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Advanced oxidation protein products and inflammatory markers in liver cirrhosis: a comparison between alcohol-related and HCV-related cirrhosis

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Advanced oxidation protein products (AOPPs) are protein markers of oxidative stress with pro-inflammatory properties that accumulated in liver cirrhosis. In the present study, we investigated the association between chronic inflammatory response triggered by AOPPs and the severity of liver disease as assessed by the Child-Pugh score. Plasma concentrations of AOPPs and inflammatory markers such as C-reactive protein, tumor necrosis factor-α, and interleukin-6 were measured in 41 patients with HCV-related cirrhosis, 43 patients with alcohol-related liver cirrhosis (ALC), and in 30 age and sex matched controls. In comparison with controls, AOPPs were increased in HCV-related compensated (Child-Pugh A) and decompensated (Child-Pugh B-C) cirrhosis and in alcohol-related compensated cirrhosis. AOPPs level positively correlated with Child-Pugh score in alcohol-related cirrhosis but not in HCV-related cirrhosis and the correlation with the indices of chronic inflammation was stronger in ALC. In turn, AOPPs in HCV-related cirrhosis was related to inflammation to a lesser extent, but a significant correlation with antioxidant defense could be noted. In summary, liver cirrhosis was associated with increased formation of AOPPs, which differed between alcohol-related and HCV-related cirrhosis with respect to the relationship between AOPPs and antioxidant defense, stage of liver cirrhosis, and inflammatory response. The significant correlation between AOPPs accumulation and indices of chronic inflammation, more specifically TNF-α, suggests that oxidative stress may be a mediator of chronic inflammatory state in the early stage of alcohol-related cirrhosis.

Keywords: advanced oxidation protein products, inflammatory markers, cirrhosis

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INTRODUCTION

Accumulating evidence suggests that liver cirrhosis is associated with chronic low-grade inflammation. Cirrhosis is characterized by inflammation of the liver, often caused by a rise in free radicals within the liver. Under normal circumstances, the liver maintains a supply of internal antioxidants to neutralize the free radicals generated by viruses and various endo- and exogenous compounds processed in the liver. However, when the liver is overwhelmed by continued oxidative insults (e.g., long-lasting alcohol abuse or infection with hepatitis C virus), the damage from free radicals increases, resulting in inflammation and the formation of scar tissue (fibrosis) (Czeczot *et al.*, 2006; Valko *et al.*, 2007). The persistence of profibrogenic factors, the progressive decrease of antioxidant reserves and the dysfunction of liver microcirculation determine the shift to liver regeneration and cirrhosis.

The contribution of oxidative stress *per se* to the chronic inflammatory state has been suggested, and consistent evidence has been afforded that both macrophage activation and a defect in antioxidant systems occur early in the course of chronic liver failure and gradually increase with its progression to end-stage liver disease (Kirkham, 2007; Videla, 2009).

Oxidative stress lead to formation of glycoxidation products, including advanced glycation end products (AGEs — among them *N*ε-(carboxymethyl)lysine (CML) is best known), and advanced oxidation protein products (AOPPs). Plasma concentrations of AGEs (closely correlating with AOPPs levels) increase with progression of chronic diseases (Witko-Sarsat *et al.*, 1996; 1998), therefore CML has been considered as a liver disease-related biomarker for oxidative stress (Sebekova *et al.*, 2002; Yagmur *et al.*, 2006).

The receptor for advanced glycation end products (RAGE) is a signal transduction receptor that binds both AGEs and AOPPs. RAGE is expressed by hepatic stellate cells and myofibroblasts, which are the relevant cells for fibrogenesis of chronic liver disease. Both AGEs and AOPPs trigger the inflammatory response *via* interaction with RAGE and by causing activation of nuclear factor NF-κB (reviewed in Hyogo & Yamagishi, 2008). *In vitro* experiments have shown that AGEs enhance transcription of genes for pro-inflammatory cytokines (e.g., interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α)) (Ekong *et al.*, 2006), and other studies have shown that they may increase C-reactive protein (CRP) production (Schwedler *et al.*, 2001).

Since advanced oxidation protein products are not only a markers of oxidative stress but also act as inflammatory mediators (Alderman *et al.*, 2002; Witko-Sarsat *et al.*, 2003; Yazici *et al.*, 2004; Baskol *et al.*, 2006; Fialova *et al.*, 2006) the knowledge of AOPPs pathophysiology

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Abbreviations: AGEs, advanced glycation end products; AOPPs, advanced oxidation protein products; CML, *N*ε-(carboxymethyl) lysine; CRP, C-reactive protein; HCV, hepatitis C virus; IL-6, interleukin-6; RAGE, receptor for advanced glycation end products; TAS, total antioxidant status; TNF-α, tumor necrosis factor-α; WBC, white blood cells

in chronic liver disease could provide valuable information with respect to the relationship between oxidative stress and the inflammatory response related to liver cirrhosis. The present study was performed to determine whether plasma concentration of AOPPs is an important determinant of circulating inflammatory markers such as CRP, TNF-α, and IL-6 in both alcohol-related and HCV-related cirrhosis.

MATERIALS AND METHODS

Patients. This study was performed in 84 patients with liver cirrhosis admitted to the Clinic of Infectious Diseases, Liver Diseases and Acquired Immune Deficiency for evaluation. Fifty-three were male and 31 female, and they were aged 29–76 years (median: 57). The control group was 30 healthy subjects (13 women, 17 men) aged 19–56 (median 55). Blood samples were collected in the Silesian Centre of Medical Diagnostics (Wroclaw, Poland).

The diagnosis of liver cirrhosis was based on clinical, laboratory and ultrasonographic findings or histological criteria. The etiology of cirrhosis in 43 cases was alcohol abuse, while chronic hepatitis C virus (HCV) infection was the cause in 41 cases. Clinical and biochemical characteristics of the study group, divided according to etiology, are reported in detail in Table 1. The Child-Pugh score was used to assess the severity of liver disease. Three biochemical variables (serum albumin, bilirubin, and prothrombin time (international normalized ratio, INR)) in addition to the two clinical characteristics (presence or absence of ascites and clinical signs of encephalopathy) determine the Child-Pugh score. Patients were scored as follows: 5–6 as class (group) A, 7–9 as class (group) B and 10–15 as class (group) C. The patients with cirrhosis were divided into compensated (Child-Pugh class A) and decompensated (Child-Pugh classes B and C) groups. At the time of the study no Child-Pugh A patients showed clinical features of decompensated liver cirrhosis (ascites or hepatic encephalopathy). The reasons for hospitalization were similar in the two groups of decompensated cirrhotic patients. In particular, 6 of 43 (14.0%) patients with alcohol-related cirrhosis (ALC) were hospitalized for encephalopathy and 8 of 43 (18.6%) for ascites. Eight (19.5%) of the 41 patients with HCV-related cirrhosis were hospitalized for encephalopathy and 15 of 41 (36.6%) for ascites (Table 1).

Exclusion criteria were concurrent use of antioxidant drugs; co-existing diseases like diabetes mellitus, chronic kidney disease, cardiovascular disease, hepatocellular carcinoma; concomitant chronic hepatitis B; alcohol use within previous two weeks; gastrointestinal bleeding or blood transfusion within previous two weeks.

The consent of the Bioethics Committee of the Wroclaw Medical University was obtained and all patients were informed about the character of analyses made. Studies were conducted in compliance with the ethical standards formulated in the Helsinki Declaration of 1975 (revised in 1983). Peripheral venous blood from fasted healthy subjects and fasted cirrhotic patients was collected in separate tubes, one containing the anticoagulant EDTA and the other without an anticoagulant. The blood was allowed to clot for 30 min at 25°C, centrifuged at 2000×*g* for 15 min at room temperature, and the serum was then separated and aliquoted into tubes for storage. To obtain plasma samples, the blood was centrifuged immediately at 2000×*g* for 10 min at 4°C, and then aliquoted into tubes. The tubes were then stored frozen at –80°C until used.

Determination of circulating AOPPs. Determination of AOPPs was based on spectrophotometric detection according to Witko-Sarsat *et al.* (1996). Two-hundred microliters of plasma diluted 1:5 in 20 mM phosphate buffer, pH 7.4 containing 0.9% sodium chloride (PBS), or chloramine-T standard solutions (0 to 100 μmol/L), were placed in each well of a 96-well microtiter plate (Becton Dickinson Labware, Lincoln Park, NJ, USA), followed by 20 μl of 10% acetic acid. Ten microliters of 1.16 M potassium iodide (Sigma) were then added, followed by 20 μl of 10% acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm in a microplate reader against a blank containing 200 μl of PBS, 10 μl of KI and 20 μl of 10% acetic acid. The chloramine-T absorbance at 340 nm was linear within the range of 0 to 100 μmol/L. The ratio of AOPPs concentration to albumin level (AOPPs/Alb) was expressed in micromoles of AOPPs per gram of albumin (μmol/g). The ratio of AOPPs to albumin content allows the evaluation of whether the proportion of oxidatively modified albumin is altered. Coefficient of variation (CV) served as an indicator of precision. Intra-day and inter-day CV values were < 10%.

Determination of circulating *N*ε-(carboxymethyl) lysine. Plasma *N*ε-(carboxymethyl)lysine (CML) levels were determined using a specific competitive ELISA kit (CircuLex CML/Nε-(carboxymethyl)lysine ELISA Kit (CycLex Co., Ltd, Nagano, Japan)). Measurements were performed in duplicate and the results were averaged. The ratio of CML concentration to albumin level (CML/Alb) was expressed in micrograms of CML per gram of albumin (μg/g).

Biochemical analysis and measurements of cytokines. Biochemical parameters were measured using routine laboratory methods. Serum C-reactive protein (CRP) level was determined with a high-sensitivity nephelometric method using the Beckman Image Immunochemistry system (Beckman Instruments, Fullerton, CA, USA), which has a minimum level of detection of 0.2 mg/L. Serum levels of TNF-α and IL-6 were assayed with enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. The minimum levels of detection were 0.038 pg/ml and <0.104 pg/ml for TNF-α and IL-6, respectively. The intra- and interassay coefficients of variation for measurements of CRP, IL-6, and TNF-α were 2.7%, 4.3%, and 5.0%, respectively, and 3.0%, 5.5, and 6.9%, respectively.

Measurement of the total antioxidant status of plasma. The plasma antioxidant capacity was measured using a commercially available total antioxidant status TAS kit (Randox Laboratories, Crumlin, UK). The TAS assay is based on the inhibition by antioxidants of the absorbance of the radical cation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) formed by the interaction of ABTS with ferrylmyoglobin radical species. Upon the addition of a plasma sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction mix are suppressed by the antioxidant components of the plasma, preventing the color change and thereby providing an effective measure of the total antioxidant capacity of the plasma. The assay has excellent precision values, lower than 3%, and the results are expressed as mmol/L.

Statistical analysis. Comparison of the parameters between two different groups was conducted with the

Table 1. Clinical and biochemical characteristics of study group

		Alcohol-related cirrhosis (n=43)	HCV-related cirrhosis (n=41)	*P*-value
Sex	Male:Female	27:16	26:15	
Age (years)	Median Range	56 32-76	58 29-74	
Child-Pugh class A B C	% 	 67.4 (29 of 43) 11.6 (5 of 43) 20.9 (9 of 43)	 43.9 (18 of 41) 29.3 (12 of 41) 26.8 (11 of 41)	
Ascites	%	18.6 (8 of 43)	36.6 (15 of 41)	
Encephalopathy	%	14.0 (6 of 43)	19.5 (8 of 41)	
Albumin (g/L)	Mean ± S.D.	28 ± 1.10	35 ± 1.0	n.s.
Total bilirubin (mg/dL)	Mean ± S.D.	5.1 ± 0.92	4.6 ± 0.81	n.s.
Creatinine (mg/dL)	Mean ± S.D.	1.14 ± 0.84	1.22 ± 0.68	n.s.
ALT (U/L)	Mean ± S.D.	34.1 ± 4.80	49.0 ± 12.50	n.s.
AST (U/L)	Mean ± S.D.	58 ± 31	82 ± 12	n.s.
Total cholesterol (g/L)	Mean ± S.D.	1.65 ± 0.12	1.40 ± 0.10	< 0.05
PLT ($\times 10^9$/L)	Mean ± S.D.	99.2 ± 11.80	84 ± 10.20	n.s.
WBC ($\times 10^9$/L)	Mean ± S.D.	5.40 ± 0.49	4.36 ± 0.37	< 0.05

n.s., not significant; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PLT, platelets; WBC, white blood cells

Mann–Whitney U test and the Kruskal–Wallis analysis of variances (ANOVA) for multiple comparisons. Coefficient correlations were evaluated using linear regression analysis or Spearman correlation. Values were expressed as means ± S.D. Statistical significance was established at $P < 0.05$.

RESULTS

Baseline characteristics of the study group

Clinical and biochemical characteristics of the study group are presented in Table 1. Total cholesterol levels and white blood cells (WBC) counts were significantly higher in patients with alcohol-related cirrhosis (ALC) than in HCV-related cirrhosis ($P < 0.05$). There was no significant difference between the two groups regarding the other clinical and biochemical parameters.

AOPPs as markers of oxidative stress in alcohol-related and HCV-related cirrhosis

We examined plasma AOPPs as markers of oxidant-mediated protein damage in both alcohol-related and HCV-related liver cirrhosis. We found higher levels of AOPPs in patients with HCV-related cirrhosis, both compensated (Child-Pugh A) and decompensated one

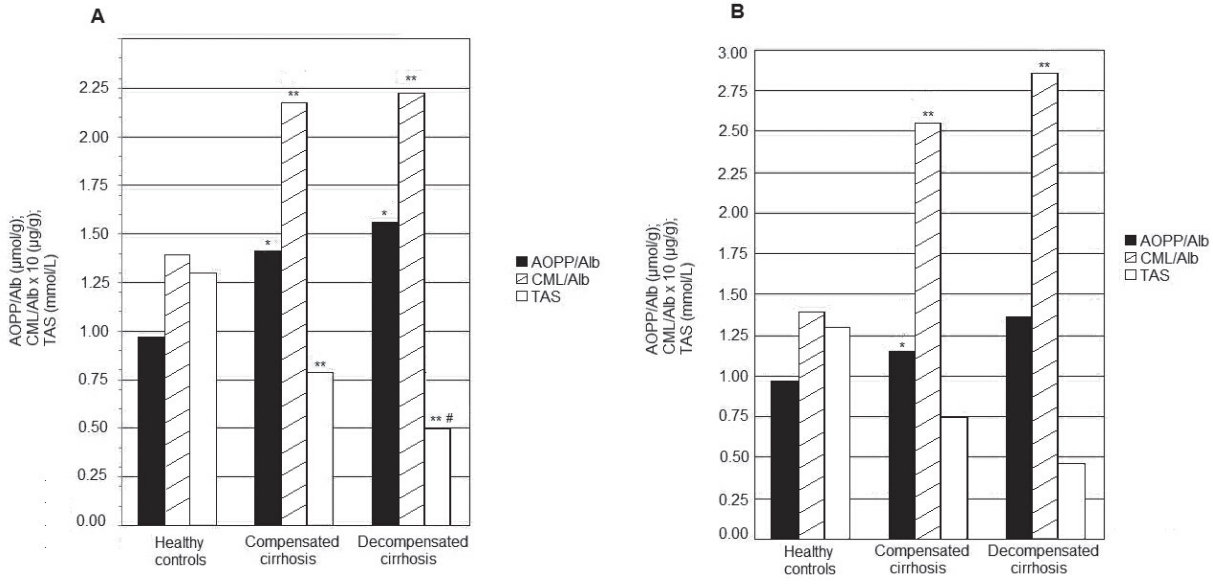

Figure 1. Serum concentrations of AOPPs, CML and TAS in patients with HCV-related (A) or alcoholic cirrhosis (B) with respect to the severity of liver cirrhosis in comparison with healthy subjects
Significance between groups: *$P < 0.05$; **$P < 0.01$ *vs*. healthy controls; #$P < 0.05$ *vs*. compensated cirrhosis.

Table 2. Plasma concentrations of AOPPs and inflammatory markers in healthy controls and in patients with HCV-related liver cirrhosis

	Healthy controls	Compensated cirrhosis	Decompensated cirrhosis
(n)	30	18	23
AOPPs/Alb (μmol/g)			
Mean ± S.D.	0.97 ± 0.77	1.41 ± 0.69*	1.56 ± 0.80*
Albumin (g/L)			
Mean ± S.D.	46 ± 10.0	35 ± 2.3*	27.82 ± 2.0*
TNF-α (pg/mL)			
Mean ± S.D.	33.11 ± 2.06	40.0 ± 2.3*	42.4 ± 4.80*
IL-6 (pg/mL)			
Mean ± S.D.	6.99 ± 0.98	7.41 ± 1.04	10.25 ± 1.25
CRP (mg/L)			
Mean ± S.D.	1.27 ± 0.64	5.96 ± 1.05*	6.80 ± 1.20*+
WBC (×10⁹/L)			
Mean ± S.D.	4.6 ± 0.40	4.85 ± 3.75	5.40 ± 2.4*+
Child-Pugh score			
Mean ± S.D.	5–6	5.6 ± 1.86*	8.7 ± 3.62*++

Note: All patients with compensated cirrhosis were Child-Pugh's A classification, whereas those with decompensated cirrhosis were Child-Pugh's B and C. Significance levels between groups: *$P<0.05$; **$P<0.01$ *vs.* healthy controls; +$P<0.05$; ++$P<0.001$ *vs.* compensated cirrhosis. Alb, albumin; AOPPs, advanced oxidation protein products; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; WBC, white blood cells.

(Child-Pugh B–C), than in healthy subjects (Fig. 1A). In turn, for alcohol-related cirrhosis AOPPs formation was significantly increased only in the compensated cirrhosis ($P<0.05$) (Fig. 1B). There was no difference in AOPPs level between alcohol-related and HCV-related cirrhosis when comparing compensated ($P=0.24$) or decompensated cirrhosis ($P=0.34$).

We compared the AOPPs level in patients with compensated cirrhosis with that in those with decompensated cirrhosis. Neither in HCV-related cirrhosis (Fig. 1A) nor in ALC (Fig. 1B) did AOPPs level significantly differ between groups. As for patients with alcohol-related decompensated cirrhosis, the lack of statistical significance may have resulted from the small number of subjects included in this group. However, plasma AOPPs levels in alcohol-related cirrhosis were increased, respectively, to the disease severity. This was demonstrated by a significant positive correlation with the Child-Pugh score ($r=0.39$, $P<0.01$). No correlation between AOPPs levels and the Child-Pugh score could be found in HCV-related cirrhosis ($r=0.19$, $P=0.48$).

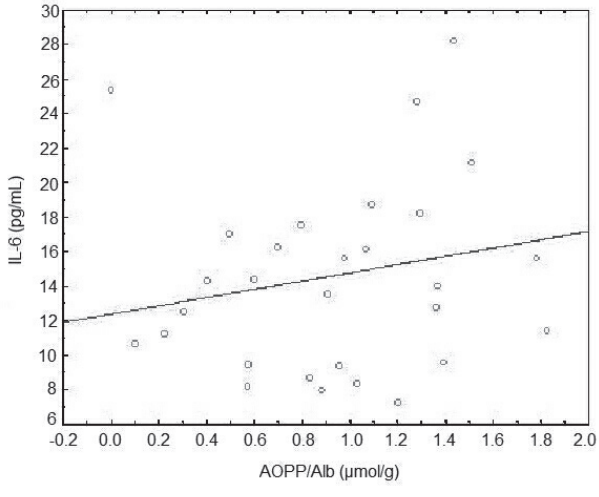

Figure 2. Correlation between AOPPs and IL-6 in alcoholic cirrhotic patients; $r=0.42$, $P<0.05$

The levels of plasma *Nε*-(carboxymethyl) lysine (CML) in all liver cirrhotic patients were higher than those of the controls and this difference was statistically significant ($P<0.01$) (Fig. 1). The levels of plasma CML in alcohol-related decompensated cirrhosis were higher than those in compensated cirrhosis, but this difference was not statistically significant (Fig. 1B). There was no statistically significant correlation between CML levels and the Child-Pugh score or AOPPs level in alcohol-related or HCV-related cirrhosis.

There was a markedly decreased total antioxidant status (TAS) in patients with HCV-related decompensated cirrhosis compared to those with compensated cirrhosis or controls ($P<0.05$, $P<0.01$, respectively) (Fig. 1A). A significant negative correlation between AOPPs levels and TAS ($r=-0.29$, $P<0.05$) was observed among the HCV cirrhotic patients belonging to all three Child-Pugh classes.

AOPPs and chronic inflammatory state in cirrhotic patients

We assessed the levels of several inflammatory markers and their association with the levels of AOPPs. Serum C-reactive protein (CRP) levels and white blood cells (WBC) counts were significantly elevated in alcoholic (Table 3) and HCV (Table 2) cirrhotic patients. Serum TNF-α levels were higher in the alcohol-related decompensated cirrhosis than in the compensated cirrhosis ($P<0.01$) (Table 3). Moreover, TNF-α concentrations were positively correlated with Child-Pugh score in ALC group ($r=0.28$, $P<0.05$). TNF-α levels were significantly increased in HCV cirrhotic patients as compared to controls ($P<0.05$), but no correlation was observed between AOPPs and TNF-α ($r=0.18$, $P=0.26$). The levels of serum Il-6 in the ALC group were higher than those of the control group and this difference was statistically significant ($P<0.05$) (Table 3). The levels of serum IL-6 in patients with alcohol-related decompensated cirrhosis were higher than those in compensated cirrhosis, but this difference was not statistically significant. The patients with HCV-related cirrhosis showed high circulating levels of IL-6 but the mean of this group was not significantly greater than for the control group (9.01 ± 1.18 pg/mL *vs.* 6.99 ± 0.98 pg/mL) (Table 2).

The association study revealed only a tendency toward a weak correlation between AOPPs and WBC in alcohol-related cirrhosis ($r=0.25$, $P<0.05$). In turn, a moderate positive correlation between AOPPs levels and CRP was observed among the alcohol-related

Table 3. Plasma concentrations of AOPPs and inflammatory markers in healthy controls and in patients with alcohol-related liver cirrhosis

	Healthy controls	Compensated cirrhosis	Decompensated cirrhosis
(n)	30	29	14
AOPPs/Alb (μmol/g)			
Mean ± S.D.	0.97 ± 0.77	1.15 ± 0.38*	1.36 ± 0.80
Albumin (g/L)			
Mean ± S.D.	46 ± 10.0	30.2 ± 2.10*	24.5 ± 1.9*+
TNF-α (pg/mL)			
Mean ± S.D.	33.11 ± 2.06	41.06 ± 4.57*	53.5 ± 4.8*+
IL-6 (pg/mL)			
Mean ± S.D.	6.99 ± 0.98	20.07 ± 13.90*	24.9 ± 15.0*
CRP (mg/L)			
Mean ± S.D.	1.27 ± 0.64	6.57 ± 1.10*	8.50 ± 1.65*+
WBC ($\times 10^9$/L)			
Mean ± S.D.	4.6 ± 0.40	5.14 ± 2.38*	5.43 ± 2.76**+
Child-Pugh score			
Mean ± S.D.	5–6	6.9 ± 2.25*	16.8 ± 7.04*++

Note: All patients with compensated cirrhosis were Child-Pugh's A classification, whereas in those with decompensated cirrhosis were Child-Pugh's B and C. Significance levels between groups: *$P < 0.05$; **$P < 0.01$ *vs.* healthy controls; +$P < 0.05$, ++$P < 0.001$ *vs.* compensated cirrhosis. Alb, albumin; AOPPs, advanced oxidation protein products; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; WBC, white blood cells.

cirrhosis patients belonging to all three Child-Pugh classes ($r = 0.37$, $P < 0.05$). The similar relationship was observed in respect to HCV-related cirrhosis ($r = 0.33$, $P < 0.05$). There was no statistically significant correlation between CML level and CRP or cytokines levels in alcohol-related or HCV-related liver cirrhosis (not shown). A significant positive correlation was found between AOPPs levels and IL-6 levels in patients with ALC ($r = 0.42$, $P < 0.05$) (Fig. 2), but not in HCV cirrhotic patients ($r = 0.29$, $P = 0.31$). As expected, a positive correlation was observed with TNF-α in alcohol-related compensated cirrhosis ($r = 0.48$, $P < 0.05$) (Fig. 3).

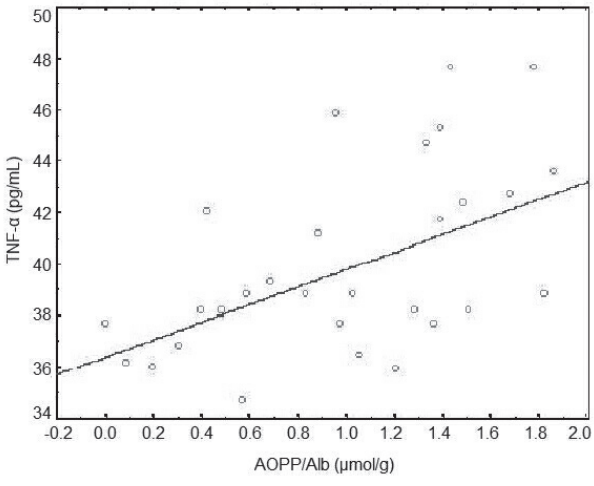

Figure 3. Correlation between AOPPs and TNF-α in compensated alcoholic cirrhotic patients; $r = 0.48$, $P < 0.05$.

DISCUSSION

The present study provides several lines of evidence to suggest that advanced oxidation protein products that we previously biochemically characterized in plasma from cirrhotic patients (Zuwała-Jagiełło *et al.*, 2009) act as mediators of the chronic inflammatory state associated with alcohol-related compensated cirrhosis.

First, plasma levels of AOPPs are elevated in both HCV-related cirrhosis and alcohol-related cirrhosis compared with those in controls. Some differences in AOPPs concentration could be observed, however, between alcohol-related and HCV-related cirrhosis. Second, and more importantly, AOPPs level positively correlated with Child-Pugh score in alcohol-related but not in HCV-related cirrhosis and the correlation with some inflammatory markers was stronger in alcoholic cirrhosis. In turn, AOPPs in HCV-related cirrhosis were related to inflammation to a lesser extent, but a significant correlation with the antioxidant defense state could be noted.

The present finding that AOPPs accumulation coexists with decreased TAS, while the plasma concentration of *Nε*-(carboxymethyl)lysine-modified advanced glycation end products remains stable, supports the contention that AOPPs are more accurate markers of oxidative stress than glycoxidation products in HCV-related cirrhosis. Chronic HCV infection of the liver leads to hepatocellular necrosis, inflammation and liver regeneration, all of which are associated with the infiltration of immune cells that produce reactive oxygen species (Murakami *et al.*, 1998; Okuda *et al.*, 2002; Korenaga *et al.*, 2005). An increase in free radical formation, manifested by increased hepatic and serum levels of lipid peroxidation products (Farinati *et al.*, 1995; De Maria *et al.*, 1996), advanced glycation end products (Sebekova *et al.*, 2002; Yagmur *et al.*, 2006; Zuwała-Jagiełło *et al.*, 2006; Górka *et al.*, 2008) and as well as decreased antioxidant levels (Jain *et al.*, 2002) have been reported in patients with HCV infection. Finally, our previous study found that the patients with cirrhosis were exposed to oxidative stress and the level of AOPPs was significantly related to the severity of liver cirrhosis of various etiologies (Zuwała-Jagiełło *et al.,* 2009).

Functionally, CRP provides a downstream integration of overall cytokine activation. Unlike upstream cytokines, CRP has a long half-life, affording stability of its level with no observable circadian variation, and has proved to be a very useful marker of inflammation in clinical studies (Pepys & Berger, 2001; Meier-Ewert *et al.*, 2001). Experiments on cell cultures showed that CRP itself participates in the inflammatory response. For example, CRP is capable of stimulating IL-6 and TNF-α production by monocytes (Ballou & Lozanski, 1992) and reactive oxygen species formation (Wang *et al.*, 2003). Advanced oxidation protein products, as pro-inflammatory factors, accumulated in diabetic (Kalousova *et al.*, 2002; Martin-Gallan *et al.*, 2003) and chronic renal failure patients, and played an important role in the occurrence and progression of complications such as dysfunction of

endothelial cells (Witko-Sarsat *et al.*, 1998) and accelerated atherosclerosis (Woods & Davies, 2003; Marsche *et al.*, 2009). Our results show that the enhanced formation of AOPPs was dependent on the inflammation status because in cirrhotic patients with normal CRP levels, AOPPs concentration was not significantly elevated in comparison with healthy subjects (not shown). Of note, higher levels of CRP and AOPPs together with lower concentrations of albumin, the main substrate in AOPPs formation, in alcohol-related cirrhosis could be also observed in the examined all cirrhotic patients. CRP is primarily produced by hepatocytes, and its chief inductor is the pro-inflammatory cytokine IL-6 (Black *et al.*, 2004). Furthermore, results from a recent study demonstrate that glycosylated and oxidized proteins indirectly up-regulate CRP expression in hepatocytes by stimulating monocytes to produce IL-6 (Li *et al.*, 2007). It seem, based on our study, even though there was functional loss of hepatocytes in patients with hepatic cirrhosis, the serum CRP level was a still maintained in high level and dependent of AOPPs level with its significant correlation. The remaining viable hepatocytes may still contribute to this result. Cytokines (IL-6 and TNF-α) have an important role not only in stimulating the CRP production but also in inhibiting albumin synthesis in the liver (Black *et al.*, 2004). The albumin synthesis in liver cirrhosis is influenced by many factors, such as change in colloid osmotic pressure and nutritional states (Romiti *et al.*, 1990; Caregaro *et al.*, 1996; Saitoh *et al.*, 1999). Finally, the significant correlation between the levels of AOPPs and IL-6, a potent procachectic cytokine (Strassmann *et al.*, 1992; Plauth & Schutz, 2002), supports the existence of a link between AOPPs and malnutrition in patients with alcohol-related cirrhosis. Nevertheless, additional studies based on other markers of malnutrition are required.

Witko-Sarsat *et al.* (1996) favor the opinion that lipids are not necessary but may enhance the *in vivo* process of AOPPs formation. Taking into account the fact that lipid peroxidation is an important factor in the development of cirrhosis (Paradis *et al.*, 1997), it is highly likely that AOPPs and lipid peroxides act in concert in this process. Very recently, advanced glycation end products (AGEs) have been found to act as pro-inflammatory factors (Sparvero *et al.*, 2009). Nevertheless, AOPPs are believed to be more closely related to inflammation (Alderman *et al.*, 2002; Witko-Sarsat *et al.*, 2003; Yazici *et al.*, 2004; Baskol *et al.*, 2006; Fialova *et al.*, 2006) than AGEs, whose receptor for advanced glycation end products (RAGE) participates in AOPPs-mediated signal transduction (Kalousová *et al.*, 2005). These interactions enhance reactive oxygen species formation, with activation of nuclear factor NF-κB and release of pro-inflammatory cytokines (Saito & Ishii, 2004; Bierhaus *et al.*, 2006; Hyogo & Yamagishi, 2008). Moreover, the macrophage RAGE can be up-regulated by TNF-α (Vlassara *et al.*, 1989; Miyata *et al.*, 1994). Increased TNF-α production in alcohol-related liver cirrhosis has been observed (Tilg *et al.*, 1992). Peripheral blood monocytes showed activity and elevated expression of TNF-α which correlated with liver disease severity (Hanck *et al.*, 2000). The concentrations of advanced oxidation protein products are high in alcohol-related compensated cirrhosis and can reflect hemodynamic alterations in the liver (Guo *et al.*, 2008). This is accompanied by the activation of macrophages and increased expression of TNF-α (Girón-González *et al.*, 2004). Moreover, it has been shown that TNF-α is involved in the progression from alcoholic liver disease to alcohol-related cirrhosis, since it promotes activation of stellate cells and matrix-gene expression (Brenner *et al.*, 1989). However, additional factors must also be present. It could therefore be consistent with observed increase levels of both AOPPs and TNF-α at an early stage of alcohol-related cirrhosis.

In conclusion, results from this study show that elevated AOPPs levels are common in both ALC and HCV-related cirrhosis and are related to the severity of alcohol-related cirrhosis. The present results provide new evidence for an association between plasma levels of AOPPs and indices of chronic inflammation, more specifically TNF-α, in patients with early-stage alcohol-related cirrhosis. Further investigation will be needed to elucidate the mechanisms underlying the regulation of advanced oxidation protein products, TNF-α and IL-6 in normal subjects and in patients suffering from alcoholic cirrhosis-related complications.

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