MEASUREMENT OF THE TOTAL ANTIOXIDANT RESPONSE IN ACUTE PANCREATITIS WITH A NOVEL AUTOMATED METHOD

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ABSTRACT

***Aim**: The aim of this study was to determine the anti-oxidative status of patients with acute pancreatitis and compare with healthy controls.*

***Materials and methods**: Thirty patients with acute edematous pancreatitis and thirty healthy volunteers were enrolled in the study. To determine the anti-oxidative status of patients and healthy individuals, serum total antioxidant status was measured by using a novel automated method. Blood samples were taken once from the healthy controls and three times from the patients before treatment, in the third and fifth days of the treatment.*

***Results**: Total antioxidant capacity of serum was significantly lower in patients with acute edematous pancreatitis than in healthy persons (P<0.01). The assessment that is made in terms of total antioxidant capacity in patients with acute edematous pancreatitis, there were significant differences between before treatment and 3 and 5 days post-treatment values. Whereas, the significant differences were not detected between 3 and 5 days after treatment.*

***Conclusions**: Evaluating of total antioxidant status by this novel assay is feasible, and fully automated in patients with acute edematous pancreatitis.*

***Key words**: Acute pancreatitis, Antioxidative status, Ranson criterion, Total antioxidant capacity.*

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Introduction

The participation of oxidative stress in the pathogenesis and development of human acute (AP)[1-3] and chronic[4, 5] pancreatitis (CP) has been suggested in various reports. The oxidative stress has also been widely examined in animal models of pancreatitis - most often cerulean-[6], taurocholate-[7] or alcohol-induced[8] and in vitro models (pancreatic acinar cell damage)[2, 8,9]. In many organs with high oxygen metabolism, biochemical reactions that permit the continuity of life produce reactive oxygen species (ROS) that damage the cell membrane, DNA and the intracellular organelles.

Oxidative stress is the resulting condition in which these harmful molecules cannot be neutralized, and it plays an important role in the aetiopathogenesis of various diseases[10,11]. Oxidative stability is defined as the equilibrium between production and elimination of ROS. Any increase in the formation rate or decrease in the elimination rate of ROS can cause an imbalance leading to oxidative stress. Oxidative stress is one of the primary reasons of tissue injury or molecular damage in the cells[12].

It has been showed that oxygen free radicals play an important role in pancreatitis with different etiologies: biliary, alcoholic, and drug- and/or xenobiotic-induced pancreatitis[2, 5, 13].

Oxidative stress is also likely to have a central role in the development of pancreatic inflammation and the extra pancreatic complications. Antioxidant activity is known to reflect the altered redox balance of affected fluids, tissues or organs in several pathologic states. Plasma is an important vehicle that can either act as a protective factor for oxidative damage to different blood components or distribute dietary antioxidants to the rest of the body[14]. Plasma concentrations of antioxidants can be measured separately in the laboratory, but these measurements are time-consuming, labour-intensive and costly. Since antioxidative effects of antioxidant components of plasma are additive, the measurement of total antioxidant response reflects the antioxidative status of plasma. We evaluated, in this study, the total antioxidative status of plasma by using a more recently developed measurement method by Erel[15]. Using this method, we aimed to compare total antioxidative status of acute pancreatitis patients with healthy controls and also to evaluate patient's response to the treatment by measuring their total antioxidative response in the third and fifth days of the treatment by using this novel automated method[16].

Materials and methods

This study included a total of 23 PD patients whSubjects

The study was conducted at the Harran University Medical Faculty, Departments of General surgery and Clinical Biochemistry. After having written informed consent and the approval of the Intuitional Review Board, 30 patients with acute edematous pancreatitis and 30 healthy volunteers were included in this study. Demographic data of all patients and controls were similar (Table 1).

Acute edematous pancreatitis was diagnosed by physical examination, clinical routine biochemical parameters, abdominal ultrasound, and tomography findings.

Exclusion criteria

Exclusion criteria included the use of supplemental vitamins and iron, smoking, history of diabetes mellitus, coronary artery disease, cancer, systemic or local infection, alcohol intake, pregnancy, concomitant chronic active hepatitis or other well known liver diseases such as metabolic or autoimmune disorders and various infectious states of the liver.

Samples

Blood samples from the patients before and in the third and fifth days of the treatment, and from the controls were withdrawn into tubes containing EDTA after overnight fasting. Plasma was separated from cells by centrifugation at 1500 g for 10 min. The samples were stored at –80 °C until use.

Measurement of the total antioxidant status of plasma

The total antioxidant status (TAS) was measured in plasma by using a novel automated colorimetric method for the total antioxidant response (TAR) developed by Erel[17].

In this method, the hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction, and reacts with the colorless substrate o-dianisidine to produce the dianisyl radical, which is bright yellowish brown in color. Upon addition of a plasma sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction medium are suppressed by the antioxidant components of the plasma, preventing the color change and thereby providing an effective measure of the total antioxidant status of the plasma. The assay results are expressed as mmol Trolox Eq/L, and the precision of this assay is excellent, the variation being lower than 3%[17].

Statistical analysis

All data were expressed as mean ± standard deviation (SD). The significance of difference was determined using One-Way ANOVA F test. Post Hoc multiple comparison test LSD was used to compare the groups with each other. The statistical significance was defined as $p < 0.05$. Data were analyzed using SPSS® for Windows computing program (Version 11.0).

Results

Table 1 and 2 presents the demographic characteristics and laboratory findings of the patients with acute pancreatitis and the controls. No significant differences were found between the groups regarding demographic characteristics such as age, sex, and body mass index. All patients included in the study were hemodynamically stable. There were no significant differences between the two groups in terms of pulse, respiratory rate and blood pressure. Mean Ranson criteria value of the patients were 2.1: ± 0.83.

Parameters	Controls(n: 30)	AEP (n: 30)
Age (years)	49±12	50±11^{ns}
Sex (Female/male)	9/16	11/14
Body mass index (kg/m^2)	22.5±3.8	21.8±2.9^{ns}

Table 1: Demographic data in controls and acute edematous pancreatitis (AEP) groups.
Values are mean ± SD;
^{ns} *= non significant*

	Controls (n: 30)	AEP (n: 30)	AEP (3rd day) (n: 30)	AEP (5th day) (n: 30)
TAS (mmol Trolox Eq/L)	2.15±0.33^{a**}	1.83 ± 0.3^{a**,b**,c*}	1.44± 0.28$^{b**,d\,ns}$	1.47± 0.19$^{c*,d\,ns}$

Table 2: Total Antioxidant Status (TAS) in controls and acute edematous pancreatitis (AEP) groups.
a: Difference between control and patient groups
b: Difference between patient groups in the 1st and 3rd days
c: Difference between patient groups in the 1^{s}t and 5th days
d: Difference between patient groups in the 3rd and 5th days
^{ns} *= non significant*
**** = P<0.001*
*** = p<0.01*
** = p<0.05*

There were significant differences between the patients with acute edematous pancreatitis and the controls regarding to plasma TAS levels (Table 2). Plasma TAS levels of the patients before treatment were significantly lower than those of the healthy persons (P<0.01) (Table 2). Whereas there was no significant difference between the third- and fifth-day TAS levels in the patients, there were significant differences between before treatment and third-day TAS levels, and between before treatment and fifth-day TAS levels (respectively; P<0.01, P<0.05).

Discussion

Acute pancreatitis led to various degrees of interstitial edema, acinar cell damage, hemorrhage and necrosis. Although the inflammation initiates in pancreas, the disease may lead to systemic multi-organ failure. Several factors (complement activation, cytokines, oxygen free radicals, ischemia, and auto digestion of pancreatic enzymes) have been known to be involved in the pathogenesis of acute pancreatitis, but the role of these factors remains still unclear. Among them, oxygen free radicals could damage extracellular tissue by degrading hyaluronic acid and collagen in the intercellular matrix and directly attack biological membrane through the peroxidation of structurally and functionally important lipids. Furthermore, they could denature enzymes and other important proteins, and damage nucleic acid. In addition, they could indirectly trigger the accumulation of polymorphonuclear (PMN) leukocytes in the tissue. Activated PMN leukocytes could secrete various enzymes such as myeloperoxidase and elastase[18]. As a result, the inflammatory reaction accelerated. Moreover, oxygen free radicals could indirectly stimulate arachidonic acid metabolism with increased production of prostaglandins, thromboxane, and leukotrienes, and eventually could lead to micro circular derangement and cellular damage[18].

There are many reports about the mechanism to production of oxygen free radicals. Xanthine oxidase, PMN leukocyte, and cytochromes P-450 have been introduced as the source of oxygen free radicals[18, 19]. However, the true sources of oxygen free radicals have not yet been identified up to the present[20, 21]. Since the first study by Sanfey et al[22], many studies have demonstrated the role of oxygen free radicals in the pathogenesis of acute pancreatitis in experimental models and patients. Oxygen free radicals have been known to mediate an important step in the initiation of acute pancreatitis[18]). However, there are many limitations on studying patients with acute pancreatitis in clinical settings. Because of the nature of their high reactivity, oxygen free radicals are difficult to measure directly. Direct measurement of oxygen free radicals with electron spin resonance (ESR) technique was limited to in vitro studies due to short half time of free radicals and toxicity of compounds[21]. For these reasons, the alternative of measuring stable metabolites was met with broad acceptance[15]. The measurements of the effects of radical reaction with biological substances (i.e., lipid peroxides and changes of glutathione metabolism) and/or body response to these free radicals called antioxidant activity, like we did in this study, have replaced the assessment of oxygen free radicals.

Plasma contains various antioxidant molecules. One of them is proteins, particularly albumin, constitute the main antioxidant component of serum. Free sulfhydryl groups of proteins are mainly responsible for antioxidant response of them. It was calculated that proteins compose about 49% of the measured serum TAS against potent free radical reactions in healthy persons as

cited by Aslan et al[24]. The others are uric acid and bilirubin which serve as potent antioxidants by radical scavenging and reducing activities. All of these antioxidants are measured as TAS in our study and found to be reduced significantly in the patients with acute pancreatitis compared to the healthy persons.

The most widely used methods for measurement of plasma TAS are either colorimetric, fluorescence-based, or chemiluminescence[25-27]. But these methods are not appropriate for routine usage. There is as yet no accepted ''gold standard'' reference method, but the novel reported assay has several major advantages over the other currently available techniques. It is simple and inexpensive, and can easily be fully automated. It is also reliable and sensitive, and is not subjected to interference by commonly occurring serum components such as bilirubin, serum lipids, and anticoagulants such as heparin or oxalate[15]. Accurate measurements of the total serum antioxidant response can be obtained in as little as 10 min, making this assay eminently suitable for the clinical biochemistry laboratory.

In this study, we have used this novel measurement method to evaluate the extent of oxidative stress in acute pancreatitis patients, and compared these indices with those of healthy volunteers. It provides a useful method for the rapid evaluation of the TAS, a parameter valuable not only in the diagnosis of this condition, but also for other disorders involving oxidative stress. In terms of acute pancreatitis, however, routine measurement of the TAS may provide a useful tool to assess patient's oxidative stress status, and in the determination of an appropriate treatment management plan. Routine screening of the TAS during acute pancreatitis may also prove useful in terms of early recognition of acute pancreatitis oxidative stress, which could then be treated with appropriate dietary supplementation, and more careful follow-up of patients at risk of developing pancreatitis. Our findings support previous studies[27-29]. The mentioned reports explain that some components of antioxidative status are deficient and oxidative injury occurs in patients with acute pancreatitis. We determined that the TAS of the patients decreased.

In the light of these findings it is possible to conclude that the patients with acute edematous pancreatitis are exposed to oxidative stress and this may play a role in the etiopathogenesis of the disease. The novel assay is rapid, easy, stable, reliable, sensitive, inexpensive and fully automated, and may be used for both diagnosis and follow-up of these patients. Routine measurement of the TAS may provide a useful tool to aid in the assessment of the patient's oxidative stress status, and in the determination of an appropriate treatment management AP plan.

In conclusion, detecting plasma TAS levels during AP as a routine and rapid test may be useful in the management of disorders coexisting with oxidative damage

References

1) Hilker R, Schweitzer K, Coburger S, Ghaemi M, 1. Tsai K, Wang SS, Chen TS, et al. *Oxidative stress: an important phenomenon with pathogenetic significance in the progression of acute pancreatitis*. Gut 1998; 42: *850-855*.
2) Schultz HU, Niderau C, Klonowski-Stumpe H, et al. *Oxidative stress in acute pancreatitis*. Hepatogastroenterology 1999; 46: *2736-2750*.
3) Telek G, Regoly-Merei J, Kovacs GC, et al. *The first histological demonstration of pancreatic oxidative stress in human acute pancreatitis*. Hepatogastroenterology 2001; 48: *1252-1258*.
4) Uden S, Schofield D, Miller PF, et al. *Antioxidant therapy for recurrent pancreatitis: biochemical profiles in placebo-controlled trial*. Aliment Pharmacol Ther 1992; 6: *229-240*.
5) Wallig MA. *Xenobiotic metabolism, oxidant stress and chronic pancreatitis*. Digestion. 1998; 59: *13-24*.
6) D&browski A, Konturek SJ, Konturek JW, et al. *Role of oxidative stres in the pathogenesis of caerulein-induced acute pancreatitis*. Eur J Pharmacol 1999; 377: *1-11*.
7) Rau B, Poch B, Gansauge F, et al. P*athophysiologic role of oxygen free radicals in acute pancreatitis. Initiating event or mediator tissue damage?*. Ann Surg 2000; 231: *352-360*.
8) Aleynik SI, Leo MA, Aleynik MK, et al. A*lkohol-induced pancreatic oxidative stress: protection by phospholipid repletion*. Free Radic Biol Med 1999; 26: *609-619*.
9) Kim H, Seo JY, Roh KH, et al. *Suppression of NF-0B activation and cytokine production by N-acetylcysteine in pancreatic acinar cells*. Free Radic Biol Med 2000; 29: *674-683*.
10) Aksin M, Incebiyik A, Vural M, Gul Hilali N, Camuzcuoglu A, Camuzcuoglu H, Aksoy N. *Does a risky outcome of antenatal screening test indicate oxidative stress?* J Matern Fetal Neonatal Med. 2014; 27: *1033-1037*.
11) Gupta S, Agarwal A, Banerjee J, Alvarez JG. *The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: a systematic review*. Obstet Gynecol Surv 2007; 62: *335-347*.
12) Buyukhatipoglu H, Kirhan I, Vural M, et al. *Oxidative stres increased in healthcare workers working 24-hour on-call shifts*. Am J Med Sci. 2010; 340: *462-467*.

13) Banerjee AK, Steele RCJ. *Current views on the pathophysiology of acute biliary pancreatitis*. Gut 1995; 36: *803-805*.
14) CzakF L, Takacs T, Varga IS, et al. *Oxidative stress in distant organs and the effects of allopurinol during experimental acute pancreatitis*. Int J Pancreatol 2000; 27: *209-216*.
15) Erel O. *A novel automated method to measure total antioxidant response against potent free radical reactions*. Clin Biochem 2004; 37: *112-119*.
16) Steenland K, Thun M, Lally C, et al. *Environmental tobacco smoke and coronary heart disease in the American Cancer Society CPS-II cohort*. Circulation 1996; 94: *622- 628*.
17) Cao G, Prior RL. *Comparison of different analytical methods for 378 assessing total antioxidant capacity of human serum*. Clin Chem 379 1998; 44: *1309-1315*.
18) Byung Kyu Park, Jae Bock Chung, Jin Heon Lee, Jeong Hun Suh, Seung Woo Park, Si Young Song, Hyeyoung Kim, Kyung Hwan Kim, Jin Kyung Kang, *Role of oxygen free radicals in patients with acute pancreatitis*. World J Gastroenterol 2003; 9(10): *2266-2269*.
19) Schulz HU, Niederau C, Klonowski-Stumpe H, Halangk W, Luthen R, Lippert H. *Oxidative stress in acute pancreatitis*. Hepato-Gastroenterol 1999; 46: *2736-2750*.
20) Schoenberg MH, Birk D, Beger HG. *Oxidative stress in acute and chronic pancreatitis*. Am J Clin Nutr. 1995; 62: *1306-1314*.
21) Uruñuela A, Manso MA, de la Mano AM, de Dios I. *Cholecystokinin blockade triggers earlier and enhanced intra-acinar oxygen free radical generation in acute pancreatitis induced by pancreatic duct obstruction in rats*. Clin Sci. 2003; 105: *203-212*.
22) Sanfey H, Bulkley GB, Cameron JL. *The role of oxygen-derived free radicals in the pathogenesis of acute pancreatitis*. Am Surg 1984; 200: *405-413*.
23) Schoenberg MH, Büchler M, Beger HG. *Oxygen radicals in experimental acute pancreatitis*. Hepato-Gastroenterol 1994; 41: *313-319*.
24) Aslan M, Sabuncu T, Kocyigit A, et al. *Relationship between total oxidant status and severity of diabetic nephropathy in type 2 diabetic patients*. Nutr Metab Cardiovasc Dis. 2007; 17: *734-740*.
25) Schlesier K, Harwat M, Bohm V, Bitsch R. *Assessment of antioxidant activity by using different in vitro methods*. Free Radic Res 2002; 36: *177-87*.
26) Janaszewska A, Bartosz G. *Assay of total antioxidant capacity: comparison of four methods as applied to human blood plasma*. Scand J Clin Lab Invest 2002; 62: *231-6*.
27 Prior RL, Cao G. *In vivo total antioxidant capacity: comparison of different analytical methods*. Free Radic Biol Med 1999; 27: *1173-81*.
28) Schulz HU, Niederau C, Kolnowski-Stumpe H, et al. *Oxidative stress in acute pancreatitis*. Hepatogastroenterology 1999; 46: *2736-50*.
29) Bilton D, Schofield D, Mei G, et al. *Placebo controlled trials of antioxidant therapy including S-adenosylmethionine in patients with recurrent non-gallstone pancreatitis*. Drug Invest 1994; 8: *10-20*.

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