-containing tubes after 8 h overnight fasting.

RESULTS: Compared to the control group, patients with ALD showed significantly lower erythrocytic catalase (11.1 ± 0.7 U/mg Hb *vs* 8.0 ± 0.7 U/mg Hb, $P < 0.05$) and superoxide dismutase (9.5 ± 1.6 U/mg Hb *vs* 3.0 ± 0.2 U/mg Hb, $P < 0.05$) activities. Furthermore, the erythrocytic reduced glutathione/oxidized glutathione ratio was significantly lower in ALD patients than that in the control group (38.1 ± 5.4 *vs* 15.7 ± 1.9, $P < 0.05$). The results revealed that patients with ALD experienced more oxidative stress than those in the control group. The non-aboriginal, but not the aboriginal, ALD group had higher erythrocytic glutathione peroxidase (GPX) activity than that in the control group (46.1 ± 7.8 U/g Hb *vs* 27.9 ± 2.2 U/g Hb, $P < 0.05$). Hepatitis, but not cirrhosis, ALD patients had higher erythrocytic GPX activity than that in the control group (44.3 ± 8.6 U/g Hb *vs* 27.9 ± 2.2 U/g Hb, $P < 0.05$).

CONCLUSION: Our results indicate that both ethnicity and the severity of ALD may cause different erythrocytic antioxidative enzyme activities especially GPX activity.

Key words: Alcoholic liver disease; Antioxidative status; Aborigines; Hepatitis; Cirrhosis

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INTRODUCTION

Alcohol is one of the major causes of liver disease worldwide. In Taiwan, the prevalence of alcohol dependence has increased 80-fold compared to three decades ago[1,2]. Except for alcoholic liver disease (ALD), most liver diseases and cirrhosis are due to chronic infection with hepatitis viruses in Taiwan[3]. Rates of chronic hepatitis B virus and hepatitis C viral infections can reach as high as 15%-20% and 1%-5% respectively[4]. Evidence shows that chronic hepatitis C viral hepatitis can increase liver damage in alcoholic patients with liver disease[3], which indicates that a chronic hepatitis virus infection may aggravate the severity of ALD.

It was found that Taiwanese aborigines, who are belong to the Malayo-Polynesia group, are more susceptible to alcohol abuse than ethnic Han Chinese[5]. In the 1990s a higher prevalence of alcoholism in aborigines was reported (44.2%-55.2%)[6]. Viral infections and alcohol consumption play important roles in the development of chronic liver diseases in Taiwanese aborigines[7]. Therefore, ALD is an important issue for aborigines in Taiwan.

Alcohol is mainly metabolized in the liver. Hepatocytes metabolize alcohol in three ways: (1) alcohol dehydrogenase (ADH) which produces acetaldehyde from alcohol in the cytosol; (2) the microsomal ethanol-oxidizing system (MEOS) which catalyzes alcohol to acetaldehyde in the endoplasmic reticula is highly induced by chronic alcohol consumption; and (3) aldehyde dehydrogenase which catalyzes acetaldehyde to acetate in mitochondria. The ADH pathway reduces nicotinamide adenine dinucleotide (NAD) to its reduced form (NADH) and an imbalance in the NAD/NADH ratio causes a number of metabolic disorders, including inhibition of the Krebs cycle and fatty acid oxidation in ALD[8-10].

It is well-established that oxidative stress is one of the pathogenic mechanisms of ALD, and oxidative stress is mainly caused by the generation of reactive oxygen species (ROS). ROS associated with alcohol toxicity are generated by the mitochondrial respiratory chain, by ethanol-metabolizing cytochrome P4502E1 (CYP2E1) which is involved in the MEOS of hepatocytes and by NADPH oxidase of Kupffer's cells and liver-infiltrating granulocytes[11]. Besides hydroxyethyl radicals produced during ethanol oxidation by CYP2E1, nitric oxide (NO) produced by Kupffer's cell NO synthetase and alterations in hepatic iron homeostasis may further result in oxidative damage[12,13]. Oxidative stress in ALD leads to lipid peroxidation and protein oxidation[14]. To protect the body from oxidative stress, there are several antioxidant defense mechanisms. The most important antioxidant enzymes involved in these mechanisms are glutathione peroxidase (GPX), glutathione reductase (GRD), superoxide dismutase (SOD) and catalase (CAT)[15].

The pathological process of ALD can be characterized by different stages of liver damage. The first stage of ALD is hepatic steatosis which is obviously caused by abnormal lipid metabolism[16-18]. In this stage, steatosis is reversible after alcohol abstinence[19,20]. The progressive stage after steatosis is hepatitis. In this stage, steatosis is accompanied by inflammation and cytokine production. The presence of hepatitis indicates initiation of liver cirrhosis, the terminal stage of alcoholic liver disease[20,21].

Although a few studies discussed ALD in Western countries, the clinical data of patients with ALD in Taiwan, especially data on Taiwanese aborigines, are not well-established. In addition, there are different genetic types of alcohol metabolic enzymes between Western and Asian people[22]. Therefore it is necessary to establish clinical data on aboriginal patients with ALD in Taiwan. Taitung, situated in southeastern Taiwan, contains the highest proportion of aboriginal residents. There are approximately 240 000 people in Taitung, one-third of whom are aborigines, and the rest are ethnic Han Chinese[5]. The aim of this study was to compare the antioxidative status between aboriginal and non-aboriginal patients with ALD in the Taitung area. Furthermore, the effect of different severities of ALD, i.e. hepatitis and cirrhosis, on the antioxidative status is also discussed in this study.

MATERIALS AND METHODS

Subjects

This study examined 27 patients with ALD recruited from Taitung Mackay Memorial Hospital. Patients with ALD included 12 non-aboriginal patients (the non-aboriginal ALD group) (12 men) and 15 aboriginal patients (the aboriginal ALD group) (11 men and 4 women). According to the severity of ALD, patients with ALD consisted of 10 patients with alcoholic hepatitis (the hepatitis ALD group) (9 men and 1 woman) and 17 patients with alcoholic cirrhosis (the cirrhosis ALD group) (14 men and 3 women). Twenty-two age- and gender-matched healthy adults served as the control group in this study. The Institutional Review Board for Human studies approved this study. Patient consent was obtained prior to blood collection. Venous blood (10 mL) of each subject was drawn into an EDTA-containing tube after an overnight fasting period of 8 h. Plasma samples were obtained by centrifugation at 1200 × *g* for 15 min at 4℃. After removing the plasma, erythrocytes were obtained by washing twice with ice-cold physiological saline. Plasma and erythrocyte samples were stored at -80℃ until being analyzed.

Biochemical analysis

Plasma aspartate aminotransferase (AST) activity, alanine aminotransferase (ALT) activity, γ glutamyltransferase (γ-GT) activity, total cholesterol (TC) concentration, triglyceride (TG) concentration, high-density lipoprotein-cholesterol (HDL-C) concentration, low-density lipoprotein-cholesterol (LDL-C) concentration, albumin concentration, uric acid concentration, and total bilirubin concentration were measured in each patient and control group using standard procedures on an autoanalyzer (SYNCHRON CX System, Hitachi 7170, Tokyo, Japan).

Deionized water (300 μL) was added to 100 μL of the erythrocyte sample and mixed well. The mixture was centrifuged at 4℃, and 8000 × *g* for 10 min. Then the supernatant fractions were used to determine antioxidant enzymes and the reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio.

GPX activity

The GPX activity of erythrocytes was determined with a commercial kit (RS 504; Randox Laboratories, Antrim, UK). First, 20 μL of the diluted sample was added to 1 mL of a mixed substrate (4 mmol/L GSH, 0.5 U/L GRD, and 0.34 mmol/L NADPH dissolved in 50 mmol/L phosphate buffer, at pH 7.2, 4.3 mmol/L EDTA). Then, 40 μL of cumene hydroperoxide (diluted in deionized water) was added to the mixture. The reaction mixture was incubated at 37℃, and the absorbance at 340 nm was determined every minute for 3 min using a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

GRD activity

The GRD activity of erythrocytes was measured with a commercial kit (GR 2368; Randox Laboratories, Antrim, UK). First, 200 μL of the diluted sample and 400 μL of 2.4 mmol/L GSSG buffer (dissolved in 125 mmol/L potassium phosphate buffer, at pH 7.5, with 2.5 mmol/L EDTA) were added to 400 μL of 0.55 mmol/L NADPH (dissolved in deionized water). The absorbance was measured at 340 nm every minute for 5 min using a microplate reader (Molecular Devices).

SOD activity

The SOD activity of erythrocytes was measured with a commercial kit (SD 125; Randox Laboratories, Antrim, UK). First, 50 μL of the diluted sample and 1.7 mL of the mixed substrate (50 μmol/L xanthine and 25 μmol/L 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride, INT) were added to 250 μL of xanthine oxidase. The reaction mixture was incubated at 37℃, and the absorbance was measured at 340 nm every minute for 3 min using a microplate reader (Molecular Devices).

CAT activity

The CAT activity of erythrocytes was determined according to the method reported by Beers and Sizer[23]. First, 100 μL of the diluted sample and 1 mL of 59 mmol/L H_2O_2 (dissolved in 50 mmol/L potassium phosphate buffer, at pH 7.0) were added to 1.9 mL of deionized water. The absorbance was measured at 240 nm every minute for 3 min using a Hitachi U-2000 ultraviolet-visible (UV-VIS) spectrophotometer. One unit of CAT activity was defined as mmol of H_2O_2 degraded/min.

Determination of the GSH/GSSG ratio in erythrocytes and liver tissue

GSH concentration: The concentration of GSH in erythrocytes was determined according to the method of Tietze[24], using GSH (0-100 μmol/L) as the standard. The diluted sample solution or standard (10 μL) was mixed with 95 μL of the reagent (2 U/mL glutathione reductase, 200 μmol/L NADPH, and 2 mmol/L EDTA in 50 mmol/L phosphate buffer, at pH 7.2), followed by the addition of 100 μL of the reagent (10 mmol/L DTNB in 50 mmol/L phosphate buffer, at pH 7.2). The reaction mixture was then incubated at room temperature, and the absorbance at 405 nm was determined every minute for 5 min using an enzyme-linked immunosorbent assay (ELISA) reader (Molecular Devices). The concentration was expressed as GSH (μmol/L) in erythrocytes.

GSSG concentration: The concentration of GSSG in erythrocytes was measured according to the method of Tietze[24], using GSSG (0-100 μmol/L) as the standard. The diluted sample solution or standard (70 μL) was mixed with 4 μL of 1-methyl-2-vinylpyridinium trifluoromethanesulfonate (M2VP). The mixture was allowed to stand at room temperature for 1 h. The reaction mixture (10 μL) was mixed with 95 μL of the reagent (2 U/mL glutathione reductase, 200 μmol/L NADPH, and 2 mmol/L EDTA in 50 mmol/L phosphate buffer, at pH 7.2), followed by the addition of 100 μL of the reagent (10 mmol/L DTNB in 50 mmol/L phosphate buffer, at pH 7.2). The reaction mixture was then incubated at room temperature, and the absorbance at 405 nm was determined every minute for 5 min using an ELISA reader (Molecular Devices). The concentration was expressed as GSSG (μmol/L) in erythrocytes.

The GSH/GSSG ratio: The GSH/GSSG ratio was then calculated by dividing the difference between the total GSH and GSSG concentrations (reduced GSH) by the concentration of GSSG. GSH/GSSG = (total GSH - 2GSSG)/GSSG.

Measurement of lipid peroxidation in plasma

Lipid peroxidation was quantitatively measured by measuring the concentration of thiobarbituric acid reactive substances (TBARS) in plasma using the method of Ohkawa *et al*[25] with minor modifications. 20 μL plasma or various levels of TMP (1,1,3,3-tetramethoxypropane; as a standard) were shaken with 800 μL of 0.22% H_2SO_4 in a 2 mL centrifuge tube. Phosphotungstic acid (100 μL; 10%) and 200 μL of 0.67% TBA (in H_2O: glacical acetic acid = 1:1, v/v) were added to the mixture, shaken, and warmed for 60 min in a boiling water bath followed by rapid cooling. Then 600 μL of an *n*-butyl-alcohol layer was shaken in a separation tube, and the MDA content in the plasma or liver homogenates was determined fluorometrically (with respective excitation and emission wavelengths of 531 and 590 nm) using a Wallace Victor-2 1420 Multilabel Counter (Perkin-Elmer, Waltham, MA, USA).

Statistical analysis

All data are expressed as the means ± SE. Student's *t* test was used to compare differences of means between the control group and ALD group using EXCEL software

Table 1 Biochemical parameters of control subjects and patients with alcoholic liver disease (mean ± SE)

	Control	ALD
n	22	27
Male/female	19/3	23/4
Age (yr)	41.2 ± 2.1	45.3 ± 2.1
Height (cm)	169.7 ± 1.2	165.3 ± 1.3[a]
Weight (kg)	66.1 ± 1.8	65.4 ± 2.3
BMI (kg/m^2)	23.9 ± 0.6	23.9 ± 0.8
AST (U/L)	20.7 ± 0.9	63.6 ± 8.7[a]
ALT (U/L)	22.6 ± 1.7	39.1 ± 5.2[a]
AST/ALT	1.0 ± 0.06	2.86 ± 0.86[a]
γ-GT (U/L)	29.2 ± 3.5	271.1 ± 81.3[a]
TC (mg/dL)	185.6 ± 5.5	153.7 ± 13.2[a]
TG (mg/dL)	99.4 ± 13.2	193.6 ± 61.2
HDL-C (mg/dL)	50.5 ± 3.1	36.7 ± 2.6[a]
LDL-C (mg/dL)	125.5 ± 6.8	72 ± 8.2[a]
Albumin (gm/dL)	4.75 ± 0.06	3.33 ± 0.18[a]
Uric acid (mg/dL)	6.26 ± 0.29	6.81 ± 0.45
Total bilirubin	0.56 ± 0.04	5.53 ± 2.21[a]

[a]$P < 0.05$ *vs* the control group (by Student's *t*-test). ALD: Alcoholic liver disease; BMI: Body-mass index; AST: Aspartate aminotransferase; ALT: Alanine transaminase; γ-GT: γ-glutamyltransferase; TC: Total cholesterol; TG: Triglycerides; HDL-C: High-density lipoprotein-cholesterol; LDL-C: Low-density lipoprotein-cholesterol.

Table 2 Erythrocytic antioxidative enzymes activities, erythrocytic reduced glutathione/oxidized glutathione ratio and plasma thiobarbituric acid reactive substances concentration of control subjects and patients with alcoholic liver disease (mean ± SE)

	Control	ALD
GPX (U/g Hb)	27.9 ± 2.2	34.4 ± 4.2
GRD (U/g Hb)	0.55 ± 0.06	0.62 ± 0.05
CAT (U/mg Hb)	11.1 ± 0.7	8.0 ± 0.7[a]
SOD (U/mg Hb)	9.5 ± 1.6	3.0 ± 0.2[a]
GSH/GSSG	38.1 ± 5.4	15.7 ± 1.9[a]
TBARS (μmol/L)	3.5 ± 0.2	4.1 ± 0.6

[a]$P < 0.05$ *vs* the control group (by Student's *t*-test). ALD: Alcoholic liver disease; GPX: Glutathione peroxidase; GRD: Glutathione reductase; CAT: Catalase; SOD: Superoxide dismutase; GSH: Reduced glutathione; GSSG: Oxidized glutathione; TBARS: Thiobarbituric acid reactive substances.

(Redmond, WA, USA). Statistical significance was assigned at the $P < 0.05$ level. To evaluate differences among the three groups in this study, one-way analysis of variance with Fisher's post hoc test was used. The SAS software (vers. 8.2, SAS Institute., Cary, NC, USA) was used to analyze all data. Differences were considered statistically significant at $P < 0.05$.

RESULTS

The biochemical parameters of the control and ALD group are shown in Table 1. No significant difference was observed with regard to age, weight, body-mass index (BMI), or plasma uric acid concentration between the control and ALD group. The plasma AST activity, ALT activity, AST/ALT ratio, γ-GT activity and total bilirubin concentrations were significantly higher in the ALD group than those in the control group ($P < 0.05$). The plasma TC, HDL-C, and LDL-C concentrations were significantly lower in the ALD group than those in the control group ($P < 0.05$). Furthermore, patients with ALD had significantly lower plasma albumin concentrations than that in the control subjects ($P < 0.05$). As shown in Table 2, no significant difference in erythrocytic GPX or GRD activities was observed between the control and ALD groups. Patients with ALD showed significantly lower erythrocytic CAT and SOD activities when compared to those in the control group ($P < 0.05$). Furthermore, the erythrocytic GSH/GSSG ratio was significantly lower in patients with ALD than that in the control group ($P < 0.05$). But no significant difference was observed with regard to the plasma TBARS concentration between the control and ALD group.

Table 3 Biochemical parameters of control subjects, and non-aboriginal and aboriginal patients with alcoholic liver disease (mean ± SE)

	Control	Non-aboriginal ALD	Aboriginal ALD
n	22	12	15
Male/female	19/3	12/0	11/4
Age (yr)	41.2 ± 2.1[a]	51.3 ± 2.7[b]	40.5 ± 2.4[a]
Height (cm)	169.7 ± 1.2[a]	167.6 ± 1.6[a,b]	163.6 ± 1.7[b]
Weight (kg)	66.1 ± 1.8[a]	66.5 ± 3.7[a]	64.5 ± 3.1[a]
BMI (kg/m^2)	23 ± 0.6[a]	23.7 ± 1.2[a]	24.1 ± 1.1[a]
AST (U/L)	20.7 ± 0.9[a]	58.0 ± 10.7[b]	68.1 ± 13.4[b]
ALT (U/L)	22.6 ± 1.8[a]	42.8 ± 10.0[b]	36.1 ± 5.1[a,b]
AST/ALT	1.0 ± 0.1[a]	3.0 ± 1.4[a]	2.7 ± 1.1[a]
γ-GT (U/L)	29.2 ± 3.5[a]	335.9 ± 173.4[b]	219.3 ± 53.0[a,b]
TC (mg/dL)	185.6 ± 5.5[a]	157.3 ± 15.8[a]	150.6 ± 21.0[a]
TG (mg/dL)	99.4 ± 13.2[a]	136.4 ± 66.3[a]	239.4 ± 109.3[a]
HDL-C (mg/dL)	50.5 ± 3.1[a]	39.8 ± 4.5[b]	34.3 ± 3.1[b]
LDL-C (mg/dL)	125.5 ± 6.8[a]	88.8 ± 13.4[b]	58.5 ± 9.2[c]
Albumin (gm/dL)	4.75 ± 0.06[a]	3.68 ± 0.23[b]	2.93 ± 0.25[c]
Uric acid (mg/dL)	6.26 ± 0.29[a]	6.74 ± 0.45[a]	6.89 ± 0.89[a]
Total bilirubin	0.56 ± 0.04[a]	3.74 ± 1.43[a,b]	7.31 ± 4.23[b]

Values in the same row with different letters (a, b, c) significantly different at $P < 0.05$ (by one-way analysis of variance). ALD: Alcoholic liver disease; BMI: Body-mass index; AST: Aspartate aminotransferase; ALT: Alanine transaminase; γ-GT: γ-glutamyltransferase; TC: Total cholesterol; TG: Triglycerides; HDL-C: High-density lipoprotein-cholesterol; LDL-C: Low-density lipoprotein-cholesterol.

In order to understand the antioxidant status in aboriginal patients with ALD (aboriginal ALD group) in the Taitung area, patients were divided into an aboriginal ALD, and a non-aboriginal ALD (non-aboriginal ALD group). Biochemical parameters of the control, non-aboriginal ALD, and aboriginal ALD group are given in Table 3. No significant difference was observed in the weight, BMI, AST/ALT, plasma TC, TG or uric acid concentrations among the three groups. Both the non-aboriginal and aboriginal ALD groups had significantly higher plasma AST activity than the control group ($P < 0.05$). The non-aboriginal, but not the aboriginal, ALD group had significantly higher plasma ALT and γ-GT activities than that in the control group ($P < 0.05$). Both the non-aboriginal and aboriginal ALD groups had significantly lower plasma

Table 4 Erythrocytic antioxidative enzymes activities, erythrocytic reduced glutathione/oxidized glutathione ratio and plasma thiobarbituric acid reactive substances concentration of control subjects, and non-aboriginal and aboriginal patients with alcoholic liver disease (mean ± SE)

	Control	Non-aboriginal ALD	Aboriginal ALD
GPX (U/g Hb)	27.9 ± 2.2[a]	46.1 ± 7.8[b]	25.0 ± 0.2[a]
GRD (U/g Hb)	0.55 ± 0.06[a]	0.68 ± 0.07[a]	0.58 ± 0.07[a]
CAT (U/mg Hb)	11.1 ± 0.7[a]	8.5 ± 0.9[b]	7.6 ± 1.1[b]
SOD (U/mg Hb)	9.5 ± 1.6[a]	2.9 ± 0.3[b]	3.1 ± 0.3[b]
GSH/GSSG	38.0 ± 5.4[a]	16.5 ± 3.8[b]	15.0 ± 1.6[b]
TBARS (μmol/L)	3.5 ± 0.2[a]	3.4 ± 0.5[a]	4.6 ± 1.0[a]

Values in the same row with different letters (a, b) significantly different at $P < 0.05$. (by one-way analysis of variance). ALD: Alcoholic liver disease; GPX: Glutathione peroxidase; GRD: Glutathione reductase; CAT: Catalase; SOD: Superoxide dismutase; GSH: Reduced glutathione; GSSG: Oxidized glutathione; TBARS: Thiobarbituric acid reactive substances.

HDL-C, LDL-C and albumin concentrations than those in the control group ($P < 0.05$). In addition, the aboriginal ALD group had lower plasma LDL-C and albumin concentrations than those in the non-aborigine ALD group ($P < 0.05$). The aboriginal, but not non-aboriginal, ALD had significantly higher plasma total bilirubin than that in the control group ($P < 0.05$).

As shown in Table 4, the non-aboriginal, but not the aboriginal ALD group had significantly higher erythrocytic GPX activity than the control group ($P < 0.05$). In addition, no significant difference was observed in the erythrocytic GRD activities and plasma TBARS concentration among the three groups. Both the non-aboriginal and aboriginal ALD group had significantly lower erythrocytic CAT activity, erythrocytic SOD activity and erythrocytic GSH/GSSG ratio than those in the control group ($P < 0.05$).

The antioxidant status with different severities of alcoholic liver disease was investigated. Patients with ALD were divided into those with hepatitis ALD (hepatitis ALD) and those with cirrhosis (cirrhosis ALD). In Table 5, no significant difference was observed in the weight, BMI or uric acid concentration among the control, hepatitis ALD, and cirrhosis ALD groups. Both the hepatitis ALD and cirrhosis ALD groups had significantly higher plasma AST activity compared to the control group ($P < 0.05$). The hepatitis, but not the cirrhosis, ALD group had significantly higher plasma ALT activity and AST/ALT ratio than those in the control group ($P < 0.05$). The cirrhosis, but not the hepatitis, ALD group had significantly higher plasma γ-GT activity compared to the control group ($P < 0.05$). The cirrhosis, but not the hepatitis, ALD group had lower plasma HDL-C concentrations than the control group ($P < 0.05$). Both the hepatitis and cirrhosis ALD groups had significantly lower plasma LDL-C concentrations when compared to the control group ($P < 0.05$). In addition, the cirrhosis ALD group had significantly lower plasma albumin concentrations than the hepatitis ALD group ($P < 0.05$). The cirrhosis, but not the hepatitis, ALD group had significantly higher plasma total bilirubin concentrations than the control group ($P < 0.05$).

As shown in Table 6, the hepatitis ALD, but not the cirrhosis ALD group, had significantly higher erythrocytic

Table 5 Biochemical parameters of control subjects, and patients with hepatitis and cirrhosis alcoholic liver disease (mean ± SE)

	Control	Hepatitis ALD	Cirrhosis ALD
n	22	10	17
Male/female	19/3	9/1	14/3
Age (yr)	41.2 ± 2.1[a]	45.4 ± 4.2[a]	45.3 ± 2.2[a]
Height (cm)	169.7 ± 1.2[a]	167.4 ± 2.3[a,b]	164.2 ± 1.5[b]
Weight (kg)	66.1 ± 1.8[a]	70.4 ± 3.4[a]	62.6 ± 3.0[a]
BMI (kg/m^2)	23.0 ± 0.6[a]	25.2 ± 1.3[a]	23.2 ± 1.0[a]
AST (U/L)	20.7 ± 0.9[a]	66.9 ± 18.6[b]	61.7 ± 9.1[b]
ALT (U/L)	22.6 ± 1.8[a]	46.2 ± 12.7[b]	34.9 ± 3.8[a,b]
AST/ALT	1.0 ± 0.06[a]	4.49 ± 2.28[b]	1.9 ± 0.22[a,b]
γ-GT (U/L)	29.2 ± 3.5[a]	207.8 ± 71.7[a,b]	308.4 ± 123.1[b]
TC (mg/dL)	185.6 ± 5.5[a]	188.9 ± 26.2[a]	131.8 ± 11.5[b]
TG (mg/dL)	99.4 ± 13.2[a]	293.5 ± 153.5[b]	134.9 ± 35.8[a,b]
HDL-C (mg/dL)	50.5 ± 3.1[a]	45.1 ± 3.8[a]	31.8 ± 3.0[b]
LDL-C (mg/dL)	125.5 ± 6.8[a]	94.4 ± 15.8[b]	58.8 ± 8.0[c]
Albumin (gm/dL)	4.75 ± 0.06[a]	3.87 ± 0.38[b]	3.08 ± 0.17[c]
Uric acid (mg/dL)	6.26 ± 0.29[a]	6.68 ± 0.71[a]	6.86 ± 0.59[a]
Total bilirubin	0.56 ± 0.04[a]	1.22 ± 0.17[a]	7.48 ± 3.06[b]

Values in the same row with different letters (a, b, c) significantly differ at $P < 0.05$. (by one-way analysis of variance). ALD: Alcoholic liver disease; BMI: Body-mass index; AST: Aspartate aminotransferase; ALT: Alanine transaminase; γ-GT: γ-glutamyltransferase; TC: Total cholesterol; TG: Triglycerides; HDL-C: High-density lipoprotein-cholesterol; LDL-C: Low-density lipoprotein-cholesterol.

Table 6 Erythrocytic antioxidative enzyme activities, erythrocytic reduced glutathione/oxidized glutathione ratio and plasma thiobarbituric acid reactive substances concentration of control subjects, patients with hepatitis and cirrhosis alcoholic liver disease (mean ± SE)

	Control	Hepatitis ALD	Cirrhosis ALD
GPX (U/g Hb)	27.9 ± 2.2[a]	44.3 ± 8.6[b]	28.5 ± 3.8[a]
GRD (U/g Hb)	0.55 ± 0.06[a]	0.74 ± 0.06[a]	0.56 ± 0.07[a]
CAT (U/mg Hb)	11.1 ± 0.7[a]	10.8 ± 1.2[a]	6.4 ± 0.6[b]
SOD (U/mg Hb)	9.5 ± 1.6[a]	3.7 ± 0.2[b]	2.6 ± 0.2[b]
GSH/GSSG	38.1 ± 5.4[a]	18.3 ± 4.2[b]	14.2 ± 1.7[b]
TBARS (μmol/L)	3.5 ± 0.2[a]	4.2 ± 1.1[a]	4.0 ± 0.7[a]

Values in the same row with different letters (a, b) significantly different at $P < 0.05$ (by one-way analysis of variance). ALD: Alcoholic liver disease; GPX: Glutathione peroxidase; GRD: Glutathione reductase; CAT: Catalase; SOD: Superoxide dismutase; GSH: Reduced glutathione; GSSG: Oxidized glutathione; TBARS: Thiobarbituric acid reactive substances.

GPX activity than the control group ($P < 0.05$). There was no significant difference in erythrocytic GRD activity and plasma TBARS concentration among the three groups. The cirrhosis, but not the hepatitis, ALD group had significantly lower erythrocytic CAT activity than the control group ($P < 0.05$). Both the hepatitis and cirrhosis ALD groups had significantly lower erythrocytic SOD activities and GSH/GSSG ratio than those in the control group ($P < 0.05$).

DISCUSSION

In most patients with liver injury, the ratio of AST to ALT is 1 or less, whereas in alcoholic hepatitis it is generally

about 2[26]. An AST to ALT ratio > 1 was reported in patients with alcoholic cirrhosis[27]. Our observation agreed with the previous study, the AST to ALT ratio was 2.86 in patients with ALD (Table 1) and the AST to ALT ratio in hepatitis ALD was higher than that in cirrhosis ALD (Table 5).

Erythrocytes can easily suffer oxidative damage due to the presence of polyunsaturated fatty acid, heme, iron, and oxygen. The antioxidant enzymes, GPX, GRD, CAT and SOD, located in erythrocytes protect the body from oxidative stress[28]. In an animal study, decreased activity of Cu, Zn SOD was demonstrated in the liver of rodents after chronic ethanol exposure[29]. However, both increased and decreased SOD activities in the blood of alcoholics were reported[30-33], and this may have been due to different durations of alcohol dependence. Free radical scavenging enzymes such as SOD, CAT, and GPX are known to be the first line cellular defense against oxidative damage, disposing of superoxide anion and H_2O_2 before their interaction to form the more harmful hydroxyl radical. In the present study, both CAT and SOD activities decreased significantly in the ALD groups compared with the control group, and it is assumed that excessive superoxide anions might elicit lipoperoxide formation and induce cell damage before being converted to H_2O_2 by SOD[34]. In the absence of adequate SOD activity, superoxide anion is not converted into H_2O_2, which is the substrate for the H_2O_2 scavenging enzyme CAT[35]. As a result, there is an inactivation of the H_2O_2 scavenging enzyme CAT, leading to a decrease in its activity[36].

GPX produces GSSG from GSH, while GRD maintains the cellular level of GSH by reducing oxidized glutathione to its reduced form. Change in activities of these two antioxidant enzymes are not consistent in ALD[37]. In the present study, erythrocytic GPX and GRD activities showed no difference between patients with ALD and the control group (Table 2). The limited size of the study populations may have contributed to this anomaly.

Glutathione, a tripeptide (γ-glutamylcysteinylglycine), plays an important role in coordinating the body's antioxidant defense processes[35]. Lower hepatic GSH levels were reported in alcoholic cirrhosis[38,39]. GSH is also present in erythrocytes[40]. GSH can be exported from hepatocytes to the sinusoidal blood, where it is rapidly broken down to dipeptides and amino acid by γ-GT and dipeptidases[40,41]. Erythrocytes can resynthesize GSH after taking up the precursor amino acid[30]. In agreement with a previous study[42], the erythrocytic GSH/GSSG ratio, an indicator of the antioxidant status, was significantly lower in the ALD group than the control group in this study (Table 2). The lower erythrocytic GSH concentrations may be due to impaired biosynthesis or decreased release from damaged liver cells[42]. The TBARS concentration reflects the level of malondialdegyde (MDA) which is the end product of lipid peroxidation. As shown by Peng *et al*[15], the MDA concentration not only significantly increased in the ALD group than in the control group, but was also significantly correlated with the duration of alcohol dependence. However, our present study is not in accordance with a previous study. The plasma TBARS concentration showed a slight increase over that of the control, but it did not reach significance. We believe that the dietary lipid composition may play an important role in lipid peroxidation. Evidence showed that the effect of saturated fatty acids may reduce endotoxemia and lipid peroxidation[43]. In addition, rats fed diets high in polyunsaturated fatty acids and ethanol could increase the susceptibility of peroxidation[44]. Therefore, the dietary lipid composition of control subjects and patients with ALD in southeastern Taiwan should be investigated in the future.

Poor health knowledge, a relatively low socioeconomic status, high rates of alcohol abuse, and poor control of chronic diseases are the characteristics of Taiwanese aborigines[45]. It is believed that both poor health knowledge and relatively low socioeconomic status may lead to malnutrition in Taiwanese aborigines. As shown in this study, the significantly lower albumin concentration, which is an index of malnutrition, was found in the aboriginal ALD group (Table 3). On the other hand, the non-aboriginal, but not the aboriginal, ALD group had higher erythrocytic GPX activities than the control group (Table 4). The higher erythrocytic GPX activity can be explained as an adaption to the oxidative stress of ALD. However, the aboriginal ALD group did not show an adaptive capacity.

As shown in table 5, the cirrhosis ALD group had significantly lower plasma TC, HDL-C, and LDL-C concentrations than those in the hepatitis ALD and control groups. A previous study indicated that patients with alcoholic cirrhosis with Child-Pugh score C showed lower plasma TC concentrations than patients with alcohol abuse without cirrhosis[46]. This confirms that the severity of liver impairment may affect plasma TC concentrations. In addition, lower plasma HDL-C and LDL-C concentrations with alcoholic cirrhosis were also reported in previous studies[47,48]. The lower lipoprotein results from impairment lipoprotein synthesis can be explained by decreased protein secretion and increased protein retention in the liver under ethanol exposure[49].

Changes in erythrocytic antioxidant enzymes differed between the hepatitis and cirrhosis ALD groups compared to the control group (Table 6). The hepatitis, but not cirrhosis, ALD group had higher erythrocytic GPX activity than the control group. The higher GPX activity in hepatitis ALD patients can be explained by adaptation to oxidative stress. In addition, the hepatitis ALD group showed significantly higher erythrocytic CAT activity than the cirrhosis ALD group. The lower erythrocytic CAT activity of cirrhosis ALD patients is consistent with a previous study[50]. This result reveals that cirrhosis ALD patients had lower erythrocytic enzyme activities than hepatitis ALD patients. Although there were different changes in erythrocytic antioxidant enzymes between the two ALD groups, both the hepatitis and cirrhosis ALD groups showed a lower erythrocytic GSH/GSSG ratio than the control group.

In conclusion, this study indicated that both ethnicity and the severity of alcoholic liver disease can cause different erythrocytic antioxidative enzyme activities especially GPX activity. This finding can not only establish the clinical data of patients in Taiwan but also provide a few clues for the treatment of alcoholic liver disease. Our research also showed no significant difference in plasma TBARS level between control subjects and ALD patients. This different result from studies in Western countries might be attributed to the dietary lipid composition in the Taitung area of southeastern Taiwan.

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COMMENTS

Background

Alcohol abuse is one of the major causes of liver disease worldwide. The prevalence of alcohol dependence is increasing in Taiwan. Besides the hepatic viral infection, another high rate prevalence liver disease in Taiwan may increase liver damage in alcoholic liver disease. Thus, more research should be done on establishing the clinical data of alcoholic liver disease in Taiwan area.

Research frontiers

A higher prevalence of alcoholism in aborigines was reported in Taiwan. Besides, both viral infection and alcohol consumption play important roles in the development of chronic liver diseases in Taiwanese aborigines. Therefore, alcoholic liver disease is an important issue for aborigines in Taiwan.

Innovations and breakthroughs

In this study, we indicated that both ethnicity and the severity of alcoholic liver disease may cause different erythrocytic antioxidative enzyme activities, especially erythrocytic glutathione peroxidase (GPX) activity.

Applications

By understanding the different changes of antioxidative enzyme activities between either different ethnicity or severity of alcoholic liver disease, we can not only establish the clinical data of patients in Taiwan but also provide a few clues for treatment of alcoholic liver disease.

Peer review

The manuscript describes the antioxidative condition of 27 alcoholic liver disease (ALD) patients in southeastern Taiwan by biochemical analysis, such as erythrocytic catalase, superoxide dismutase, reduced glutathione/oxidized glutathione ratio and GPX, and plasma low-density lipoprotein-cholesterol concentration. The authors concluded that both ethnicity and the severity of ALD may cause different erythrocytic antioxidative enzyme activities especially GPX activity and the findings are of interest.

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