Association between circulating inflammatory molecules and alcoholic liver disease in men

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Abstract The association between alcoholic liver disease (ALD) and the inflammatory response remains controversial. The aim of this study was to explore this association between ALD and inflammation. We enrolled 214 male participants, who were divided into three age-matched groups: ALD ($n = 135$), chronic alcohol ingestion without ALD (non-ALD; $n = 42$), and control ($n = 37$). The BMI was significantly higher in the ALD group than in the non-ALD and control groups (all $P = 0.000$). Further, the constituent ratio of the liver inflammatory level was significantly higher in the ALD group than in the non-ALD and control groups ($P = 0.002$ and $P = 0.000$, respectively). In addition, the median serum ALT, AST, and GGT levels were significantly higher in the ALD group than in the control group ($P = 0.023$, $P = 0.008$, and $P = 0.000$, respectively); these levels were also significantly higher in the ALD group than in the non-ALD group ($P = 0.013$, $P = 0.010$, and $P = 0.000$, respectively). The median serum CRP level was significantly higher in the ALD group than in the non-ALD and control groups ($P = 0.006$ and $P = 0.000$, respectively). Further, the median serum TNF- α level was significantly lower in the ALD group than in the non-ALD and control groups ($P = 0.004$ and $P = 0.000$, respectively). The median serum sOX40L and HSP70 levels were significantly lower in the ALD group than in the control

group ($P = 0.008$ and $P = 0.018$, respectively). In addition, the ALT, AST, and GGT levels were positively correlated with the CRP level ($r = 0.211$, $P = 0.002$; $r = 0.220$, $P = 0.001$ and $r = 0.295$, $P = 0.000$, respectively), and the GGT level was negatively correlated with the TNF- α ($r = -0.225$, $P = 0.001$), sOX40L ($r = -0.165$, $P = 0.016$), and HSP70 levels ($r = -0.178$, $P = 0.009$). Further, the Cr level was negatively correlated with the IL-10 level ($r = -0.166$, $P = 0.015$). Logistic regression analysis verified that the BMI (OR = 1.637, 95%CI: 1.374–1.951, $P = 0.000$) and GGT level were significantly higher (OR = 1.039, 95%CI: 1.020–1.059, $P = 0.000$) and that the TNF- α (OR = 0.998, 95%CI: 0.996–1.000, $P = 0.030$) and HSP70 levels were significantly lower (OR = 1.017, 95%CI: 1.003–1.031, $P = 0.029$) in the ALD group than in the non-ALD group. Further, the moderate-to-severe ALD patients had a significantly higher serum CRP level (OR = 1.349, 95%CI: 1.066–1.702, $P = 0.013$) and significantly lower HSP60 (OR = 0.965, 95%CI: 0.938–0.993, $P = 0.014$) and HSP70 levels (OR = 0.978, 95%CI: 0.962–0.995, $P = 0.010$) than the mild ALD patients. These results suggest that ALD patients may present with obesity, liver damage, and an imbalanced inflammatory immune response, mainly manifesting as decreased levels of immune inflammatory cytokines. In addition, they suggest that certain liver and kidney function parameters and ALD severity are either positively or negatively correlated with certain inflammatory cytokines. Hence, ALD patients may be at increased risks of obesity- and inflammation-related diseases. Accordingly, to control the inflammatory response, preventative measures for patients with this disease should include weight control and protection of liver and kidney function.

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Keywords Alcoholic liver disease · Inflammatory cytokines · Body mass index · Hepatorenal function

Abbreviations

ALD	Alcoholic liver disease
BMI	Body mass index
ALT	Alanine transaminase
AST	Aspartate aminotransferase
GGT	Gamma-glutamyltransferase
BUN	Blood urea nitrogen
Cr	Creatinine
CRP	C-reactive protein
IL-6	Interleukin-6
IL-10	Interleukin-10
TNF- α	Tumor necrosis factor-alpha
ADP	Adiponectin
HSP	Heat shock protein
sOX40L	Soluble OX40 ligand
ELISA	Enzyme-linked immunosorbent assay

Introduction

Alcoholic liver disease (ALD) is a major cause of acute and chronic liver diseases worldwide and thus represents a major healthcare challenge (Thomes et al. 2015a). It is a multifaceted disease characterized by hepatic steatosis, fat deposition, hepatitis, and inflammation (Ambade and Mandrekar 2012). It also causes hepatocyte damage, inflammatory cell activation, and increased intestinal permeability, leading to the clinical manifestations of alcoholic hepatitis (Szabo and Satishchandran 2015). Cytokines are proteins made and released by one cell that affect the behaviors of other cells (e.g., activate other cells) and cell–cell interactions (Molina et al. 2010a). The initiation of ALD is a complex process involving different liver cell types, including both parenchymal and non-parenchymal cells (Molina et al. 2010a), such as macrophages and Kupffer cells. In ALD, activated macrophages, including M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages, are present in the liver (Saha et al. 2015). The activation of liver-resident macrophages or Kupffer cells, at least in part by increased exposure to gut-derived bacterial wall components such as endotoxins, results in the increased production of pro-inflammatory cytokines (Wree et al. 2013; Lewis and Mohanty 2010) and numerous other factors. These factors include products of ethanol-induced inflammation, ethanol metabolism, and the indirect reactions stimulated by ethanol metabolites (Schaffert et al. 2009). Several studies (Albano 2006; Viitala et al. 2000; Szabo et al. 2012) have demonstrated relationships between ALD, the inflammatory response, and oxidative stress. For example, one previous study has reported that chronic alcohol use has profound modulatory effects on the immune system (Franchi et al. 2010), and another study has proposed that ALD is a common form of acquired immunodeficiency (Pimentel-Nunes et al. 2010). However, there is a gap in the understanding of the association of ALD with the inflammatory

response. Therefore, the precise mechanism responsible for the downregulation of pro-inflammatory cytokines in ALD requires further investigation. For this purpose, we investigated the correlation between ALD and the inflammatory response by measuring the body mass index (BMI) and serum levels of hepatorenal function parameters and inflammatory cytokines.

Materials and methods

Ethics statement

Written informed consent was obtained from all patients prior to their participation. The protocol was approved by the clinical research ethics committee of Taishan Hospital in Shandong Province.

Participants

From January 2012 to May 2015, we recruited 214 age-matched male participants from Taishan Hospital in Shandong Province based on the results of health examinations. Among these participants, 135 met the criteria for ALD established by the Fatty Liver and Alcoholic Liver Disease Group, Hepatology Branch, Chinese Medical Association (The group of Chinese medical association branch of hepatology fatty liver and alcoholic liver disease 2006). The degree of hepatic steatosis, as assessed by ultrasound, was categorized as mild or moderate-severe (Ahn et al. 2015). These 135 participants also met the criteria for diagnosis of chronic alcohol ingestion (daily ethanol intake of greater than 40 g in men for a period of longer than 5 years) (Galustian et al. 2003). The remaining 79 participants did not have ALD. The participants were divided into the following three groups: ALD ($n = 135$), chronic alcohol ingestion without ALD (non-ALD; $n = 42$), and control ($n = 37$). A cross-sectional study was performed to explore the associations between the levels of circulating inflammatory molecules and ALD. In particular, we measured BMI and the serum levels of hepatorenal function parameters, including alanine transaminase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), blood urea nitrogen (BUN), creatinine (Cr), C-reactive protein (CRP), interleukin (IL)-6, IL-10, tumor necrosis factor-alpha (TNF- α), E-selectin, adiponectin (ADP), soluble OX40 ligand (sOX40L), heat shock protein (HSP) 60, and HSP70. According to these hepatorenal function parameters, the liver inflammatory status was classified as follows: an abnormal liver inflammatory status was defined as an abnormal level of at least one liver enzyme, i.e., an ALT level of greater than or equal to 40 mmol/l, an AST level of greater than or equal to 40 mmol/l, or a GGT level of greater than or equal to 50 mmol/l, and a normal liver

inflammatory status was defined as normal liver enzyme levels, including an ALT level of less than 40 U/L, an AST level of less than 40 mmol/l, and a GGT level of less than 50 U/L. The exclusion criteria used in this study have been previously described (Qu et al. 2015a, b), and subjects with other complications, such as ascites, pancreatitis, encephalopathy, coagulopathy and anemia, were also excluded.

Experimental apparatus

A color ultrasound system (GEV7 and LOG7; GE, USA), an automatic biochemical analyzer (7080; Japan), and a standard plate reader (ANTHOS 2010; Austria) were utilized in this study.

Reagents

The inflammatory response was measured using kits to detect CRP (provided by the Beijing JiuQiang Company), IL-6, IL-10, TNF- α , E-selectin, ADP, sOX40L, HSP60, and HSP70 (provided by Shanghai Enzyme-Linked Immune Co. Ltd.). All of the kits were manufactured by R&D Systems (USA).

Laboratory tests

Venous blood was obtained from just above the elbow after the subjects had fasted overnight for at least 10 h. Serum was collected by centrifugation at 3000 rpm for 10 min and then stored at -70°C . Serum levels of hepatorenal function parameters were detected using an automatic biochemistry analyzer. Serum CRP levels were detected by immunoturbidimetry. Serum levels of IL-6, IL-10, TNF- α , E-selectin, ADP, sOX40L, HSP60, and HSP70 were measured by enzyme-linked immunosorbent assay (ELISA). All tests were performed according to the manufacturer's instructions.

Statistical analysis

SPSS 19.0 statistical package (SPSS Inc., Chicago, IL, USA) was utilized for all statistical analyses. Categorical variables were expressed as a sample size (number of cases) and percentage (%) and were compared using the chi-square (χ^2) test. All continuous variables were expressed as a median and range. Kruskal-Wallis ANOVA was used for comparisons among the three groups, and the Mann-Whitney U test was used for comparisons between two groups. Spearman rank correlation was used to analyze the correlations between hepatorenal function and inflammatory cytokine levels. Odds ratios (ORs) for the presence of ALD, as well as the severity, classified as mild ALD or moderate-to-severe ALD, were calculated with 95 % confidence intervals (CIs) using binary logistic regression models with the backward LR method. P values of less than 0.05 were considered significant.

Results

Comparisons of age, median alcohol drinking history, median daily alcohol consumption, BMI, constituent ratio of the liver inflammatory status, liver function, renal function, and inflammatory cytokine levels among the three groups are presented in Table 1. No significant differences in age, median alcohol drinking history, or median daily alcohol consumption were observed between pairs of groups (all $P > 0.05$). However, a significant difference in BMI was detected among the three groups ($P = 0.000$). In particular, the BMI was significantly higher in the ALD group than in the non-ALD and control groups (all $P = 0.000$).

The constituent ratio of the liver inflammatory status significantly differed among the three groups ($P = 0.000$); this ratio was significantly higher in the ALD group than in the non-ALD and control groups ($P = 0.002$ and $P = 0.000$, respectively). The median serum ALT, AST, and GGT levels also significantly differed among the three groups ($P = 0.010$, $P = 0.004$, and $P = 0.000$, respectively); these levels were significantly higher in the ALD group than in the control group ($P = 0.023$, $P = 0.008$, and $P = 0.000$, respectively), and they were significantly higher in the ALD group than in the non-ALD group ($P = 0.013$, $P = 0.010$, and $P = 0.000$, respectively).

The median serum BUN and Cr levels were observed among the three groups or between sets of two groups (all $P > 0.05$).

The median serum CRP levels were significantly different among the groups ($P = 0.000$); this level was significantly higher in the ALD group than in the non-ALD and control groups ($P = 0.006$ and $P = 0.000$, respectively). However, no significant differences in the median serum IL-6 or IL-10 level were observed among the three groups or between sets of two groups (all $P > 0.05$).

The median serum TNF- α levels were significantly different among the groups ($P = 0.000$); this level was significantly lower in the ALD group than in the non-ALD and control groups ($P = 0.004$ and $P = 0.000$, respectively). The median serum E-selectin and ADP levels did not significantly differ among the three groups or between sets of two groups (all $P > 0.05$).

The median serum sOX40L and HSP70 levels were significantly different among the three groups ($P = 0.010$ and $P = 0.033$, respectively); these levels were significantly lower in the ALD group than in the control group ($P = 0.008$ and $P = 0.018$, respectively). However, no significant differences in the median serum HSP60 level were observed among the three groups or between sets of two groups (all $P > 0.05$).

The correlations detected between hepatorenal function and inflammatory cytokine levels are presented in Table 2. The ALT, AST, and GGT levels were positively correlated with the CRP level ($r = 0.211$, $P = 0.002$; $r = 0.220$, $P = 0.001$ and $r = 0.295$, $P = 0.000$, respectively). Further, the GGT level was negatively correlated with the TNF- α level ($r = -0.225$, $P = 0.001$), as well as with the sOX40L and HSP70 levels ($r = -0.165$, $P = 0.016$;

Table 1 Comparisons of age, median alcohol drinking history, median daily alcohol consumption, BMI, liver function, renal function, and inflammatory cytokine levels among two or three groups (medians plus ranges)

Group	ALD	Non-ALD	Control	Chi square	P
N	135	42	37		
Age (years)	45.00 (25.00–65.00)	48.00 (35.00–61.00)	47.00 (35.00–58.00)	1.734	0.420
Median alcohol drinking history (years)	5.00 (5.00–20.00)	6.00 (5.00–27.00)		0.578	0.563
Median daily alcohol consumption (g)	56.50 (42.00–99.00)	56.00 (40.00–101.00)		0.007	0.994
BMI (kg/m ²)	27.40 *§ (21.30–35.90)	24.55 (19.50–29.50)	23.40 (20.20–28.41)	63.356	0.000
Constituent ratio of liver inflammatory status (n, %)					
Normal	54 (40)	28 (66.67)	30 (81.08)	23.949	0.000
Abnormal	81 (60)&#	14 (33.33)	7 (18.92)		
Liver function (U/L)					
ALT	31.00*§ (2.00–156.00)	27.00 (5.00–126.00)	27.00 (10.00–49.00)	9.229	0.010
AST	26.00 * § (2.00–97.00)	24.00 (12.00–78.00)	23.40 (14.00–36.00)	11.114	0.004
GGT	45.00 *§ (2.00–403.00)	26.00 (9.00–72.00)	24.00 (9.00–65.00)	54.077	0.000
Renal function					
BUN (mmol/l)	5.60 (3.70–9.70)	5.73 (4.19–8.50)	5.54 (3.60–8.90)	0.857	0.652
Cr (μmol/L)	63.00 (53.00–102.00)	64.00 (55.00–103.00)	65.20 (56.00–102.00)	4.850	0.088
Inflammatory cytokines (ng/L)					
CRP	3.48 *§ (2.26–29.94)	2.89 (2.13–8.22)	2.87 (2.18–20.58)	21.573	0.000
IL-6	17.90 (7.00–103.00)	16.70 (7.00–109.00)	22.30 (7.00–100.00)	3.285	0.193
IL-10	395.40 (10.00–2921.00)	367.50 (38.00–1998.00)	460.80 (10.00–1773.00)	0.560	0.756
TNF-α	248.50† (1.00–1774.00)	430.40 (124.00–2078.00)	646.20 (169.00–1676.00)	22.866	0.000
E-selectin	36.10 (4.00–240.00)	46.65 (16.00–265.00)	77.70 (15.00–213.00)	1.923	0.382
ADP	1441.00 (96.00–10412.00)	1591.50 (738.00–7663.00)	2042.00 (110.00–6354.00)	0.542	0.763
SOX40L	8.57 # (1.01–80.00)	11.80 (0.40–68.50)	15.90 (0.50–80.00)	9.256	0.010
HSP60	70.40 (1.70–400.00)	75.20 (29.70–400.00)	103.80 (25.70–400.00)	0.458	0.795
HSP70	48.00& (9.10–369.90)	64.80 (27.10–350.60)	93.80 (21.40–328.90)	6.795	0.033

ALD alcoholic liver disease, Non-ALD non-alcoholic liver disease, BMI body mass index, ALT alanine transaminase, AST aspartate aminotransferase, GGT gamma-glutamyltransferase, BUN blood urea nitrogen, Cr creatinine, CRP C-reactive protein, IL-6 interleukin-6, IL-10 interleukin-10, TNF-α tumor necrosis factor alpha, ADP adiponectin, SOX40L soluble OX40 ligand, HSP60 heat shock protein 60, HSP70 heat shock protein 70

BMI: *§ $P=0.000$, all (ALD group vs non-ALD group and control group)

Constituent ratio of liver inflammatory status: & $P=0.002$ and # $P=0.000$, respectively (ALD group vs non-ALD group and control group)

ALT, AST and GGT: * $P=0.023$, $P=0.008$, and $P=0.000$, respectively (ALD group vs control group)

ALT, AST and GGT: § $P=0.013$, $P=0.010$, and $P=0.000$, respectively (ALD group vs non-ALD group)

CRP: * $P=0.006$ (ALD group vs non-ALD group), and § $P=0.000$ (ALD group vs control group)

TNF-α: $P=0.004$ (ALD group vs non-ALD group), and † $P=0.000$ (ALD group vs control group)

SOX40L: # $P=0.008$ (ALD group vs control group)

HSP70: & $P=0.018$ (ALD group vs control group)

and $r=-0.178$, $P=0.009$, respectively). In addition, the Cr level was negatively correlated with the IL-10 level ($r=-0.166$, $P=0.015$).

The results of binary logistic regression analysis are shown in Table 3, and they revealed that the ALD patients had a significantly higher BMI (OR = 1.637, 95%CI: 1.374–1.951,

Table 2 Correlations between hepatorenal function and inflammatory cytokines

Comparison of two variables	Correlation coefficient	<i>P</i> value
ALT and CRP	0.211	0.002
AST and CRP	0.220	0.001
GGT and CRP	0.295	0.000
GGT and TNF- α	-0.225	0.001
GGT and SOX40L	-0.165	0.016
GGT and HSP70	-0.178	0.009
Cr and IL-10	-0.166	0.015

$P = 0.000$) and GGT level (OR = 1.039, 95%CI: 1.020–1.059, $P = 0.000$) and significantly lower TNF- α (OR = 0.998, 95%CI: 0.996–1.000, $P = 0.030$) and HSP70 levels (OR = 1.017, 95%CI: 1.003–1.031, $P = 0.029$) than the non-ALD patients. Further, the moderate-to-severe ALD patients had a significantly higher serum CRP level (OR = 1.349, 95%CI: 1.066–1.702, $P = 0.013$) and significantly lower HSP60 (OR = 0.965, 95%CI: 0.938–0.993, $P = 0.014$) and HSP70 levels (OR = 0.978, 95%CI: 0.962–0.995, $P = 0.010$) than the mild ALD patients.

Discussion

The results of this study demonstrated that the BMI and levels of biochemical indices of liver inflammation (ALT, AST, and GGT) were obviously increased in the ALD patients compared with the non-ALD and control subjects. According to the above hepatorenal function parameters, the constituent ratio of the liver inflammatory status was significantly higher in the ALD group than in the non-ALD and control groups. In addition, binary logistic regression analysis verified that the ALD patients had a significantly higher BMI and GGT level. Hence, ALD is associated with the BMI and liver functional impairment, supporting the viewpoint that this disease is related to

Table 3 Results of binary logistic regression analysis of the presence and severity of ALD

Variable	Coefficient	SE	Wald	OR (95 % CI)	<i>P</i> value
Associations between the presence of ALD and all parameters					
BMI	0.493	0.089	30.378	1.637 (1.374–1.951)	0.000
GGT	0.038	0.010	16.136	1.039 (1.020–1.059)	0.000
TNF- α	-0.002	0.001	4.682	0.998 (0.996–1.000)	0.030
HSP70	0.017	0.007	5.505	1.017 (1.003–1.031)	0.029
Associations between ALD severity and inflammatory cytokines					
CRP	0.298	0.119	6.229	1.349 (1.066–1.702)	0.013
HSP60	-0.035	0.014	6.007	0.965 (0.938–0.993)	0.014
HSP70	-0.022	0.009	6.715	0.978 (0.962–0.995)	0.010

overweight (Qu et al. 2015a, b) and obesity (Lu et al. 2004). The serum GGT level may be elevated in hepatobiliary disease, with or without elevations in the levels of other liver enzymes. Many previous studies have demonstrated that both low and moderate levels of chronic alcohol consumption are associated with an increased BMI (Conigrave et al. 2003a) and that they cause varying degrees of liver damage, with elevated ALT (Conigrave et al. 2003b), AST (Conigrave et al. 2003c), ALP (Das and Vasudevan 2005) and GGT levels (Conigrave et al. 2003d; Hietala et al. 2005; Das and Vasudevan 2005; Grasselli et al. 2014) and even moderate/severe hepatic steatosis (Conigrave et al. 2003e). However, another study has reported conflicting results, showing that heavy drinkers have a significantly lower body weight, percent body fat, and BMI compared with non-heavy drinkers (Das and Vasudevan 2005). Therefore, the influences of the severity of drinking on the body weight, percent body fat, and BMI differ, but the cause of these differences remains unclear and requires further elucidation. In addition, ALD is a potentially avoidable disease because chronic excess alcohol consumption is required for its development. In the Chinese population, the ethanol risk threshold for developing ALD is 20 g per day, and the risk increases with increased daily ethanol intake (Lu et al. 2004). ALD occurs in 80 % of unselected heavy drinkers who consume an excess of 80 g of alcohol a day (Levene and Goldin 2012). Patients with alcoholic hepatitis typically have a moderately elevated AST level, an AST:ALT ratio of two or greater, and an elevated serum bilirubin level (Stewart et al. 2007; Lucey et al. 2009). Thus, liver functional impairment, especially an elevated GGT level, may be an indicator of alcohol abuse or ALD (Sangsu et al. 2015).

With regard to cytokines, the present study revealed that the level of the pro-inflammatory cytokine CRP was markedly increased in the ALD patients compared with the non-ALD and control patients, in support of a previous study showing that CRP expression is upregulated in alcohol-induced acute liver injury (Redwine et al. 2003); however, the levels of all of the other pro-inflammatory cytokines measured, including IL-6, TNF- α , E-selectin, and ADP, as well as the anti-inflammatory cytokine IL-10, were decreased. Among these cytokines, TNF- α in particular was significantly decreased in the subjects with ALD and chronic alcohol ingestion compared to the control subjects. Notably, binary logistic regression analysis revealed this level was significantly lower in the ALD patients compared with the non-ALD patients only. Hence, a decreased level of the pro-inflammatory cytokine TNF- α may be an indicator of ALD; however, this conclusion is not completely consistent with those of previous studies. In support of our findings, one previous study has demonstrated that cytokines released from immune cells control immune processes by activating lymphocytes and phagocytes, coordinating the cell-mediated and humoral immune responses, mediating the process of inflammation, and directly killing cells (Molina et al.

2010b). In addition, both acute and chronic alcohol abuse have been shown to result in significant abnormalities in the body's defense against pathogenic microorganisms by interfering with multiple aspects of the immune response. Acute alcohol exposure generally suppresses cytokine activities (Pruett et al. 2004). Chronic alcohol ingestion may decrease the frequency of lymphocytes and increase the risks of both bacterial and viral infections (Barr et al. 2016), as well as the number of dendritic cells (Laso et al. 2007; Siggins et al. 2009a), and interfere with their differentiation and functioning (Szabo et al. 2004). In addition, chronic alcohol ingestion may interfere with TNF- α expression by inhibiting the normal processing of newly produced TNF that is necessary for its normal functioning (Zhao et al. 2003). Ethanol exposure may impact each level of the molecular regulation of TNF- α expression in macrophages, resulting in enhanced liver inflammation (Nagy 2003). Further, alcoholic patients have a lower IL-6 level, IL-6/IL-10 ratio, and natural killer (NK) cell activity (Gupta et al. 1995). Other studies have presented opposing viewpoints that chronic alcohol ingestion may enhance the expression of inflammation-promoting (i.e., pro-inflammatory) cytokines (Mandrekar et al. 2009), particularly TNF (Nagy 2004). Inflammation is characterized by the activation of innate immune cells and production of the pro-inflammatory cytokines IL-1 α , IL-1 β , and TNF- α (Szabo et al. 2012). The mechanism of inflammation resulting from chronic alcohol ingestion may involve disruption of the intestinal epithelial tight junction complex (Thomes et al. 2015b), which increases intestinal permeability and decreases fecal bifidobacteria and lactobacilli levels (Chiu et al. 2015a, b). Additionally, alcohol intake increases gut permeability (Dogru et al. 2013) and strongly upregulates the serum TNF- α (Siggins et al. 2009), IL-6 (Siggins et al. 2009c), and E-selectin levels (Bertola et al. 2013). These changes permit the paracellular translocation of gut-derived pathogenic molecules to the liver, where they cause inflammation and progressive liver injury (Thomes et al. 2015). Further, chronic ethanol feeding sensitizes Kupffer cells to activation by lipopolysaccharides (LPS), leading to increased production of reactive oxygen species (ROS) and TNF- α (Park et al. 2006a). Notably, chronic alcoholics have increased IL-13 and IL-10 levels but a decreased IL-4 level (González-Reimers et al. 2012). IL-10 mediates anti-inflammatory activity via the induction of IL-10-inducible genes, including heme oxygenase-1 (HO-1), which is an important downstream mediator of the anti-inflammatory effects of IL-10 in macrophages (O'Shea and Murray 2008). In addition, chronic alcoholic patients undergoing cardiac and gastric surgery have a higher level of the anti-inflammatory cytokine IL-10, as well as a lower ratio of pro-inflammatory IL-6 to anti-inflammatory IL-10. These changes are associated with a marked increase in the infection rate after surgery (Sander et al. 2002). Few studies have examined the association between ALD and inflammation; thus, the factors underlying this association remain unclear. Enhanced liver inflammation due to

ethanol exposure is an important contributor to liver injury (Molina et al. 2010b). Chronic alcoholism complicated by ALD is characterized by activation of the inflammatory response (Park et al. 2006b; El-Taouky et al. 2006), mediated in part by the activation of Kupffer cells (Park et al. 2006c). Moreover, the progression of ALD is thought to involve a hepato-protective effect of ADP (Park et al. 2006d). Hence, further study is needed to elucidate the mechanisms underlying the inflammatory changes that occur in chronic alcoholism.

The relationship between ALD and the inflammatory immune response remains controversial. Innate immune activation has been postulated to be a central mechanism of disease progression from hepatic steatosis to steatohepatitis in obesity-related fatty liver disease (Navarro et al. 2015). In addition, emerging evidence suggests that innate immunity drives the development of ALD and that interferon regulatory factor 3 (IRF3), a transcription factor that regulates innate immune responses, is indispensable for the development of this disease (Petrasek et al. 2013). TLRs are key molecules of the innate immune system that play a major role in mediating inflammatory processes by promoting the production of several circulating inflammatory molecules, including cytokines, chemokines, and other molecules that participate in tissue repair or exacerbate tissue damage in several diseases (Lin et al. 2011). Patients with stable, chronic ALD exhibit attenuation of the TLR2-mediated innate immune responses in peripheral blood monocytes (Pimentel-Nunes et al. 2010). sOX40L is a key component involved in the generation of adaptive memory responses, and it provides the necessary co-stimulatory signals for activation of effector T cells (Laustsen et al. 2014). In addition, a positive relationship of sOX40L with CRP and a negative relationship with IL-10 have been associated with atherosclerosis (Peng et al. 2010). HSPs have been reported to play important roles in the activation and maturation of dendritic cells (Tsan and Gao 2009). Increased HSP60 expression occurs during chronic active hepatitis, suggesting that immune reactions to HSP60 may play roles in the immunopathogenesis and perpetuation of chronic inflammatory liver disease (Lohse et al. 1993). Our previous study has shown that the median serum HSP70 level in patients with moderate-to-severe ALD is significantly lower than that in patients with mild ALD or in individuals without ALD who consume alcohol (QU et al. 2015). Our present study demonstrated that the levels of inflammatory immune response markers, including sOX40L, HSP60, and HSP70, were all decreased in the ALD patients compared to the control subjects; furthermore, the median serum sOX40L and HSP70 levels were significantly lower in the subjects with ALD than in those with chronic alcohol ingestion and in the controls. Binary logistic regression analysis revealed that the ALD patients had a significantly lower HSP70 level than the non-ALD patients; in addition, the moderate-to-severe ALD patients had a significantly higher serum CRP level and significantly lower serum HSP60 and HSP70 levels than the

mild ALD patients, suggesting that the inflammatory immune response is downregulated in ALD. Downregulation of inflammatory immune responses occurs with the aggravation of ALD. Presently, the literature lacks information on the relationships between liver and kidney function and inflammatory cytokines. Our study revealed that median ALT, AST, and GGT levels were positively correlated with the CRP level and that the GGT level was negatively correlated with median TNF- α , sOX40L, and HSP70 levels. In addition, the median Cr level was negatively correlated with median IL-10 level. Hence, the relationships between liver and kidney function and inflammatory cytokines need to be further investigated.

In conclusion, ALD patients may present with obesity, liver damage, and an abnormal inflammatory immune response, mainly manifesting as decreased levels of immune inflammatory cytokines. In addition, certain liver and kidney function parameters and ALD severity were either positively or negatively correlated with the levels of certain inflammatory cytokines; hence, ALD patients may be at increased risks of obesity- and inflammation-related diseases. Accordingly, to control the inflammatory response, preventative measures for patients with this disease should include weight control and protection of liver and kidney function.

Our study has certain limitations. First, the study sample was small and biased by a disproportionate number of male subjects. Second, confounding factors, such as drug use and other risk factors, were not considered. Third, this study did not distinguish the effects of the variety or quantity of drinking on the levels of the inflammatory markers in the ALD patients.

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Compliance with Ethical Standards

Conflict of Interest The authors have no conflicts of interest to disclose.

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