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Oxidative Stress Parameters in Predicting the Severity of Acute Pancreatitis

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Abstract

Aim: The aim of our study was to investigate the importance of oxidative stress levels in the diagnosis and prognosis of acute pancreatitis.

Materials and Methods: In total, 55 patients with acute pancreatitis and 47 healthy volunteers were included in the study. The study group was divided into three subgroups according to the computed tomography (CT) severity index. Group 1 consisted of patients with CT severity index score of 0-2, Group 2 consisted of patients with CT severity index of 3-6, and Group 3 consisted of patients with CT severity index 7-10. Total oxidant stress (TOS), total antioxidant stress (TAS), and oxidative stress index (OSI) were compared between study and control groups.

Results: The mean age of the patients in the study group was significantly higher compared to the control group ($p<0.001$). No significant difference was found between groups in terms of sex ($p=0.999$). TOS ($p<0.001$), TAS ($p<0.011$), and OSI ($p<0.001$) levels were significantly higher in the study group compared to the control group. No significant difference was found between subgroups of the study group (Group 1-good prognosis, Group 2-moderate prognosis, Group 3-poor prognosis) with respect to TOS ($p=0.844$), TAS ($p=0.600$), and OSI ($p=0.846$) levels.

Conclusion: Although our study does not support the use of oxidative stress parameters for the prediction of the severity and prognosis in patients with acute pancreatitis, additional and larger studies are warranted.

Keywords: Pancreatitis, oxidative stress, prognosis, diagnosis

Introduction

Acute pancreatitis is an inflammatory process of the pancreas characterized by abdominal pain and increase of blood pancreatic enzymes in clinical practice (1). The diagnosis of acute pancreatitis requires meeting two of the following three criteria: (i) persistent and severe epigastric pain with acute onset frequently radiating to the back, (ii) increase in serum lipase or amylase levels to more than three-fold of the upper limit of normal, and (iii) demonstration of characteristic acute pancreatitis findings with imaging.

Abdominal computed tomography (CT) is the gold standard in the differential diagnosis of patients with suspected pancreatitis that cannot be diagnosed by laboratory methods and clinical findings. It is also used to confirm the diagnosis already made with laboratory tests and clinical examination. Imaging tests may also be needed to ensure the patient does not have any complications of pancreatitis, which require a surgical consultation as well.

Although there has been important improvement recently in the diagnosis and treatment of acute pancreatitis, it remains a significant disease with a mortality rate of 5%-10% (2). In severe acute pancreatitis, the mortality rate can increase to as high as 30% (1). To recognize patients with a severe disease course that requires early intensive care support various laboratory and clinical scoring methods (Ranson, Acute physiology and chronic health evaluation [APACHE II], etc.) have

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been described (3). According to the revised Atlanta classification, acute pancreatitis cases can be divided into two categories based on morphology (interstitial edematous pancreatitis and necrotizing acute pancreatitis) and three categories based on the clinical manifestation (mild, moderate, and severe) (4, 5).

Computed tomography is important in the early diagnosis and classification of acute pancreatitis and in the selection of a suitable treatment, hence in decreasing morbidity and mortality. Balthazar et al. (6) stratified the severity of pancreatitis into five categories according to CT appearance. The presence of necrosis and acute inflammation are the most important prognostic factors in the determination of pancreatitis severity. Balthazar et al. (7) developed a CT severity index using these two prognostic factors. According to CT severity index, morbidity and mortality rates increase in direct proportion to the degree of pancreatic necrosis. If CT index score is 0 or 1, mortality and morbidity rate is 0%; if it is 2-6, mortality is 0% and morbidity is 4%; and if it is between 7-10, mortality is 17% and morbidity is 92% (8, 9).

In previous studies, it has been shown that free oxygen radicals play an important role in the onset and progression of acute pancreatitis (10). Oxidative stress emerges when there is imbalance between the production and elimination of free oxygen radicals. It is a result of an increase in the formation of oxidative products and depletion of antioxidant endogenous defense mechanisms (6, 11). After the original demonstration of the role of free oxygen radicals in acute pancreatitis, many experimental and clinical studies have been conducted (12, 13). Determination of patients with the probability of morbidity and mortality using oxidative stress parameters may help the early triage of patients requiring intensive care and early selection of those requiring special procedures (14).

In this study, our goal was to determine the relation between oxidative stress and acute pancreatitis. We studied whether oxidative stress levels can be used in the early diagnosis of acute pancreatitis and in predicting the severity of disease.

Materials and Methods

This was a prospective, observational, clinical study. After the institutional review board approved the study protocol, it was conducted in the emergency department of a Training and Research Hospital between April and November 2013.

Patients diagnosed with acute pancreatitis in the emergency department were included in the study group, whereas healthy volunteers were assigned to the control group. The control group volunteers included emergency department personnel who had no history of chronic disease. Patients who meet at least two of the following criteria were diagnosed with acute pancreatitis: (i) persistent and severe epigastric pain with acute onset frequently radiating to the back, (ii) increase in the serum lipase or amylase levels to more than three-fold of the upper limit of normal, and (iii) demonstration of characteristic acute pancreatitis findings with imaging. Patients who did not meet at least two of these criteria, who had chronic pancreatitis, and with markedly high creatinine values (>1.8 mg/dL) were excluded from the study.

Researchers have followed the ethical rules and written informed consent was obtained from each patient participating in the study.

Blood samples were drawn from all cases at presentation to the emergency service and oxidative stress levels (total oxidant stress [TOS] and total antioxidant stress [TAS]) were measured in the biochemistry laboratory of the Yildirim Beyazit University Faculty of Medicine. The ratio of TOS to TAS was regarded as oxidative stress index (OSI). TOS and TAS were measured using commercially available kits (Relassay, Turkey). TOS unit is μmol H_2O_2Eqv/L, while TAS unit is Troloxequivalent/L. The OSI calculation formula is as follows: OSI (arbitrary unit)=TOS (mol H_2O_2equivalent/L)/TAS (molTroloxequivalent/L). Oxidative stress levels were also measured in the blood samples collected from 47 healthy volunteers.

All cases underwent contrast-enhanced abdominal CT examination. Radiology consultants evaluated the CT images. The study group was divided to three subgroups according to the CT severity index: Group 1 (good prognosis): patients with CT severity index score 0-2; Group 2 (moderate prognosis): patients with CT severity index score 3-6; and Group 3 (poor prognosis): patients with CT severity index score 7-10.

The TOS, TAS, and OSI were compared between the study and control groups. In addition, TOS, TAS, and OSI levels of subgroups of study group (Groups 1, 2, and 3) were compared to determine the role of oxidative stress in predicting the prognosis of pancreatitis.

Statistical analysis

The Statistical Package for Social Sciences (SPSS Inc.; Chicago, IL, USA) 17.0 program was used for analysis. Categorical variables were expressed as number and percentage and continuous measurements as mean and standard deviation (when necessary as median and minimum-maximum). For the comparison of categorical variables, the Chi square test was used. For the comparison of continuous measurements, between-group distribution was verified and as variables are nonparametric, the Mann-Whitney U test was used. The Kruskal–Wallis test was used for the comparison of subgroups formed according to the CT severity index. In all tests, 0.05 was considered statistically significant. Results were evaluated with 95% confidence interval and a p value <0.05 was interpreted as significant.

Results

In total, 55 patients with acute pancreatitis and 47 healthy subjects were included in the study. Of the 55 patients, 24 were males (43.6%) and 31 were females (56.4%). The age range of the patients was 17-87 years; the median age was 59 years. Of the 47 healthy volunteers, 21 were males (44.7%) and 26 were females (55.3%); the age range was 25–51 years and the median age was 34 years. The mean age of the study group was significantly higher than that of control group (p<0.001). No significant difference was found between groups in terms of sex (p=0.999).

In the study group, the white blood count (WBC; p<0.001), alanine transaminase (ALT; p<0.001), aspartate transaminase (AST; p<0.001), direct bilirubin (p<0.001), amylase (p<0.001), and lipase values (p<0.001) were found to be significantly higher than those of the control group. The mean time between the onset of symptoms and CT examination was 3 days.

In the study group, TOS, TAS, and OSI levels were found to be significantly higher than those in the control group (Table 1).

Table 1. Comparison of TOS, TAS, and OSI levels between the study and control groups

	Study Group (n=55)		Control Group (n=47)		
	Mean±SD	Med (min-max)	Mean±SD	Med (Min-Max)	p
TOS	7.45±8.76	4.78 (0.51-46.80)	3.27±3.45	2.18 (0.21-21.77)	0.001
TAS	2.24±0.36	2.23 (1.46-3.24)	2.06±0.28	2.04 (1.51-2.62)	0.011
OSI	3.23±3.48	1.95 (0.23-17.13)	1.57±1.52	1.18 (0.09-9.28)	0.001

TOS: Total oxidant stress; TAS: Total antioxidant stress; OSI: Oxidative stress index; SD: standard deviation

Table 2. Comparison of age, TOS, TAS, and OSI levels between subgroups determined according to the CT severity index

	Group 1 (n=24) (Good prognosis)		Group 2 (n=21) (Moderate prognosis)		Group 3 (n=10) (Poor prognosis)		
	Mean±SD	Med (min-max)	Mean±SD	Med (min-max)	Mean±SD	Med (min-max)	p
Age, years	59.96±14.17	59 (35-87)	53.14±19.58	50 (22-87)	60.60±23.95	59 (17-87)	0.392
TOS	7.38±8.52	4.95 (0.56-35.43)	6.87±6.27	3.92 (0.51-23.16)	8.86±13.57	5.05 (1.80-46.80)	0.844
TAS	2.29±0.40	2.23 (1.66-3.24)	2.22±0.31	2.28 (1.55-2.78)	2.16±0.36	2.16 (1.46-2.73)	0.600
OSI	3.20±3.52	2.02 (0.23-15.98)	3.01±2.66	1.75 (0.23-8.33)	3.79±4.96	2.17 (0.95-17.13)	0.846

TOS: Total oxidant stress; TAS: Total antioxidant stress; OSI: Oxidative stress index; SD: standard deviation

No significant difference was found between the subgroups of the study group (Groups 1, 2, and 3) with respect to TOS, TAS, and OSI levels (Table 2).

We have not calculated the sample size before the study started, we checked similar studies and chose a convenient sample size. A post hoc power analysis revealed a power of 0.88 for TAS, and 1 for both TOS and OSI with 0.05 type 1 error level.

Discussion

Acute pancreatitis is a complex disease with high rates of morbidity and mortality. There is large body of evidence regarding the role of free oxygen radicals in pancreatitis (12, 13, 15, 16). After Sanfley et al. (12) demonstrated the role of free oxygen radicals in acute pancreatitis, many experimental and clinical studies have been conducted (13). It was shown in different experimental pancreatitis models that lipid peroxidation triggered by free oxygen radicals and changes in glutathione metabolism occur in the early course of the disease (15, 17). Rau et al. (18) indicated that changes in oxidized glutathione and lipid peroxidation products occur with pancreatitis and it is correlated to acinary cell damage. In the experimental acute pancreatitis models developed by Schoenberg et al. (13) and Sajewicz et al. (19), it was demonstrated that oxidative stress occurs in the initial period of the disease.

As oxidant and antioxidant factors have additive interaction, instead of measuring oxidant and antioxidant components separately, the measurement of overall TOS and TAS gives a better idea of the degree of oxidant stress. Since the measurement of these components separately will lead to higher cost and time consumption, a combined measurement is more reasonable (20). In this study, TOS and TAS levels in blood samples collected from patients at the time of referral to the emergency service were compared with those obtained from healthy volunteers. In addition, OSI obtained by the ratio of TOS to TAS was compared in the patient and control groups. The TOS, TAS, and OSI values were found to be significantly higher in the patient group. This finding is consistent with previous findings that oxidative stress is present in the course of pancreatitis.

Many grading systems and serum markers are utilized in the prediction of the severity of acute pancreatitis among clinical judgment. These are based on several clinical, laboratory, and radiological risk factors. For this purpose, laboratory results, such as hemoconcentration, blood C-reactive protein, blood urea nitrogen, creatinine, procalcitonin test, urinary trypsinogen activation peptide ELISA test, and urinary anionic trypsinogen test, and imaging methods, such as chest films, CT screening, magnetic resonance imaging, and magnetic resonance cholangiopancreatography can be used. Robert et al. (21) stated that these predictive models have low specificity. Among clinical predictors, we can mention advanced age, alcoholic pancreatitis, short period after symptom onset, obesity, and organ failure to be poor prognostic factors. Sex has not been found to be a prognostic factor. In our study, WBC ($p<0.001$), ALT ($p<0.001$), AST ($p<0.001$), direct bilirubin ($p<0.001$), amylase ($p<0.001$), and lipase ($p<0.001$) values were found to be significantly higher in the patient group compared to the control group.

In the determination of the severity of pancreatitis, the presence of necrosis and acute inflammation are the most important prognostic factors in CT. Balthazar et al. (8) developed a CT severity index using these two prognostic CT factors. Morbidity and mortality rates increase in direct proportion to the degree of necrosis in the CT severity index. The CT criteria and severity index of Balthazar were found to be more valuable than Ranson criteria, APACHE II criteria, and simplified acute physiological scoring.

Although CT severity index is quite successful in predicting the prognosis of the disease and CT is very useful in the diagnosis and follow-up acute pancreatitis, it is an expensive and time consuming,

and the patient is exposed to risks of ionized radiation and contrast media. These limitations of CT led to conducting a study to determine the role of oxidative stress parameters in the prediction of severity and prognosis of pancreatitis. In our study, patients were divided into three subgroups based on the CT severity index. These subgroups were compared with respect to TAS, TOS, and OSI values. Although TOS and OSI values were higher in the poor prognosis subgroup, the difference was not statistically significant. (p=0.844, p=0.846) The TAS values were not significantly different between the three subgroups. (p=0.600) Based on the data, we may not use oxidative stress parameters for the prediction of prognosis in patients with acute pancreatitis yet, but further studies including larger patient series should be conducted.

Study limitations

Our study was a single-center study. We did not compare the oxidative stress levels with the real prognosis of patients but have compared it with a CT prognostic index instead. We could not determine a cut-off value for the oxidative stress parameters in the diagnosis of acute pancreatitis, but we think our findings might help further researches in the area. The mean age of the study group was significantly higher than that of that control group, and aging is related to increased oxidative stress, and this may have an affected our results.

Conclusion

In this study, we measured oxidative stress levels in patients diagnosed with acute pancreatitis to determine whether these parameters have any role in the early diagnosis or prediction of severity in acute pancreatitis. We compared oxidative stress parameters with CT severity index in diagnosis and severity prediction of acute pancreatitis. Findings obtained in this clinical study based on the measurement of TOS and TAS levels and calculation of OSI levels corroborate the relation between acute pancreatitis and oxidative stress. In view of these findings, oxidative stress levels are significantly higher in the cases of acute pancreatitis. Based on our data, we may not use oxidative stress parameters for the prediction of severity in patients with acute pancreatitis, yet but further studies including larger patient series should be conducted.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Yıldırım Beyazıt University Faculty of Medicine (Date: 25.3.2013- Number: 38).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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