ORIGINAL ARTICLE

Serum Ischemia-Modified Albumin Levels in Experimental Model of Acute Pancreatitis

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ABSTRACT

Objective: To establish whether the Ischemia-Modified Albumin (IMA), a new parameter of oxidative stress, has diagnostic role in experimental acute pancreatitis.

Study Design: Randomized controlled trial.

Place and Duration of Study: Experimental Animal Center, Çanakkale Onsekiz Mart University, Çanakkale, Turkey, from May to September 2013.

Methodology: Sixteen Sprague-Dawley rats were randomly divided into two groups (n=8 each): Sham and AP groups. AP was induced by ligation of pancreatic duct. Serum IMA, amylase, lipase, AST, ALT and CRP were determined. The severity of pancreatitis was scored by a blinded pathologist under microscope.

Results: Serum IMA levels in the AP group increased significantly compared with the control group ($p < 0.05$). There was also a strong positive correlation between amylase and IMA levels.

Conclusion: The present study showed in a rodent model that serum IMA might serve as an additional marker to monitor inflammation during pancreatitis.

Key Words: *Amylase. Acute pancreatitis. Rodent. Ischemia-modified albumin.*

INTRODUCTION

Free oxygen radicals that occur during ischemia result in a conformational change of the N-terminus of albumin leading to decreased binding of cobalt. This newly formed albumin is called Ischemia-Modified Albumin (IMA). It is well known that IMA rises within minutes of the ischemic events and clinically useful in the early phases of ischemia before necrosis occurs.[1]

Acute Pancreatitis (AP) is an inflammatory process caused by the activation of digestive enzymes in the pancreas that normally synthesized and found in inactive forms. This activation causes self-digestion of the pancreas.[2] Recently, it was shown that excessive production of free oxygen radicals and variations of cytokines are effective in the pathogenesis of acute pancreatitis.[3] The most common used markers for diagnosis in AP are serum amylase and lipase levels. Phospholipase A2, trypsin, tripsinogen activation peptide and elastase are also increase in pancreatitis but not used widely in clinical conditions.[4] Although IMA was used as an ischemic marker primarily, previous studies have shown that it can also be used as a marker in oxidative stress conditions.[5,6]

This animal experiment was designed to determine the plasma levels of IMA as an inflammatory marker in acute pancreatitis.

METHODOLOGY

The study was done from May to September 2013 in Çanakkale Onsekiz Mart University (ÇOMU) Experimental Animal Center after animal care and all procedures were approved by the Animal Care Committee of ÇOMU. Sixteen Sprague-Dawley rats, weighing 200 - 250 g were housed under standard animal care conditions with free access to food and water and kept under controlled environmental conditions with a 12-hour light and dark cycle. The rats were randomly divided into two groups (n=8), one rat from case group, two rats from control group died and were excluded from the study. Group 1 was the Sham operation (Sham) group in whom only laparotomy was made had six rats. Group 2 was the acute pancreatitis group caused by pancreatic duct ligation and included seven rats.

All surgical procedures were performed using 50 mg/kg ketamine hydrochloride and 10 mg/kg xylazine, given intramuscularly before the procedures. Only median laparotomy was performed in group 1 whereas acute pancreatitis was made by ligation of pancreatic duct in group 2.[7] After 48 hours, animals were sacrificed by taking blood from the heart. Blood samples were

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separated immediately by centrifugation at 4000 rpm for 5 minutes and stored at -20°C for further analysis.

The albumin cobalt binding test was analyzed according to the method defined by Bar-Or *et al*.[8] In this method 200 μL serum was added to 50 μL of 0.1% (w/v) cobalt chloridine water solution. It was mixed gently and waited for 10 minutes for sufficient cobalt-albumin binding. Then 50 μL dithiothreitol (DTT) (1.5 mg/mL H_2O) was added as a colorizing agent. After waiting for two minutes 1.0 mL 0.9% NaCl was added to stop the cobalt binding process of albumin. Afterwards, the absorbance was measured in a spectrophotometer at 470 nm. A sample without DTT was used as a blank. The results were reported as absorbance units (ABSU). AST, ALT, amylase, lipase and CRP levels were also studied by standard biochemical methods.

Specimens from the pancreas were fixed in 10% formalin, embedded in paraffin and stained with hematoxylin-eosin. Pathological findings were assessed by one of the author blinded to group allocations. Acute pancreatitis was evaluated and documented in each tissue sections. Edema, inflammation, vacuolization and necrosis of pancreatic tissue were scored between 0 and 4 using a scale.[9]

Data were presented as mean and median (minimum-maximum) values. Data were analyzed using SPSS version 19.0 software (SPSS, Chicago, IL, USA.). Differences between the groups were compared using non-parametric Mann-Whitney U-test. Spearman correlation coefficient was used to determine the strength of the association between two continuous variables. A p-value < 0.05 was considered statistically significant.

RESULTS

One rat from case group, two rats from control group died and excluded from the study. Biochemical results of rats were summarized in Table I. The IMA level was higher significantly in the AP group compared to control group (p=0.004). Mean amylase, lipase and ALT values were also significantly higher in the group 2 (p < 0.05, Table I).

There was a strong positive correlation between IMA levels and amylase levels in AP group (Spearman's rho=+0.821, p=0.023).

In histopathological evaluation, normal pancreatic tissue was present in group 1 tissue samples. There was necrosis, severe neutrophilic infiltration and edema in large acinar cells and peripancreatic fat tissue in group 2 tissue samples (Figure 1).

DISCUSSION

The present study demonstrated that serum IMA levels were significantly higher in AP group compared to the control group. And also there was strong positive correlation between IMA and amylase levels.

Table I: Biochemical analysis of rats in groups.

Variables	Sham group (n=6) Median (min-max)	AP group (n=7) Median (min-max)	p*
ALT (U/L)	143.37 (127.64-201.15)	1272.85 (280.43-1941.51)	0.003
AST (U/L)	291.25 (109.10-430.50)	272.70 (132.40-569.40)	0.886
Amylase (U/L)	1398.50 (1032.00-1682.00)	2069.00 (921.00-2218.00)	0.032
Lipase (U/L)	11.90 (9.90-17.80)	17.50 (13.20-27.00)	0.032
CRP (mg/dl)	15.05 (12.80-22.30)	15.06 (11.02-23.05)	0.943
IMA	781.50 (715.00-933.00)	991.00 (854.00-1297.00)	0.004

**Mann-Whitney U-test.*

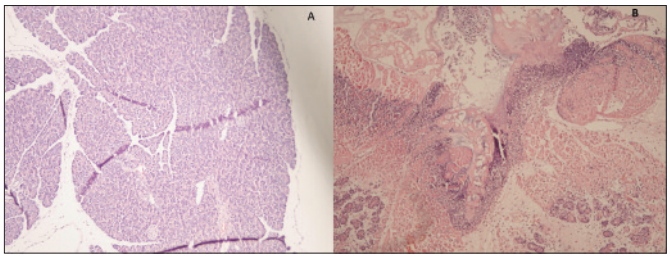

Figure 1: (A) Pathological slice of normal pancreatic tissue of Sham group. **(B)** Pathological specimen from acute pancreatitis group. There is necrosis and severe neutrophilic infiltration and edema in large acinar cells and peripancreatic fat tissue.

The pathogenesis of AP is not clearly enlightened yet. The most widely accepted hypothesis is self-digestion theory that argues activation of inactive proenzymes within the pancreas. These enzymes either directly harm the gland or the inflammatory response to them can cause systemic effects such as shock, respiratory distress, renal failure, cardiac arrhythmias and death. The first inflammatory events can cause acinar cell apoptosis or free oxygen radical production that lead to antioxidant loss and increase in acinar cell damage from ischemia reperfusion. Free oxygen radicals can also be directly toxic to acinar cells.[3]

The most common diagnostic criteria in AP are acute abdominal pain with three-fold increase in amylase levels.[10] Urine amylase, serum lipase, serum elastase 1, trypsin, phospholipase A_2, CRP, IL-6, IL-8, procalcitonin, ESR, AST and ALT levels can also rise in AP.

Amylase is still the most commonly used marker and preserves its diagnostic value. It increases in 6-12th hours and its half-life is 10 hours. Its sensitivity in the diagnosis of AP is 67 - 100% and specificity is 85 - 98%.[11,12] In non-complicated cases, elevation continues for 3-5 days. In this study, amylase levels were significantly higher in the AP group in the 48th hour. IMA levels were also significantly higher and had strong positive correlation with amylase levels.

The other common diagnostic marker, lipase, elevates in 4 - 8th hours, reach maximum at 24th hour and decreases at 8 - 14th days.[13] The sensitivity and the specificity both are 82 - 100%.[11,12] In a lot of study its specificity in AP was found superior to amylase

levels.[14,15] In this study, lipase levels were significantly higher in the AP group. In terms of IMA, there was no significant correlation with the lipase.

CRP is a non-specific but useful test in the identification of the severity AP. Zrnic *et al*.[16] showed that there is a correlation between CRP levels and the severity of the disease and it could be useful in prediction of the complications. Similarly, Schütte *et al*.[17] showed that CRP values together with ESR are predictive of the disease severity in first 24-hour of the disease. Generally, CRP levels reach the highest level at 48th hour. Its half-life is 19-hour. Therefore, it was suggested that the levels after 48th hour of symptoms are more significant from earlier values.[18] In this study, the 48th hour CRP levels in both AP group and control group increased but there was no statistical difference between groups. It was predicted to be higher in AP group but this situation was explained by excess inflammation due to laparotomy in control group.

In AP, hyperglycemia and increased transaminases can be detected. Transaminase increase along with amylase or lipase can be more useful in diagnosis.[4] Johnson *et al*. found that if the ALT levels are more than 150 IU/L it is compatible 95% with the biliary pancreatitis.[19] Coskun *et al*. found that mortality rates are higher in the cases with hyperglycemia and increased ALT.[20] Serum ALT levels of AP group were significantly higher in this study and IMA levels has significant and strong positive correlation with ALT.

In recent years, it has been suggested that free oxygen radicals are important in the beginning and progression of the AP.[3,9] When the balance between production and elimination of these free oxygen radicals is broken oxidative stress arises. It is now known that oxidative stress plays a primary role in the pathogenesis of AP.[9,21,22] According to the severity of the oxidative stress the pathologic changes can include cellular modulation, fibrosis, inflammation and irreversible cellular injury.[23] Recently, IMA is being considered as an oxidative stress marker. It is shown that IMA levels increased in diseases causing non-cardiac hypoxia and oxidative stress such as chronic kidney disease, hypercholesterolemia, systemic sclerosis and type 2 diabetes mellitus although the most common utilization is in early myocardial injury.[5,6,8,24,25] Therefore, increased IMA levels in AP group in this study was thought to be related with increased oxygen radicals rather than organ specificity.

CONCLUSION

IMA could be used as a diagnostic marker in AP, however, due to lack of tissue specificity it would be more convenient to use as a marker in the progression of diagnosed cases.

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