Evaluation of oxidant/anti-oxidants status in patients with mild acute pancreatitis

Husniye Baser[1], Ummugulsum Can[2], Deniz Karasoy[1], Abdulhalim Serden Ay[3], Salih Baser[1], Fatma Humeyra Yerlikaya[4], Samil Ecirli[1]

(1) The Department of Internal Medicine, (2) The Department of Biochemistry, (3) The Department of General Surgery, Konya Education and Research Hospital, Konya, Turkey ; (4) The Department of Biochemistry, Meram Medical School of Necmettin Erbakan University, Konya, Turkey.

Abstract

Background and aim : **Acute pancreatitis (AP), an inflammatory disorder of the pancreas, is associated with significant morbidity and mortality. The pathogenesis of AP has been suggested to involve high oxidative stress (OS), combined with inadequate anti-oxidant status.**

We aimed to investigate the levels of serum total anti-oxidant status (TAS), total oxidant status (TOS) and ischemia-modified albumin (IMA) in patients with mild AP.

Methods : **Thirty subjects with mild AP and 29 healthy controls were enrolled into the study. The levels of TAS, TOS and IMA, C-reactive protein (CRP), high sensitivity CRP (hs-CRP) and fibrinogen were measured in both groups.**

Results : **TAS levels were significantly lower ($p = 0.037$), while IMA levels were significantly higher ($p < 0.001$) in patients, compared to controls. TOS levels were similar between two groups. Fibrinogen, CRP and hs-CRP levels were significantly higher in patients than those of controls ($p < 0.001$ for all parameters). IMA levels were positively correlated with amylase and lipase levels ($r = 0.448$, $p = 0.001$ and $r = 0.469$, $p < 0.001$, respectively). There was a negative correlation between TAS levels, and amylase and lipase levels ($r = -0.277$, $p = 0.035$ and $r = -0.278$, $p = 0.034$, respectively).**

Conclusions : **OS is reported to be associated with the inflammatory process and the severity of AP. In our study, among OS parameters, an increase in IMA levels and a decrease in TAS levels were observed in mild AP patients.** (Acta gastroenterol. belg., **2016**, 79, **23-28**).

Key words : pancreatitis, oxidative stress, total anti-oxidant status, total oxidant status, ischemia-modified albumin.

Introduction

Acute pancreatitis (AP) is known as a potentially life-threatening disease with a wide spectrum of severity. While the overall mortality of AP is about 5%, the rate can reach up to 20 to 30% in patients with severe AP (1,2). Many studies have reported an increase in the incidence for the past few decades (3,4).

The pathogenesis of AP was proposed as multifactorial, and recent studies have focused on oxidative stress (OS) (5,6). In light of the available experimental and clinical evidences of increased lipid peroxidation, glutathione depletion and enhanced free radical activity, the incompletely elucidated pathophysiological association between pancreatitis and systemic inflammatory response syndrome (SIRS) shows that OS is critically involved in the development of complications in the severity and outcome of AP (7,8), combined with poor antioxidant levels (9).

A combination of increased production of reactive oxygen species (ROS) and impaired antioxidant capacity is known to lead to OS (10-15). ROS is composed of a group of highly reactive intermediary oxygen metabolites generated in the course of oxygen metabolism. ROS have many important biological functions, such as regulation of redox-sensitive transcription factors, redox-sensitive signal transduction pathways and direct interaction with various molecules. Under normal conditions, ROS are safely neutralized by the antioxidant defense system. However, when ROS production exceeds the capacity of the antioxidant defense system, the excess ROS causes cellular injury and dysfunction by attacking biomolecules, and modulating redox-sensitive signal transduction pathways and transcription factors (16,17).

According to the revised Atlanta classification system, AP can be classified as mild, moderate or severe. Mild acute pancreatitis is characterised by the absence of an organ failure and the absence of local or systemic complications. Severe AP is associated with significant mortality, and also with poor antioxidant status (AOS). OS has been shown to affect progression of complications in these patients (18,19). The measurements of total anti-oxidant status (TAS), total oxidant status (TOS) and ischemia-modified albumin (IMA) are tests used for the prediction of OS. To our knowledge, there has been no study concerning evaluation of TAS, TOS and IMA levels in mild AP patients in literature. Here, by measuring TAS, TOS and IMA levels in mild AP patients, we aimed to assess the oxidative/antioxidative balance in mild AP patients.

Material and method

Thirty patients with mild AP were included into the study. The controls were composed of 30 age- and sex-matched healthy subjects. For the diagnosis of AP, we used the following criteria : acute onset of epigastric pain with at least 3-fold increase in levels of serum amylase or lipase and ultrasonographic evidence of pancreatitis.

Correspondence to : Husniye Baser, M.D., Ovecler, 1297 Sokak, No : 1/22, 06460 Ankara, Turkey. E-mail : drhusniyebaser@yahoo.com.tr

Submission date : 20/07/2015
Acceptance date : 02/11/2015

Computed tomography was used for diagnosis only in cases with suggestion of severe pancreatitis or necrotising pancreatitis. The Ranson criteria and Glasgow scales of patients were calculated. We included only patients displaying the first episode of AP into the study. Patients with diabetes melllitus, chronic renal failure, hepatic failure, congestive cardiac failure, chronic pancreatitis, pregnancy, hypothyroidism, hyperthyroidism or those on antioxidants were excluded. The study was approved by the ethical board of the institution, and informed consent forms were obtained from all participants.

In both groups, the levels of TAS, TOS, IMA, C-reactive protein (CRP), high-sensitivity CRP (hs-CRP) and fibrinogen were measured. Blood samples were drawn from the antecubital vein and centrifuged at 3000 rpm for 10 min. Then, the samples were stored at -80 °C until analysis. The blood samples for the measurement of the OS parameters of patients were obtained in first 24 hours of the hospital admission. Blood tests including complete blood count, glucose, urea, creatinine, sodium, potassium, calcium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), direct bilirubin (DB), alkaline phosphatase, amylase, lipase and arterial blood gas analysis were performed on admission and repeated as per requirement. Biochemical parameters were measured using commercially available kits based on routine methods on Architect C 8000 System (Abbott Laboratories, Abbott Park, Illinois, USA). Fibrinogen levels were measured with colorimetric method (BCS XP autoanalyser SIEMENS Diagnostic System), and CRP and hs-CRP levels were measured with nephelometric method (BN2 autoanalyser SIEMENS Diagnostic System).

Measurement of oxidants and antioxidants

Serum TAS was measured by using an automated measurement method based on the bleaching of characteristic color of a more stable 2,2'-azino-bis (3-ethylbenz-thiazoline 6-sulfonic acid) (ABTS) radical cation by anti-oxidants (20). In this method, the values are expressed in mmol Trolox equivalents/L (mmol Trolox equiv./L).

Serum TOS was determined by a novel automated measurement method (21). Oxidants existing in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion which is enhanced by glycerol molecules of reaction medium. In acidic medium, the ferric ion generates a colored complex with xylenol orange. The spectrophotometrically measured color intensity is a result of total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in terms of micromolar H_2O_2 equivalents per liter (μmol H_2O_2 equiv./L).

IMA level was determined by using a colorimetric assay developed by Bar-Or *et al*. (22). This method is based on the measurement of unbound cobalt after incubation with patients' sera. Increased amounts of IMA result in less cobalt binding and more unbound cobalt available for complex formation with a chromogen [dithiothreitol (DTT)]. This complex can be measured photometrically. The procedure was performed as follows : 50 μL of 0.1% cobalt chloride was mixed with 200 μL of serum, and waited for 10 min for adequate cobalt-albumin binding. Fifty microliters of DTT, at a concentration of 1.5 mg/mL, was added as a colorizing agent, and the reaction was stopped 2 min later by adding 1.0 mL of 0.9% NaCl. The colored end product was measured at 470 nm, and compared with a serum-cobalt blank without DTT and reported in absorbance units (ABSU). Adjusted IMA was calculated as (individual serum albumin concentration/median serum albumin concentration of the population) × IMA ABSU value. This formula was applied to correct IMA values for serum albumin. The median serum albumin concentration of the subjects in each group was used separately (23).

Statistical analysis

All statistical analyses were performed with SPSS 15.0 statistical soft-ware (SPSS Inc. Il. USA). The Kolmogorov-Smirnov test was used for the compliance with the normal distribution. Other parameters, except for AST, ALT, TB, DB, amylase, lipase, CRP and hs-CRP, were within the normal distribution ranges. The comparisons between groups were performed via the student's *t* test for parametric variables, and the Mann-Whitney U test for non-parametric variables. Descriptive analyses were presented using mean ± standard deviation (SD) for normally distributed variables, and median and range (min-max) for non-normally distributed variables. The chi-square test was used to investigate the differences between groups regarding the categorical variables. The Spearman's correlation analyses were performed in order to document possible associations between variables. A *p* value less than 0.05 was accepted as statistically significant.

Results

Thirty AP patients (12 female, 18 male) with mean ages of 51.15 ± 16.76 years were included into the study. The control group consisted of 29 healthy subjects (6 female, 23 male) with mean age of 45.12 ± 6.44 years. Demographic and laboratory data of the study groups are given Table 1. Of 30 patients, 18 (60%) had biliary pancreatitis, while 12 (40%) had nonbiliary pancreatitis (due to the reasons such as drug, alcohol use or hypertriglyceridemia, etc.). The Ranson critera of all patients were found as ≤ 3.

IMA levels were significantly higher while TAS levels were significantly lower in patients, compared to controls ($p < 0.001$ and $p = 0.037$, respectively). There were no statistically significant differences in TOS levels between the two groups ($p = 0.344$). In addition, CRP, hs-CRP

Table 1. — **Demographic and laboratory data of study groups**

	Patients (n = 30)	Controls (n = 29)	*p*
Age (yrs)	51.15 ± 16.76	45.12 ± 6.44	0.090
Female/Male	12/18	6/23	0.107
Hemoglobin (g/dL)	13.36 ± 1.84	15.34 ± 1.57	< 0.001
Leukocytes (10000/mm^3)	9701.00 ± 3374.71	5853.07 ± 2117.19	< 0.001
ESR (mm/h)	26.00 ± 12.58	9.53 ± 6.99	< 0.001
Glucose (mg/dL)	119.86 ± 35.21	92.25 ± 11.69	< 0.001
Blood urea (mg/dL)	36.60 ± 18.44	26.67 ± 5.55	0.008
Serum creatinine (mg/dL)	0.82 ± 0.38	0.78 ± 0.12	0.611
Serum albumin (g/dL)	3.69 ± 0.50	4.25 ± 0.18	< 0.001
Serum Ca (mg/dL)	8.73 ± 0.67	9.10 ± 0.27	0.008
AST (U/L)	119 (12-460)	18.5 (13-26)	< 0.001
ALT (U/L)	116 (7-666)	21.5 (12-45)	0.002
Total bilirubin (mg/dL)	1.30 (0.25-6.00)	0.77 (0.40-2.60)	0.070
Direct bilirubin (mg/dL)	0.68 (0.10-2.50)	0.28 (0.17-0.60)	0.029
Amylase (U/L)	1390 (131-5325)	65 (34-87)	< 0.001
Lipase (U/L)	3248 (328-12000)	21 (9-39)	< 0.001
CRP (mg/L)	26.75 (3.50-96.20)	3.19 (3.19-8.33)	< 0.001
hs-CRP (mg/L)	10.20 (3.32-93.00)	1.28 (0.34-6.42)	< 0.001
Fibrinogen (g/L)	542.67 ± 162.52	336.76 ± 95.98	< 0.001
IMA (ABSU)	0.56 ± 0.14	0.38 ± 0.16	< 0.001
TAS (mmol Trolox equiv./L)	1.32 ± 0.17	1.42 ± 0.15	0.037
TOS (μmol H_2O_2 equiv./L)	5.01 ± 3.81	4.16 ± 2.85	0.344

ESR, erythrocyte sedimentation rate ; Ca, calcium ; AST, aspartate aminotransferase ; ALT, alanine aminotransferase ; CRP, C-reactive protein ; hs-CRP, high-sensitivity C-reactive protein ; IMA, ischemia-modified albumin ; TAS, total anti-oxidant status ; TOS, total oxidant status.

and fibrinogen levels were significantly higher than those of controls ($p < 0.001$ for all parameters).

There were no statistically significant differences between IMA, TAS and TOS levels of the biliary and nonbiliary pancreatitis groups ($p = 0.722$, $p = 0.309$ and $p = 0.227$, respectively). In addition, CRP, hs-CRP and fibrinogen levels were also similar between two groups ($p > 0.05$, for all parameters) (Table 2).

A positive correlation was observed between IMA levels, and CRP, hs-CRP, amylase and lipase levels ($r = 0.512$, $p < 0.001$; $r = 0.559$, $p < 0.001$; $r = 0.448$, $p = 0.001$ and $r = 0.469$, $p < 0.001$, respectively). In addition, TAS levels were negatively correlated with hs-CRP, amylase and lipase levels ($r = -0.268$, $p = 0.046$; $r = -0.277$, $p = 0.035$ and $r = -0.278$, $p = 0.034$, respectively). No significant correlation was found between TOS levels, and CRP, hs-CRP, amylase and lipase levels ($p > 0.05$ for all parameters). There was no significant correlation between fibrinogen levels, and IMA, TAS and TOS levels (Table 3).

Discussion

TAS, TOS and IMA are among the parameters used in order to evaluate OS. Our study is the first to evaluate TAS, TOS and IMA levels in patients with mild AP, and while an increase was observed in IMA levels, a decrease was found in TAS levels in AP patients. Even so, no alteration was demonstrated in TOS levels.

AP is likely to be seen in a wide clinical entities from a mild, self-limiting localized disease to a situation of widespread multi-organ failure with high mortality rates. However mild pancreatitis is the inflammation and edema of the pancreas, severe pancreatitis has additional features of necrosis and injury to extrapancreatic organs (24,25). In the pathogenesis of pancreatitis, OS and inflammation play critical roles. A combination of increased production of ROS and impaired antioxidant capacity are known to lead to OS (10-15). Severe AP is associated with significant mortality, and along with poor antioxidant status (AOS), OS has been shown to affect the progression of complications in AP patients (18,19). The primary cause of mortality is a SIRS, which leads to multiple organ dysfunction, presumably related to increased OS. In a study performed by Booth *et al*., it was suggested that oxygen free radical (OFR) induction in acinar cells elevated apoptosis while inhibition of OFR generation caused an increase in necrosis accompanied by reduced ATP (26). In another study, Scott *et al*. demonstrated that excessive OFR in a pathologic state could cause damage in tissues and cells (27). OFR is involved in pancreas edema process in AP, and may be involved in the pancreas necrosis process. As highly reactive species, OFR directly attacks lipids and proteins in the biological membranes, thus disrupting their functions. The action of OFR includes oxidation of lipids in pancreatic cell membrane and oxidatively modified proteins, depolarization of the mitochondrial membrane and induction of DNA fragmentation (28).

In our study, we used TAS, TOS and IMA to assess OS in AP patients. The method of evaluation of OS is very important. There is no unique method that can

Table 2. — **Laboratory data of biliary and nonbiliary pancreatitis**

	Biliary pancreatitis (n = 18)	Nonbiliary pancreatitis (n = 12)	*p*
Amylase (U/L)	1503 (143-4604)	717 (131-5325)	0.075
Lipase (U/L)	5129 (328-12000)	940 (451-12000)	0.261
CRP (mg/L)	23.70 (3.50-96.20)	19.75 (4.00-62.00)	0.626
hs-CRP (mg/L)	10.20 (3.75-26.90)	10.20 (3.32-93.00)	0.151
Fibrinogen (g/L)	515.68 ± 168.31	579.49 ± 154.22	0.333
IMA (ABSU)	0.55 ± 0.12	0.57 ± 0.17	0.722
TAS (mmol Trolox equiv./L)	1.35 ± 0.16	1.28 ± 0.19	0.309
TOS (μmol H_2O_2 equiv./L)	5.74 ± 4.56	3.98 ± 2.17	0.227

CRP, C-reactive protein ; hs-CRP, high-sensitivity C-reactive protein ; IMA, ischemia-modified albumin ; TAS, total anti-oxidant status ; TOS, total oxidant status.

Table 3. — **Correlation between biochemical and oxidative stress paramaters**

	CRP		hs-CRP		Fibrinogen		Amylase		Lipase	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
IMA	0.512	< 0.001	0.559	< 0.001	0.129	0.366	0.448	0.001	0.469	< 0.001
TAS	-0.229	0.089	-0.268	0.046	-0.008	0.957	-0.277	0.035	-0.278	0.034
TOS	0.119	0.385	0.097	0.483	0.185	0.185	0.132	0.328	0.237	0.076

CRP, C-reactive protein ; hs-CRP, high-sensitivity C-reactive protein ; IMA, ischemia-modified albumin ; TAS, total anti-oxidant status ; TOS, total oxidant status.

accurately measure the OS (29). For determination of antioxidant status, one of the methods is the measurement of different antioxidant molecules individually. This approach is not practical and also may not reflect the interaction of different antioxidants. The measurement of the TAS is often used to estimate the overall antioxidative status because of two reasons. One is the effects of the the antioxidants can be additive and the other is the measurement of antioxidants seperately is time consuming (20). Likewise, TOS is measured to determine a patient's overall OS (21). IMA has been initially proposed as a marker for the diagnosis of myocardial ischemia. IMA, however, is not a tissue-specific marker of ischemia. IMA is considered a non-specific biomarker in the evaluation of OS, and the production of IMA seems to be associated with the production of ROS that modifies the metal-binding sites of albumin (30,31). In this study, we observed decreased TAS levels in AP patients. However we found increased IMA levels in AP patients, we also demonstrated similar TOS levels in both groups. There was no difference between two groups as to TOS levels may be attributed to the fact that our patients were composed of those with mild AP, and OS in these patients progresses more slightly, compared to patients with severe AP. The other reason for the presence of no difference of TOS levels between groups may be associated with delayed admission of patients to the hospital because of the slight symptoms. Unfortunately the duration between onset of symptoms and hospital admission was not evaluated in this study.

The degree of oxidant-antioxidant balance changes in the early phase of human AP, which is correlated with the clinical severity of the disease (8). Park *et al*. reported higher plasma levels of lipid peroxides and myeloperoxides and lower superoxide dismutase (SOD) activity in patients with severe AP than in those with mild AP (32). In another study performed by Thareja *et al*., it was reported that OS levels as indicated by SOD and thiobarbituric acid reactive substances were significantly higher in patients with AP, and AOS was poor. In addition, high OS was observed during the early phase of AP, and gradually improving AOS was associated with a better clinical outcome in these patients (33).

Most patients with AP develop mild disease. Many clinicians monitor serum levels of amylase and lipase in an attempt to predict the disease course, but this strategy has not been recommended by practice guidelines. Even so, a recent study reported that the percentage change in the serum level of amylase was associated with the severity of AP (34). In addition, Coffey *et al*. observed that serum lipase, performed within 24 hours of presentation, was significantly associated with the severity of AP in children and adolescents (35). A positive correlation between serum pancreatic enzyme levels and disease severity has been observed in a murine study, in which taurocholate-induced severe necrotizing pancreatitis was compared with cerulein-induced mild pancreatitis and a sham control (36). The fact that IMA was positively correlated with amylase and lipase, and TAS levels had a negative correlation with these markers in our study indicated the existence of an associaton between OS markers and pancreatic enzymes. It may be speculated that OS markers and pancreatic enzymes can be used together in evaluating the severity of pancreatitis in AP patients.

However OS parameters were evaluated in AP patients with mild disease, TAS, TOS and IMA levels of patients with severe AP were not determined in this study. Further studies with evaluation of these parameters in severe AP patients are needed in order to determine association between OS and severity of AP.

CRP has been used widely in the early risk assessment of patients with AP. Previous studies suggested that CRP with a sensitivity of 80% and a specificity of 76%, was proposed as a biochemical marker to predict the severity of AP (37,38). Cardoso *et al*. reported that CRP at 48 h after hospital admission showed a good prognostic accuracy for severe AP (39). In AP patients, hs-CRP levels were reported to be higher (40). As consistent with literature, CRP and hs-CRP levels were observed to be higher in our patients with AP. Furthermore, a correlation was seen to be present between OS markers and these parameters.

In literature, there are several studies where antioxidants were used in the treatment of AP patients. However, the clinical efficacy of antioxidant treatment in these patients remains unproven. In a study performed by Virlos *et al*., no benefits were observed following the multiple antioxidant combination (selenium, N-acetylcysteine and ascorbic acid) in severe AP patients (41). Siriwardena *et al*. also failed to show any benefits in AP patients given selenium intravenously as part of a cocktail of antioxidants including NAC and vitamin C (42). On the other hand, Sevillano *et al*. demostrated that N-acetyl cysteine showed beneficial effects in reducing the severity of disease (43). In a recent meta-analysis, the effects of antioxidant therapy in AP, chronic pancreatitis (CP) and post-endoscopic retrograde cholangiopancreatography pancreatitis (PEP) have been evaluated. The authors demonstrated that however antioxidant therapy significantly reduced the duration of hospital stay, it had no significant effect on serum CRP after 5-7 days, CP-induced pain, incidence of severe, moderate and mild PEP in AP patients. The authors also reported that antioxidant therapy had no significant effect on serum amylase when measured in 8 h blood sampling but had significantly reduced serum amylase levels when evaluated in 24-h sampling (44). In another meta-analysis, antioxidant therapy resulted in a borderline significant reduction in hospital stay, and a significant decrease in complications and a non-significant decrease in mortality rate in AP patients (45).

There are also several limitations in our study. First, our sample size was small. Second, only patients with mild AP were included into the study ; so, TAS, TOS and IMA levels could not be investigated in patients with severe AP. Finally, these measurements were not repeated after AP treatment so the effects of TAS, TOS and IMA levels in patients clinical progress were not evaluated.

In conclusion, oxidative status in patients with mild AP was evaluated via the measurements of TAS, TOS and IMA, and IMA levels were observed to increase in patients with AP, and TAS levels to decrease. Also, IMA and TAS levels were demonstrated to be correlated with amylase and lipase levels. We consider that future studies investigating the association between OS and pancreatic enzymes, and also measuring different OS parameters will be beneficial.

References

1. PAVLIDIS P., CRICHTON S., LEMMICH SMITH J., MORRISON D., ATKINSON S., WYNCOLL D. *et al*. Improved outcome of severe acute pancreatitis in the intensive care unit. *Crit. Care Res. Pract*., 2013, **2013** : 897107.
2. BANKS P.A., FREEMAN M.L. ; PRACTICE PARAMETERS COMMITTEE OF THE AMERICAN COLLEGE OF GASTROENTEROLOGY. Practice guidelines in acute pancreatitis. *Am. J. Gastroenterol*., 2006, **101** : 2379-2400.
3. SATOH K., SHIMOSEGAWA T., MASAMUNE A., HIROTA M., KIKUTA K., KIHARA Y. *et al*. Research Committee of Intractable Diseases of the Pancreas. Nationwide epidemiological survey of acute pancreatitis in Japan. *Pancreas*, 2011, **40** : 503-507.
4. ROBERTS S.E., AKBARI A., THORNE K., ATKINSON M., EVANS P.A. The incidence of acute pancreatitis : impact of social deprivation, alcohol consumption, seasonal and demographic factors. *Aliment. Pharmacol. Ther*., 2013, **38** : 539-548.
5. WINTERBOURN C.C., BONHAM M.J., BUSS H., ABU-ZIDAN F.M., WINDSOR J.A. Elevated protein carbonyls as plasma markers of oxidative stres in acute pancreatitis. *Pancreatology*, 2003, **3** : 375-382.
6. TSAI K., WANG S.S., CHEN TS., KONG C.W., CHANG F.Y., LEE S.D., LU F.J. Oxidative stress : an important phenomenon with pathogenetic significance in the progression of acute pancreatitis. *Gut*, 1998, **42** : 850-855.
7. ABU-ZIDAN F.M., BONHAM M.J., WINDSOR J.A. Severity of acute pancreatitis : a multivariate analysis of oxidative stress markers and modified Glasgow criteria. *Br. J. Surg*., 2000, **87** : 1019-1023.
8. DZIURKOWSKA-MAREK A., MAREK T.A., NOWAK A., KACPEREK-HARTLEB T., SIERKA E., NOWAKOWSKA-DULAWA E. The dynamics of the oxidant-antioxidant balance in the early phase of human acute biliary pancreatitis. *Pancreatology*, 2004, **4** : 215-222.
9. CURRAN F.J., SATTAR N., TALWAR D., BAXTER J.N., IMRIE C.W. Relationship of carotenoid and vitamins A and E with the acute inflammatory response in acute pancreatitis. *Br. J. Surg*., 2000, **87** : 301-305.
10. VAZIRI N.D. Roles of oxidative stress and antioxidant therapy in chronic kidney disease and hypertension. *Curr. Opin. Nephrol. Hypertens*., 2004, **13** : 93-99.
11. VAZIRI N.D., DICUS M., HO N.D., BOROUJERDI-RAD L., SINDHU R.K. Oxidative stress and dysregulation of superoxide dismutase and NADPH oxidase in renal insufficiency. *Kidney Int*., 2003, **63** : 179-185.
12. VAZIRI N.D., OVEISI F., DING Y. Role of increased oxygen free radical activity in the pathogenesis of uremic hypertension. *Kidney Int*., 1998, **53** : 1748-1754.
13. HIMMELFARB J., HAKIM R.M. Oxidative stress in uremia. *Curr. Opin. Nephrol. Hypertens*., 2003, **12** : 593-598.
14. HIMMELFARB J., STENVINKEL P., IKIZLER T.A., HAKIM R.M. The elephant in uremia : oxidant stress as a unifying concept of cardiovascular disease in uremia. *Kidney Int*., 2002, **62** : 1524-1538.
15. SINDHU R.K., EHDAIE A., FARMAND F., DHALIWAL K.K., NGUYEN T., ZHAN C.D. *et al*. Expression of catalase and glutathione peroxidase in renal insufficiency. *Biochim. Biophys. Acta*, 2005, **1743** : 86-92.
16. ROBERTSON R.P., HARMON J.S. Diabetes, glucose toxicity, and oxidative stress : A case of double jeopardy for the pancreatic islet beta cell. *Free Radic. Biol. Med*., 2006, **41** : 177-184.
17. ROBERTSON R.P., HARMON J., TRAN P.O., TANAKA Y., TAKAHASHI H. Glucose toxicity in beta-cells : type 2 diabetes, good radicals gone bad, and the glutathione connection. *Diabetes*, 2003, **52** : 581-587.
18. WERESZCZYÑSKA-SIEMIATKOWSKA U., DABROWSKI A., JEDYNAK M., GABRYELEWICZ A. Oxidative stress as an early prognostic factor in acute pancreatitis (AP) : its correlation with serum phospholipase A2 (PLA2) and plasma polymorphonuclear elastase (PMN-E) in different-severity forms of human AP. *Pancreas*, 1998, **17** : 163-168.
19. ABU-ZIDAN F.M., BONHAM M.J., WINDSOR J.A. Severity of acute pancreatitis : a multivariate analysis of oxidative stress markers and modified Glasgow criteria. *Br. J. Surg*., 2000, **87** : 1019-1023.

20. EREL O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin. Biochem.*, 2004, **37** : 277-285.
21. EREL O. A new automated colorimetric method for measuring total oxidant status. *Clin. Biochem.*, 2005, **38** : 1103-1111.
22. BAR-OR D., LAU E., WINKLER J.V. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia- a preliminary report. *J. Emerg. Med.*, 2000, **19** : 311-315.
23. LIPPI G., MONTAGNANA M., SALVAGNO G.L., GUIDI G.C. Standardization of ischemia-modified albumin testing : adjustment for serum albumin. *Clin. Chem. Lab. Med.*, 2007, **45** : 261-262.
24. BATCIOGLU K., GUL M., UYUMLU A.B., ESREFOGLU M. Liver lipid peroxidation and antioxidant capacity in cerulein-induced acute pancreatitis. *Braz. J. Med. Biol. Res.*, 2009, **42** : 776-782.
25. EŞREFOĞLU M., GÜL M., TURAN F. Comparative effects of several therapatic agents on hepatic damage induced by acute experimental pancreatitis. *Dig. Dis. Sci.*, 2008, **53** : 1303-1310.
26. BOOTH D.M., MURPHY J.A., MUKHERJEE R., AWAIS M., NEOPTOLEMOS J.P., GERASIMENKO O.V. *et al*. Reactive oxygen species induced by bile acid induce apoptosis and protect against necrosis in pancreatic acinar cells. *Gastroenterology*, 2011, **140** : 2116-2125.
27. SCOTT P., BRUCE C., SCHOFIELD D., SHIEL N., BRAGANZA J.M., MC CLOY R.F. Vitamin C status in patients with acute pancreatitis. *Br. J. Surg.* 1993, **80** : 750-754.
28. INOUE S., KAWANISHI S. Oxidative DNA damage induced by simultaneous generation of nitric oxide and superoxide. *FEBS Lett.*, 1995, **371** : 86-88.
29. POLJSAK B.., SUPUT D.., MILISAV I. Achieving the balance between ROS and antioxidants : when to use the synthetic antioxidants. *Oxid. Med. Cell. Longev.*, 2013, **2013** : 956792.
30. BAR-OR D., CURTIS G., RAO N., BAMPOS N., LAU E. Characterization of the Co(2+) and Ni(2+) binding amino-acid residues of the N-terminus of human albumin. *Eur. J. Biochem.*, 2001, **268** : 42-47.
31. GIDENNE S., CEPPA F., FONTAN E., PERRIER F., BURNAT P. Analytical performance of the albumin cobalt binding (ACB) test on the Cobas MIRA Plus analyzer. *Clin. Chem. Lab. Med.*, 2004, **42** : 455-461.
32. PARK B.K., CHUNG J.B., LEE J.H., SUH J.H., PARK S.W., SONG S.Y. *et al*. Role of oxygen free radicals in patients with acute pancreatitis. *World J. Gastroenterol.*, 2003, **9** : 2266-2269.
33. THAREJA S., BHARDWAJ P., SATEESH J., SARAYA A. Variations in the levels of oxidative stress and antioxidants during early acute pancreatitis. *Trop. Gastroenterol.*, 2009, **30** : 26-31.
34. KUMARAVEL A., STEVENS T., PAPACHRISTOU G.I., MUDDANA V., BHATT A., LEE P.J. *et al*. A Model to Predict the Severity of Acute Pancreatitis Based on Serum Level of Amylase and Body Mass Index. *Clin. Gastroenterol. Hepatol.*, 2015, **13** : 1496-1501.
35. COFFEY MJ., NIGHTINGALE S., OOI CY. Serum lipase as an early predictor of severity in pediatric acute pancreatitis. *J. Pediatr. Gastroenterol. Nutr.*, 2013, **56** : 602-608.
36. WITTEL UA., WIECH T., CHAKRABORTY S., BOSS B., LAUCH R., BATRA SK. *et al*. Taurocholate-induced pancreatitis. A model of severe necrotizing pancreatitis in mice. *Pancreas*, 2008, **36** : e9-21.
37. TRIANTOPOULOU C., LYTRAS D., MANIATIS P., CHRYSOVERGIS D., MANES K., SIAFAS I. *et al*. Computed tomography versus Acute Physiology and Chronic Health Evaluation II score in predicting severity of acute pancreatitis : a prospective, comparative study with statistical evaluation. *Pancreas*, 2007, **35** : 238-242.
38. PAPACHRISTOU G.I., WHITCOMB D.C. Inflammatory markers of disease severity in acute pancreatitis. *Clin. Lab. Med.*, 2005, **25** : 17-37.
39. CARDOSO F.S., RICARDO L.B., OLIVEIRA A.M., CANENA J.M., HORTA D.V., PAPOILA A.L. *et al*. C-reactive protein prognostic accuracy in acute pancreatitis : timing of measurement and cutoff points. *Eur. J. Gatroenterol. Hepatol.*, 2013, **25** : 784-789.
40. WEN T., LIU L., XIONG G.Z. Matrix metalloproteinase levels in acute aortic dissection, acute pancreatitis and other abdominal pain. *Emerg. Med. J.*, 2009, **26** : 715-718.
41. VIRLOS I.T., MASON J., SCHOFIELD D., MC CLOY R.F., EDDLESTON J.M., SIRIWARDENA A.K. Intravenous n-acetylcysteine, ascorbic acid and selenium-based anti-oxidant therapy in severe acute pancreatitis. *Scand. J. Gastroenterol.*, 2003, **38** : 1262-1267.
42. SIRIWARDENA A.K., MASON J.M., BALACHANDRA S., BAGUL A., GALLOWAY S., FORMELA L. *et al*. Randomised, double blind, placebo controlled trial of intravenous antioxidant (n-acetylcysteine, selenium, vitamin C) therapy in severe acute pancreatitis. *Gut*, 2007, **56** : 1439-1444.
43. SEVILLANO S., DE LA MANO A.M., MANSO M.A., ORFAO A., DE DIOS I. Nacetylcysteine prevents intra-acinar oxygen free radical production in pancreatic duct obstruction-induced acute pancreatitis. *Biochim. Biophys. Acta*, 2003, **1639** : 177-184.
44. GOOSHE M., ABDOLGHAFFARI A.H., NIKFAR S., MAHDAVIANI P., ABDOLLAHI M. Antioxidant therapy in acute, chronic and post-endoscopic retrograde cholangiopancreatography pancreatitis : An updated systematic review and meta-analysis. *World J. Gastroenterol.*, 2015, **21** : 9189-9208.
45. JEURNINK S.M., NIJS M.M., PRINS H.A., GREVING J.P., SIERSEMA P.D. Antioxidants as a treatment for acute pancreatitis : A meta-analysis. *Pancreatology*, 2015, **15** : 203-208.