



Original article

Association between antioxidants and mild acute pancreatitis

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ABSTRACT

Background and study aims: The mechanisms underlying acute pancreatitis have not been well elucidated. Over the last 20 years, there has been increasing awareness regarding the role played by oxidative stress in acute pancreatitis, but it is less well defined in human clinical trials. The aim of this study was to identify the relationship between antioxidants and acute pancreatitis.

Patients and methods: We performed a cross-sectional trial on patients with mild acute pancreatitis. The study population consisted of 53 patients with mild acute pancreatitis and 55 healthy controls. Serum paraoxonase, arylesterase activity, total antioxidant status, total oxidant status and thiol levels were measured, and oxidative stress index was calculated.

Results: Paraoxonase, arylesterase activity, thiol and total antioxidant status levels were significantly lower in the acute pancreatitis group than in the control group ($p = .024$, $p < .001$, $p < .001$, $p = .010$, respectively). Oxidative stress index and total oxidant status levels were higher in the acute pancreatitis group than in the control group, but the difference was not statistically significant ($p = .135$, $p = .253$, respectively).

Conclusions: This study demonstrates that decreased antioxidant levels are associated with mild acute pancreatitis. No association was observed between mild acute pancreatitis and total oxidant status.

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Introduction

Acute pancreatitis (AP) is one of the most important acute gastrointestinal disorders throughout much of the world. The incidence of AP ranges from 4.6 to 100/100,000 persons in Europe [1]. Gallstones are the most common cause of AP, and mortality ranges from 3% for patients with interstitial (oedematous) pancreatitis [2] to 15% for patients who develop necrosis [3]. The rate of hospitalisation for AP continues to increase over time [4]. The Atlanta Classification system was developed at a consensus conference in 1992 to establish standard definitions for classification of AP [5], and recently, a completed revision of the Atlanta Classification and definitions was reported by international consensus [6]. This revised classification of AP identifies two types of the disease: interstitial oedematous pancreatitis and necrotising pancreatitis [6]. Disease severity was classified as mild, moderate and severe

in this revision [6]. Mild AP, the most common form, has no organ failure or local and systemic complications and usually resolves in the first week. The major pathophysiologic processes in AP are inflammation, oedema and necrosis of pancreatic tissue [7,8]. AP is initiated by intracellular activation of pancreatic proenzymes and autodigestion of the pancreas. Destruction of the pancreatic parenchyma first induces inflammatory mediators and early organ failure. Concomitantly, anti-inflammatory cytokines and specific cytokine inhibitors are produced [9]. Over the past 150 years, many animal models of pancreatitis have been developed that have allowed researchers to study the pathogenesis and pathophysiology of AP [10]. Unfortunately, the mechanisms underlying the pathogenesis of AP remain elusive despite significant advances in the last 25 years, and there is no specific therapy because of the obscure pathogenesis [7,11]. It has been shown in many inflammatory diseases that oxygen radicals play an important role in the development of inflammation [12]. The similarity of inflammatory tissue damage in inflammatory diseases to that in pancreatitis has led many researchers to study oxidative stress (OS) in AP [12]. Over the last 20 years, there has been increasing awareness regarding the role played by OS in AP [13]. OS occurs when there is an

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imbalance between generation of reactive oxygen species (ROS) and adequate antioxidant defence systems. OS can cause cell damage either directly or by altering the signalling pathways [14]. It has been demonstrated that oxygen-derived free radicals mediate an important step in the initiation of AP in *in vivo* models of acute experimental pancreatitis [12,15]. It is suggested that depletion of ROS, which is an indirect marker of products of lipid peroxidation in the acinar cells, leads to low adenosine triphosphate (ATP) state and favours necrosis, while ROS induction favours apoptosis, thus avoiding severe pancreatic damage, and therefore seems to be protective to the acinar cell. Moreover, OS in the neutrophils leads to inflammation and may contribute to pancreatic injury. Thus, OS appears to play a dual role in pancreatitis [14,15]. Human serum paraoxonase-1 (PON1) is an ester hydrolase that has both arylesterase (ARE) and paraoxonase (PON) activities. PON1 is a high-density lipoprotein (HDL)-associated enzyme with antioxidant functions and can protect low-density lipoproteins (LDLs) from oxidation induced by either copper ion or the free radical generator azobis (amidinopropane) hydrochloride [16,17]. Thiol plays a key role in protecting cells from OS and has antioxidant effects [18]. OS in AP is less well defined in human clinical trials.

The aim of this study was to identify the role of total antioxidant status (TAS) and total oxidant status (TOS) in patients with acute pancreatitis and correlate them with Ranson/Balthazar score and C-reactive protein (CRP) levels.

Patients and methods

Patients

We performed a cross-sectional trial on patients with AP. The study population consisted of 53 patients with AP admitted to the gastroenterology unit within 24 h after onset of the disease. These patients had mild interstitial oedematous AP and were categorised as the AP group. Patients aged under 18 years with chronic underlying diseases (including cardiovascular disorders, malignancy, asthma, allergic rhinitis, cystic fibrosis, metabolic disease, renal or liver disease or immunodeficiency) and active drug or alcohol abuse were excluded from the study. Gallstones were the cause of mild AP in all patients. We included only patients with biliary acute pancreatitis. The control group consisted of 55 healthy individuals with no known history of any disease. None of the controls was a smoker or alcohol consumer, and all were matched for age and sex. The diagnosis and severity of AP were defined according to the Atlanta classification [6]. The diagnosis of AP was based on two of the following three criteria: [1] abdominal pain consistent with AP, [2] serum lipase level (or amylase level) at least three times greater than the upper limit of normal and [3] characteristic findings of AP on contrast-enhanced computed tomography or transabdominal ultrasonography. Mild AP was defined by the absence of organ failure and the absence of local or systemic complications. Ranson scores [19] and Balthazar scores [20] were calculated for each patient. The study was approved by the Ethics Committee of Yildirim Beyazit University. The study protocol was carried out in accordance with the Helsinki Declaration of 1975 (as revised in 2000). All subjects were informed about the study protocol, and written consent was obtained from each participant.

Blood sample collection and preparation

In patients with AP, blood tests including complete blood count, electrolytes, blood sugar, renal and liver function tests, serum amylase and arterial blood gas analysis were performed within 24 h after onset of AP and repeated according to the requirement. In the control group, blood samples from healthy volunteers were

collected. Peripheral venous blood samples from patients and controls were collected into empty tubes. Samples were immediately separated from the cells by centrifugation at 3000g for 10 min and then stored at -80°C until further analysis. The following biochemical parameters were analysed: PON activity, total thiol concentration, ARE activity, oxidative status through TAS measurement, TOS and oxidative stress index (OSI). The total serum thiol concentration and PON1 and ARE enzyme activities were measured using commercially available kits (Relassay®; Turkey), and one unit of PON activity is equal to 1 mol of paraoxon hydrolysed per litre per minute at 37°C [21]. Phenyl acetate was used as a substrate to measure the ARE activity, and 1 unit of ARE activity is equal to 1 mmol of phenyl acetate hydrolysed per litre per minute at 37°C [22]. Serum TAS activities were measured using TAS assay kits (Relassay®; Turkey). Serum TOS activities were measured using TOS assay kits (Relassay®; Turkey). The assay is calibrated with hydrogen peroxide, and the results are expressed in terms of micromolar hydrogen peroxide equivalent per litre ($\mu\text{mol H}_2\text{O}_2$ equivalents/L) [23]. The percentage ratio of the TOS level to the TAS level was accepted as the OSI, an indicator of the degree of OS. To perform the calculation, the resulting unit of TAS was changed to mmol/L, and the OSI value was calculated using the following formula: $\text{OSI (arbitrary unit)} = [(\text{TOS}, \mu\text{mol H}_2\text{O}_2 \text{ equivalents/L}) / (\text{TAS}, \text{mmol Trolox equivalents/L}) \times 100]$.

Statistical analysis

Data analysis was performed using SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL, United States). Kolmogorov Smirnov test was used to determine whether the distributions of continuous variables were normal. Continuous variables were expressed as mean $\pm$ standard deviation (SD) or median (IQR) where applicable.

Student's *t*-test was used to compare the mean differences between groups, and Mann Whitney *U* test was used to compare the median values. Nominal data were analysed by Pearson's chi-square test.

Wilcoxon signed-rank test was used to determine whether the differences in median levels between first and second clinical measurements were statistically significant. Degrees of association between continuous variables were evaluated by Spearman's rank correlation analyses.

Multiple linear regression analyses were used to evaluate whether the case and control groups continued to show statistically significant differences after adjustment for age and gender. Coefficient of regression and 95% confidence intervals for each independent variable were also calculated.

Multiple linear regression analyses were used to determine the best predictors that affect major clinical measurements (e.g. TAS, PON, ARE) after adjustment for all potential confounding factors. Any variable that showed a *p* value of $<.10$ in the univariable test was considered as a candidate for the multivariable model analysis along with all variables of known clinical importance.

A *p* value of $<.05$ was considered statistically significant.

Results

Fifty-three patients with mild AP (AP group) and 55 healthy subjects (control group) were enrolled in the study. Characteristics of the patients and controls are shown in Table 1. There were no significant differences between the AP group and control group with regard to gender and age ($p > .05$). The mean age of patients in the AP group was 54.0 ± 17.4 years, compared with a mean age of 49.6 ± 13.7 years in the control group. PON activity, ARE activity, thiol and TAS levels were significantly lower in the AP

Table 1
Characteristics of patients in the acute pancreatitis (AP) group and control group.

Characteristics	AP group n = 53 (%)	Control group n = 55 (%)	P value
Age (years)	54.0 ± 17.4	49.6 ± 13.7	.148
Gender			
Male	21 (39.6)	28 (50.9%)	.156
Female	32 (60.4)	27 (49.1%)	.239
TAS (mmol Trolox equivalents/L)	2.06 ± 0.28	2.18 ± 0.25	.010
TOS (μmol H ₂ O ₂ equivalents/L)	3.42 ± 2.53	2.63 ± 1.57	.253
OSi (arbitrary unit)	1.20 ± 0.95	1.10 ± 1.06	.135
PON activity (U/L)	161.56 ± 114.61	202.07 ± 118.00	.024
ARE activity (U/L)	169.61 ± 58.78	216.77 ± 63.62	<.001
Thiol concentration (μmol/L)	159.10 ± 38.42	196.39 ± 28.75	<.001
Ranson score on admission	1.1 ± 1.07	–	
Balthazar score	1.7 ± 0.96	–	
CRP (mg/L)	73.95 ± 70.9	–	

TAS: Total antioxidant status, TOS: Total oxidant status, OSi: Oxidative stress index, PON: Paraonase, ARE: Arylesterase, CRP: C-reactive protein.

group than in the control group ($p = .024$, $p < .001$, $p < .001$, $p = .010$, respectively) (Figs. 1–4). OSI and TOS levels were higher in the AP group than in the control group, but the difference was not statistically significant ($p = .135$, $p = .253$, respectively). Balthazar and Ranson scores were calculated for the AP group. The mean level of CRP in the AP group was 73.95 ± 70.9 mg/L. Mean Ranson and Balthazar scores of the AP group were 1.1 ± 1.07 and 1.7 ± 0.96 , respectively. Correlation analysis of Balthazar and Ranson scores and TAS, TOS and CRP levels was performed in the AP group. TAS, TOS, PON activity, ARE activity and thiol levels did not show a significant positive or negative correlation with Ranson score ($p = .416$, $p = .565$, $p = .530$, $p = .195$, $p = .663$, respectively) and Balthazar score ($p = .663$, $p = .666$, $p = .166$, $p = .356$, $p = .463$, respectively). In addition, serum CRP levels were not correlated with TAS, TOS, PON activity, ARE activity and thiol levels ($p = .062$, $p = .879$, $p = .333$, $p = .612$, $p = .796$, respectively).

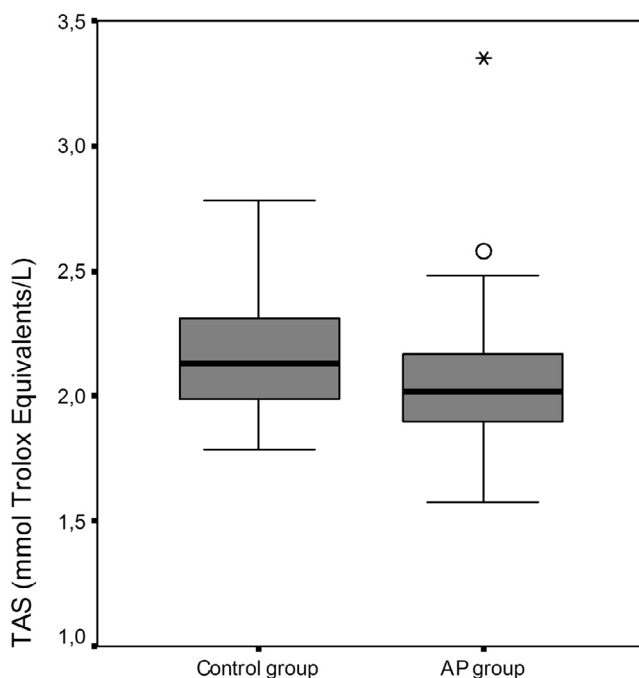


Fig. 1. Comparison of serum total antioxidant status (TAS) levels between the control and acute pancreatitis (AP) groups.

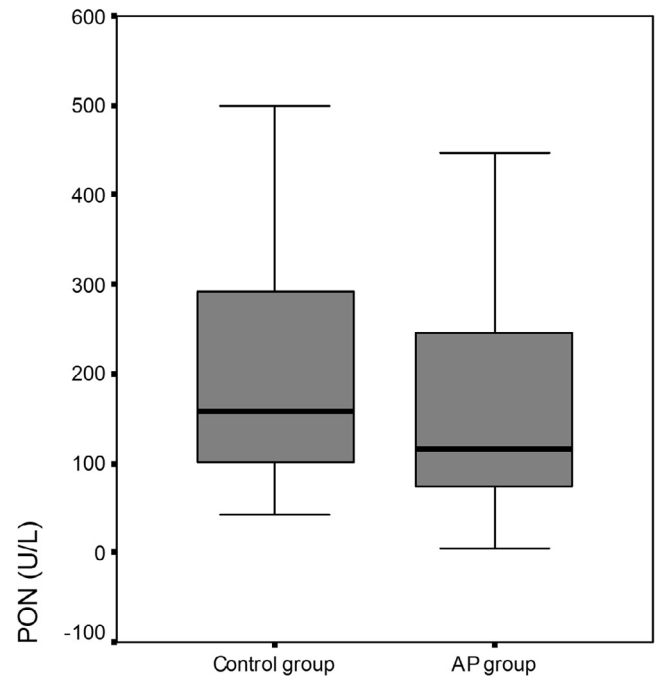


Fig. 2. Comparison of serum paraoxonase (PON) levels between the control and acute pancreatitis (AP) groups.

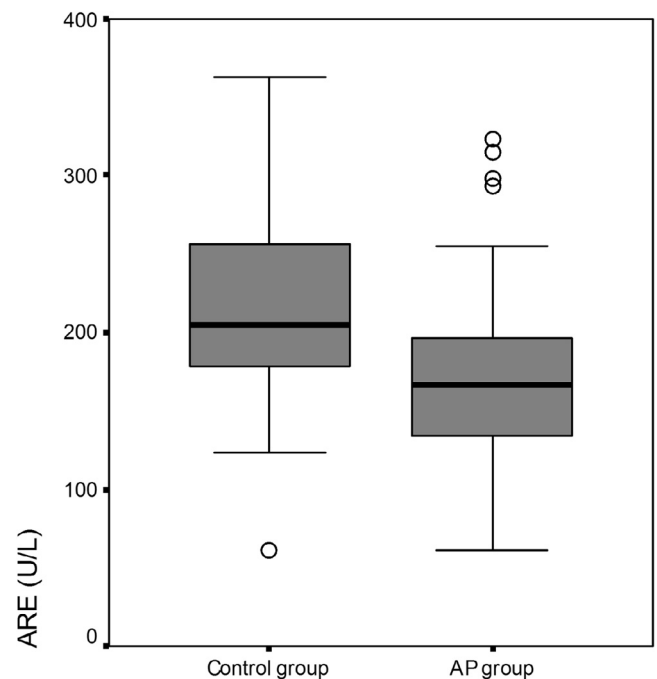


Fig. 3. Comparison of serum arylesterase (ARE) levels between the control and acute pancreatitis (AP) groups.

Discussion

AP, an acute inflammatory condition, is believed to be due to premature and inappropriately activated trypsinogen and other digestive enzymes in the pancreatic acinar cells, resulting in 'autodigestion' of the pancreas; however, the pathogenesis is still not well understood [11,24]. Many animal models have been proposed to study the pathogenesis and pathophysiology of pancreatitis [10]. Unfortunately, the mechanisms underlying the

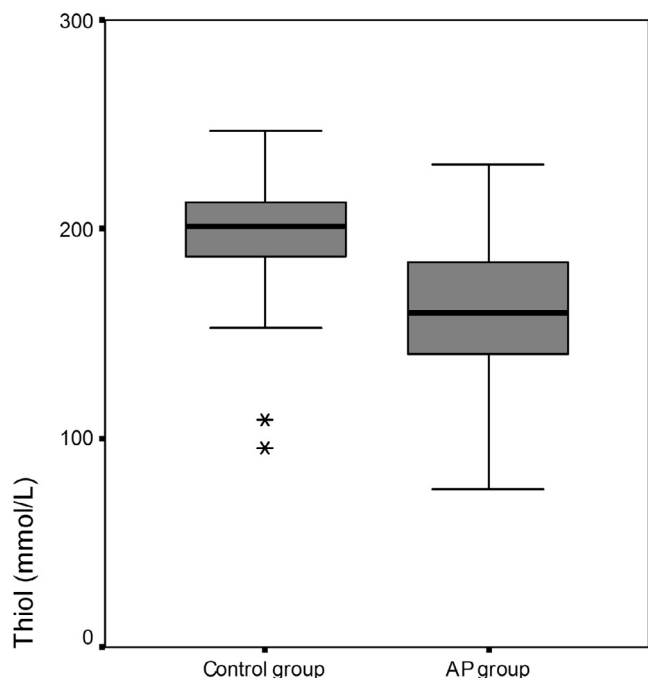


Fig. 4. Comparison of serum thiol levels between the control and acute pancreatitis (AP) groups.

pathogenesis of AP remain unclear. Mitochondrial dysfunction has increasingly been implicated as an important cellular event in AP, and OS plays an important role in mitochondrial depolarisation and loss of ATP production in pancreatic acinar cells [24]. An imbalance of oxidants, which implies the production of free oxygen radicals, and antioxidants, which implies deactivation of free radicals, leads to OS, which is associated with tissue injury and disease processes including the pathogenesis of AP [24,25]. Studies in experimental models of pancreatitis have shown that pancreatic OS was generated in AP at an early stage of induction [26,27]. In clinical trials, the role of OS was studied in humans by researchers in few studies. Telek et al. [28] histologically demonstrated persistent intrapancreatic OS in human AP. Braganza et al. [29] reported that OS spread to the vascular compartment by the time of admission in patients with AP. Curran et al. [30] reported that in AP, circulating concentrations of vitamin antioxidants decreased, which was inversely related to the increase in CRP level. In this study, 13 patients with severe AP were compared with 26 matched healthy controls. In the literature published in English, there are few clinical studies that have studied oxidants in AP. The study by Tsai et al. [31] was the first to demonstrate an association between OS and clinical disease severity. In their study, 56 healthy subjects, 30 patients with mild AP, and 23 patients with severe AP were enrolled. Patients with AP showed increased levels of superoxide radical and lipid peroxides in their blood samples. Thareja et al. [32] reported that high OS was observed in the early phase of AP. In their study, 65 patients with AP were compared with 70 healthy subjects. Superoxide dismutase levels were significantly higher in patients than in controls. Dziurkowska-Marek et al. [33] demonstrated that the oxidant-antioxidant balance rapidly changes in the early phase of human AP, and the degree of changes correlates with the clinical severity of pancreatitis. However, in this study, patients with mild and severe AP were compared, and a control group of healthy subjects was not included. Abu-Zidan et al. [34] investigated patients with mild and severe AP and reported that markers of OS were highly correlated with the severity of pancreatitis. However, the markers of OS were not compared with those in healthy subjects. In our study, TOS levels were higher

in the AP group than in the control group, but the difference was not statistically significant. We may obtain statistically significant results by increasing the number of patients and healthy individuals for TOS comparison. Compared with other studies, we examined only patients with mild AP. In clinical practice, oxidant levels may significantly increase in patients with severe AP rather than in those with mild AP. A lack of sufficient antioxidant reserve leads to OS, which is correlated with severity. Further studies with a larger sample size could evaluate the effect of OS in patients with mild and severe AP. There are a limited number of clinical trials that evaluated the effect of antioxidants on the mechanism underlying AP. Recently, Baser et al. [35] evaluated the oxidant and antioxidant status in 30 patients with mild AP and 29 healthy controls and observed the same result as our study, i.e. a decrease in TAS levels in mild AP, but there was no statistically significant difference between the control group and the AP group in serum TOS levels. Bopanna et al. [36] suggested that increased OS and antioxidant deficiency may be involved in the pathogenesis of idiopathic recurrent AP. Although oxidants are important mediators of tissue damage, perhaps oxidants alone do not induce the morphologic changes in AP [37], but may worsen inflammatory response after onset of AP [13]. We collected the blood samples for evaluating TAS and TOS levels within 24 h after onset of AP in the present study, but we did not evaluate the TAS and TOS levels in the following days. Deficiency of antioxidants may have initially impaired the pancreatic defence mechanism and led to a subsequent relative increase in oxidants. A larger clinical prospective study is needed to support this hypothesis. We hypothesise that antioxidants are associated with the mechanism underlying AP.

In conclusion, this study demonstrates that decreased antioxidant levels are associated with mild AP. It can be assumed that antioxidant levels are low in inflammation as they decrease during pancreatic injury in acute pancreatitis. Neither TAS nor TOS levels were associated with Ranson and Balthazar scores and CRP levels. Further studies are needed to better understand the role of antioxidant status in determining the severity of AP.

Conflict of interest

The authors declare that there are no conflicts of interest.

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