DOI: 10.4149/BLL_2017_027

Bratisl Med J 2017; 118 (3)

137 – 141

EXPERIMENTAL STUDY

Investigation of procalcitonin, IL-6, oxidative stress index (OSI) plasma and tissue levels in experimental mild and severe pancreatitis in rats

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ABSTRACT

AIM: This study was planned to evaluate blood and tissue levels of procalcitonin, IL-6 and Oxidative Stress Index (OSI), which have a role in the pathophysiology of inflammation, in rats with experimental mild and severe pancreatitis.

MATERIAL AND METHODS: Thirty male Wistar Albino rats were included in the study and the rats were equally divided into the three groups. After 1, 5 and 24 hours, blood was obtained from the tail of all rats and samples were taken from pancreas tissue after 24 hours. Amylase, lipase, AST, ALT, WBC, LDH, glucose, total bilirubin, direct bilirubin, GGT, ALP and TNF-α, Procalcitonin and IL-6 levels were evaluated from blood and tissue specimens.

RESULTS: The mild pancreatitis group (group 1) and the severe pancreatitis group (group 3) were compared according to the OSI, Amylase, Lipase, Pct, IL-6, AST, ALT, Glucose, WBC, LDH and Tnf-α levels. In the severe pancreatitis group, a statistically significant increase was detected compared to the mild pancreatitis group ($p < 0.05$). There was no statistically significant difference in TOS, T.Bil, D.Bil, GGT and ALP values ($p > 0.05$). Statistically significant increase was observed in severe pancreatitis group according to OSI, Amylase, Lipase, Pct, IL-6, LDH, WBC and TNF-α levels samples obtained from pancreatic tissues, compared to mild pancreatitis group ($p < 0.05$).

CONCLUSION: In conclusion, our study showed that OSI, TNF-α, IL-6 and procalcitonin levels increases proportionally with the severity of pancreatitis. This also suggests that OSI, TNF-α, IL-6 and procalcitonin are the guiding substances in acute pancreatitis, and that this damage increases as the duration and severity of the pathological process increase. However, this result must be confirmed with more detailed and broadly planned future studies *(Fig. 3, Ref. 25)*. Text in PDF *www.elis.sk.*

KEY WORDS: acute pancreatitis, procalcitonin, IL-6, TNF-α, amylase, lipase.

Introduction

Although acute pancreatitis (incidence 15–50/100,000) is not a very common disease, effective nutritional support is needed because the mortality rate is high in severe cases. With this support, the mortality rate of acute pancreatitis has been reduced from 30 % to 5–10 % over the last 30 years. While the mortality rate is 1.5 % in mild cases, it is 26 % in severe cases. According to literature, if necrosis develops in 50 % of pancreatic tissue, mortality rate is 50 %.

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Acknowledgement: This study was performed in Harran University Faculty of Medicine, Laboratory of Animal Experiments, after approval of the Ethics Committee (issue number 1184 and decree number 2011/10).

There are 300,000 new cases in the US each year, with 10–20 % serious cases and more than 3,000 deaths. Pancreatitis is responsible for 4,000 deaths annually and costs more than $ 2 million per year (1).

Most patients with pancreatitis have a relatively mild illness with parenchymal edema, no distant organ dysfunction, and recover without any problems. Beside this, severe pancreatic necrosis, systemic inflammatory response syndrome (SIRS), multiorgan failure, sudden clinical deterioration, and even death may occur in severe disease (2). Ten to twenty percent of AP patients have severe acute pancreatitis. Mortality rates in acute pancreatitis are 1–2 % in mild uncomplicated disease, 20% in sterile pancreatic necrosis and 30% in infected necrosis (3). As the main event in the pathogenesis of acute pancreatitis, early intraaciner activation of trypsinogen, which is a proteolytic enzyme, has been shown (4, 5). The potency to activate other enzymes in the zymogen granule structure allows tripsinogen to play a key role in the pathogenesis, even though it is not a strong proteolytic enzyme (4, 6).

There are many cytokines, chemokines and neuropeptides, that play an active role in pancreatitis. They demonstrate proinflammatory or antiinflammatory functions. The ones, which demonstrate proinflammatory functions, are interleukin (IL-1, IL-6), tumor

necrosis factor (TNF-α), platelet activating factor (PAF), MIP1-α; and functions as anti-inflammatory are C5a, IL-10 and IL-11. It is known that TNF-α plays a central role in sepsis, but its effect on acute pancreatitis is not fully explained. However, TNF-α secretion increases in patients who develop systemic complications. Serum levels of IL-1, IL-6 and IL-8 are significantly increased in patients with acute pancreatitis. The new diagnostic parameter that differs from the current inflammatory response indicators is procalcitonin (PCT). The induction amount and the plasma level of PCT is directly correlated with the inflammatory reaction.

Acute pancreatitis can develop in different grades. The current discussion is that acute pancreatitis begins with the activation of zymogens in the acinar cells, which cause acinar cell damage. Recent studies support that the severity of the pancreatitis pattern can be explained by the acinar cell damage. This includes the production and release of cytokines and other inflammatory mediators as well as the localization and activation of inflammatory cells (7).

Acute pancreatitis is basically considered as the activation of inactive pro-enzymes inside the pancreas and self-digestion of the gland (8). Interleukin (IL), tumor necrosis factor (TNF) and free oxygen radicals come out from the damaged acinus cells (9). Free radicals attract leukocytes to the area of inflammation.

Oxidative stress index is a proportional index, which is obtained by dividing the levels of total oxidants into the levels of total antioxidants and elevated levels of OSI occurs in situations with increased oxidative stress (10).

Mediators and cytokines released from leukocytes are destructive for the organism (11). The physiopathological processes of inflammation in experimental and clinical forms of acute pancreatitis have not been fully understood yet and there are ongoing debates and an extensive research in this field.

This study was planned to evaluate blood and tissue levels of procalcitonin, IL-6 and Oxidative Stress Index (OSI), which have a role in the pathophysiology of inflammation, in rats with experimental mild and severe pancreatitis.

Material and methods

This study was performed in the Harran University Faculty of Medicine, Laboratory of Animal Experiments, after approval of the Ethics Committee (issue number 1184 and decree number 2011/10).

Rats were kept at room temperature and in 12 hours of light and 12 hours of darkness before study. Thirty Wistar-albino rats weighing between 140–150 g were used in our study. All rats were fed with mains water and standard rat food, under standard conditions. Feeding of rats was stopped 8 hours before the intervention. Rats were divided into the 3 groups as the mild pancreatitis group (Group 1), severe pancreatitis group (Group 2) and operated control group (Group 3).

In the first group, 50 micrograms/kg and in the second group 80 micrograms/kg of cerulein was administered to the rats, one hour apart for a total of five times in the form of intraperitoneal infusion. The rats in the third group were given 0.1 ml serum physiological one hour apart for a total of five times, in the form of intraperitoneal infusion.

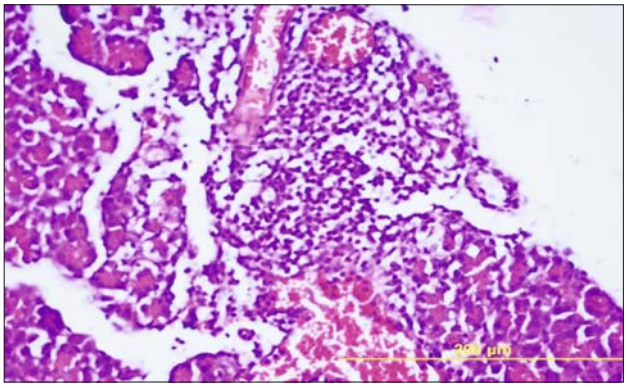

Fig. 1. Inflammatory elements are seen in the presence of mild pancreatitis (Hematoxylin–Eosin, x400).

After cerulein infusion, bloods were obtained from all rats at the end of 1, 5 and 24 hours and serums obtained were taken for biochemical analysis. The blood obtained from the rats was stored in heparinized biochemical tubes and centrifuged at 3000 rpm for 10 minutes to separate the serum and the samples were taken to the biochemical laboratory for storage at –80 °C until analysis.

At the end of 24 hours, rats were anesthetized. Ketamine and Xylazine were combined in anesthesia of rats. Ketamine was administered intravenously at a dose of 87 mg / kg intraperitoneally (Ketalar; Parke Davis, Eczacibaşi, Istanbul, Turkey) and Xylazine 13 mg/kg (Rompun; Bayer AG, Leverkusen Germany). The rats were given the right position. Laparotomy was performed after the abdominal area of the rats was disinfected.

In histopathological evaluation, the pancreatic tissue was examined in terms of acute inflammation findings. No pathological findings were observed in the pancreatic tissues of the control group. In the mild pancreatitis group, mild pancreatitis was found in all pancreatic tissues (Fig. 1). In the group with severe pancreatitis, moderate severe pancreatitis was observed in the entire pancreas.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) for Windows, version 16.0 (SPSS Inc. Chicago, IL, USA). The data were presented as the mean + standard deviation. The Kolmogorov–Smirnov test was used to assess the distribution of data. Independent samples t-test was used for comparison of the groups since the parameters were normally distributed in both groups. $p < 0.05$ was considered as statistically significant.

Results

Blood and tissue samples were studied biochemically. The results were evaluated statistically. First, the control group (group 3) and the mild pancreatitis group (group 1) was compared to evaluate if pancreatitis developed. There was a statistically significant increase ($p < 0.05$) in OSI, Amylase, Lipase, Pct, IL-6, AST, ALT, Glucose, WBC, LDH and TNF-α levels in mild pancreatitis group compared

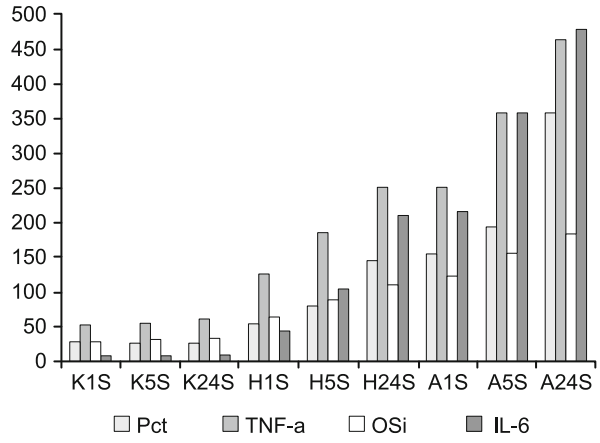

Fig. 2. Levels of Pct, TNF-α, OSI, IL-6 at 1, 5 and 24 hours in control, mild and severe pancreatitis group.

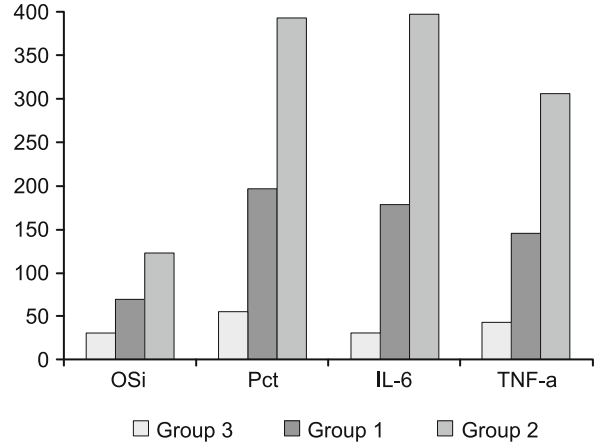

Fig. 3. Comparison of pancreatic tissue levels of OSI, Pct, IL-6 and TNF-α in control, mild and severe pancreatitis group.

to the control group. According to these results, it was observed that experimental pancreatitis was developed. There was an increase in T.Bil and D.Bil. levels in the mild pancreatitis group compared to the control group but this increase was not statistically significant ($p>0.05$). A decrease in total antioxidant status / level (TAS) was observed in mild pancreatitis group compared to the control group but this difference was not statistically significant ($p>0.05$).

As the result of evaluation of intergroup serum biochemical parameters at 1, 5 and 24 hours, the group II (mild pancreatitis) and group III (severe pancreatitis) amylase (U / L) and lipase (U / L) levels were higher compared to the control group and this was statistically significant ($p<0.05$). In comparison of the mild pancreatitis group and the severe pancreatitis group, the difference between GGT, ALP and T.Bil, D.Bil. levels was not statistically significant ($p>0.05$). In comparing the biochemical results of tissue in mild pancreatitis and severe pancreatitis, difference between T.Bil, D.Bil. and TAS levels was not statistically significant ($p>0.05$), but statistically significant increase was found in other biochemical results ($p<0.05$). There was a statistically significant increase ($p<0.05$) in OSI, Amylase, Lipase, Pct, IL-6, WBC and TNF-α levels from the samples taken from the pancreas tissues. Difference between T.Bil, D.Bil. and TOS levels were not statistically significant ($p>0.05$).

When the Pct, TNF-α, OSI, IL-6 levels of the control, mild and severe pancreatitis groups at 1, 5 and 24 hours were compared, a statistically significant increase was found ($p<0.05$) (Fig. 2). When the tissue levels of OSI, Pct, IL-6 and TNF-α were compared, a statistically significant increase was found ($p<0.05$) (Fig. 3).

Discussion

Acute pancreatitis is a localized inflammation of the pancreas at the beginning, which may lead to local and systemic complications later. Systemic inflammatory response, cytokines and oxidative stress constitutes the major components of pathophysiological events in the development of severe acute pancreatitis. However, it is still unclear why some acute pancreatitis episodes are mild while some are severe. There is a synergy between proinflammatory cytokines and oxidative stress in the development inflammatory response to acute pancreatitis. Proinflammatory cytokines and oxidative stress stimulate the inflammatory cascade by triggering similar signal transduction pathways. This is mainly due to mitogen-activated protein kinases, and nuclear factor kappa beta activation. Beyond this, proinflammatory cytokines, especially TNF-α and oxidative stress trigger each other, leading to a vicious cycle in acute pancreatitis (12).

It is known that proinflammatory mediators have an effect in the development of disease and complications. There are many cytokines, chemokines and neuropeptides that play an active role in pancreatitis. They demonstrate proinflammatory or antiinflammatory functions. The ones, which demonstrate proinflammatory functions are interleukin (IL-1, IL-6), tumor necrosis factor (TNF-α), platelet activating factor (PAF), MIP1-α; and the ones functioning as anti-inflammatory are C5a, IL-10 and IL-11. It is known that TNF-α plays a central role in sepsis, but its effect on acute pancreatitis is not fully explained. However, TNF-α secretion increases in patients with systemic complications. Serum levels of IL-1, IL-6 and IL-8 are significantly increased in patients with acute pancreatitis. A new diagnostic parameter that differs from the current inflammatory response indicators is the procalcitonin (PCT). The induction amount and the plasma level of PCT directly correlate with the inflammatory reaction.

Many theories have been suggested in the pathophysiology of acute pancreatitis. Beger et al reported that reactive O_2 radicals caused an impairment of cell membranes and functioned by its direct action on lipids and proteins in the early and late stages of acute pancreatitis, and damage to pancreatic cells by deregulation of lysosomal enzymes (13, 14). In our study, we found that pancreatic enzymes and oxidative stress factors were statistically significantly higher in the severe pancreatitis group than in the mild pancreatitis group.

Lerch et al have shown that acinar cell necrosis started within 3 hours after and fat necrosis, haemorrhage and inflammation developed within 12 hours after pancreatic duct ligation (15). His-

topathologic examination of pancreatic tissues from rats in our study revealed more intense inflammation and edema in the severe pancreatitis group than in the mild pancreatitis group.

In the study of Zilvinas et al, severe acute pancreatitis has been associated with increased pro-inflammatory cytokines [interleukin 6, interleukin 8, macrophage migration inhibitory factor (MIF)]. Concentrations of serum IL-6 and MIF are the most distinctive mediators in the early stages of severe and necrotizing acute pancreatitis. It is suggested that Interleukin 6 and macrophage migration inhibitory factor (MIF) could be used as early predictors of complications (16). We also found that IL-6 levels significantly increaseed in the severe pancreatitis group compared to the mild pancreatitis group.

Byung Kyu et al revealed that patients with acute pancreatitis have increased lipid peroxidation production and decreased vitamin C levels. A direct correlation between oxidative stress and severity of pancreatitis had been reported. Interstitial edema, acinar cell damage, hemorrhage, and necrosis cause acute pancreatitis at various degrees. The role of various factors (complement activation, cytokines, free oxygen radicals, ischemia, and pancreatic enzymes) is known in the pathogenesis of acute pancreatitis. However, the role of these factors remains unclear. The association between free oxygen radicals and disease has been shown in various experimental models and in patients with acute pancreatitis. In conclusion, oxygen-derived free radicals are closely related to inflammatory process and severity of acute pancreatitis, and lipid peroxide (LPO) plasma level may be a significant index to detect severity of the disease (17).

The study of Nurullah et al showed the importance of procalcitonin, which is the marker of systemic inflammation, in distinguishing edematous and severe acute pancreatitis and the parenchymal cells (cells of the liver, lung, kidneys, fat and muscle tissue) were found to be responsible for the increased rate of procalcitonin in circulation. Procalcitonin is a simple and practical indicator that can be used for early diagnosis and clinical prognosis of severe acute pancreatitis (18).

Anna et al revealed in their study that procalcitonin concentration was found to be significantly higher in patients with severe pancreatitis and found that there was a prognostic value between routinely measured parameters (total calcium concentration, lactic dehydrogenase activity, and glucose concentration) (19).

Reza et al showed that increased procalcitonin (PCT) levels were found to be closely related to inflammatory response of host to microbial infections and revealed that severity of procalcitonin (PCT) level was an early marker of acute pancreatitis patients with organ failure (20).

Caroline et al revealed changes in biochemical and histological parameters by induction of acute pancreatitis after application of intraperitoneal cerulein. Cerulein has also been shown to increase amylase and pancreatic weight / body weight ratio (5.944 ± 0.227 mg/g). TNF-α and IL-6 were significantly higher in cerulein-induced pancreatitis (21).

Ceranowicz et al revealed that, acute edematous pancreatitis developed in rats given intraperitoneal cerulein. Interlobuler and intralobular edema accompanied by perivascular diffuse leukocyte inflammation were observed using the light microscopy (22).

There are many experimental acute pancreatitis studies with the use of cerulein in literature. In these studies, cerulein was infused or applied i.p. to rats or mice at a dose of 5.0–7.5 g/kg / h and interstitial pancreatitis is created. In conclusion, cerulein-induced pancreatitis mimics the early period of acute edematous pancreatitis in humans (23).

Cerulein, a decapeptide, is an analog of cholecystokinin-pancreosime isolated from the skin of an amphibian (Australian frog) named Hyla caerulea (24, 25). Many studies revealed that edematous pancreatitis occurred when it was applied to rats i.p, i.v and subcutaneously. Rapidly invading lesions, noninvasiveness and similarity to human pancreatitis are shown as the reason for the selection of cerulein-induced pancreatitis models in literature and this model was used in our study.

On microscopic examination of the groups, normal pancreas tissue was observed in the group I. In the Group II, acute inflammation and edema was observed around the ductus, consisting of neutrophil polymorph and lymphocytes. In the group III, inflammation in pancreatic tissue and necrosis of aciner cells in a focal area was observed.

As already known, reasons that are responsible for etiopathogenesis of acute pancreatitis (gallstones, alcohol and trauma, drugs, infections, metabolic reasons and free oxygen radicals); create severe acinar cell damage in pancreatic tissue, intense interstitial edema and hemorrhage, and consequently inflammation occurs in the tissue. In our study, a statistically significant increase in serum and tissue Pct, OSI, IL-6, TNF-α levels at 1, 5, 24 hours in group II and group III seemed to support this data.

In this study, we investigated the relationship of TNF-a, procalcitonin, IL-6 and OSI levels with acute pancreatitis in mild and severe pancreatitis-induced rats. When the blood and tissue levels of procalcitonin, cytokine and oxidative stress index were compared in the pathophysiological processes of inflammation of acute pancreatitis, a statistically significant increase was found as the end result ($p<0.05$). While it may be used as an indicator of morbidity, clinical course and mortality of patients with acute pancreatitis, it may also allow for the intervention and understanding of pathophysiological processes of inflammation.

In order to determine the prognosis, many factors have been investigated and various scoring systems have been developed in accordance with these, but none have yielded a complete and definitive result in terms of prognosis. We believe that more extensive studies are needed for this opinion that oxidative stress factors, procalcitonin, and inflammatory markers may be another useful early prognostic factors for acute pancreatitis.

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Received November 16, 2016.
Accepted Novemver 28, 2016.