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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.

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 3000*g* 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 (μmol H_2O_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: OSI (arbitrary unit) = [(TOS, μmol H_2O_2 equivalents/L)/(TAS, mmol Trolox equivalents/L) × 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 ± 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

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