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The relation between serum cytokeratin 18 and acute pancreatitis: Can it be a serological predictive marker?

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Background/aims: *Acute pancreatitis is well defined as pancreatic inflammation due to the activation of pancreatic enzymes secondary to several etiological factors. In the majority of patients, the clinical symptoms are self-limited, but it can also cause tissue necrosis and severe organ failures. In experimental animal models, it has been shown that wide apoptotic cell death is related to a clinically mild presentation of acute pancreatitis. Cytokeratin 18, a cytoskeletal protein, is shown to be related with apoptosis. In this study, we aimed to show the relation between serum cytokeratin 18 and the clinical presentation of acute pancreatitis in humans.* ***Materials and Methods:*** *A total of 54 acute pancreatitis patients were enrolled into the study. Patients were classified as mild or severe pancreatitis according to Ranson's criteria. There were 36 (66.7%) patients in the mild pancreatitis group (score <6), and 18 (33.3%) patients in the severe pancreatitis group (score ≥6). During the first admission, blood samples were obtained for serum cytokeratin 18 levels.* ***Results:*** *Cytokeratin 18 levels in the mild pancreatitis group were significantly higher than in the severe pancreatitis group (271.2±45.5 vs. 152.6±38.2 IU/L; p<0.001). There was a negative correlation between the disease activity score (Ranson score) and the serum cytokeratin 18 levels (p<0.001; r= -0.724).* ***Conclusions:*** *This first human study suggests that cytokeratin 18 (marker of apoptosis) might be a serological predictive marker for acute pancreatitis for disease activity.*

Key words: Cytokeratin 18, acute pancreatitis, apoptosis

Serum sitokeratin 18 ile akut pankreatit arasındaki ilişki: Serolojik bir ön belirleyici olabilir mi?

Giriş ve Amaç: *Akut pankreatit farklı etyolojilere sekonder gelişen pankreatik enzimlerin aktivasyonu sonucu ortaya çıkan pankreatik inflamasyondur. Çoğu zaman kendini sınırlayan ve hafif klinik bulgularla seyreden bir tablo iken doku nekrozu ve ciddi organ yetmezliklerine de yol açabilir. Deneysel hayvan çalışmalarında apopitozisin akut pankreatit klinik seyri ile ilişkili olduğu ve apopitozisin arttığı durumlarda kliniğin hafif seyrettiği ve bir hücre iskelet proteini olan sitokeratin 18'in apopitozis ile ilişkili olduğu gösterilmiştir. Bu çalışmada insanlarda sitokeratin 18 ile akut pankreatit klinik seyri arasındaki ilişkiyi araştırdık.* ***Gereç ve Yöntem:*** *Toplam 54 akut pankreatitli hasta çalışmaya dâhil edildi. Ranson kriterleri kullanılarak hastalar hafif pankreatit (hafif pankreatit grup; skor<6) ve ağır pankreatit (ağır pankreatit grup; skor≥6) olmak üzere iki gruba ayrıldı. 36 hasta (%66,7) hafif pankreatit; 18 hasta (%33,3) ağır pankreatit grubunda yer aldı. Hastaların ilk müracaatında sitokeratin 18 için serum örnekleri alındı.* ***Bulgular:*** *Hafif pankreatit grubunda serum sitokeratin 18 düzeyleri ağır pankreatit grubuna göre anlamlı olarak daha yüksekti (271,2±45,5 vs. 152,6±38,2 IU/L; p<0.001). Ayrıca serum sitokeratin 18 düzeyleri ile Ranson skoru arasında da negatif korelasyon gözlendi (p<0.001; r= -0.724).* ***Sonuç:*** *İnsanlarda yapılan bu ilk çalışma apopitozisin bir göstergesi olan serum sitokeratin 18 düzeylerinin akut pankreatit klinik seyrini önceden belirlemede bir parametre olabileceğini düşündürmektedir.*

Anahtar kelimeler: Sitokeratin 18, akut pankreatit, apopitozis

INTRODUCTION

Acute pancreatitis is well-defined as pancreatic inflammation due to the activation of pancreatic enzymes secondary to several etiological factors (1,2). In the majority of patients, the clinical symp-

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toms are self-limited, and it heals without any systemic or local complications. The other cases present as severe clinical presentation, and organ failures can be seen in such situations. Pancreatic necrosis is a result of this type of pancreatitis (3). Biliary stones and alcohol are the major etiological factors. A few classification criteria and system are used for acute pancreatitis clinically. The most commonly used for defining the clinical presentation of acute pancreatitis are Ranson's criteria (4) (Table 1). There is a relationship between the Ranson score and disease severity and prognosis. A Ranson score of <3 indicates the expected mortality is very low, of 3-6 indicates an expected mortality between 10-20%, and of ≥6 indicates an expected mortality of about 50% (5).

Apoptosis is defined as genetically controlled and programmed cell death. It was first described by Kerr et al. in 1972 (6). Apoptosis may be induced by intrinsic as well as extrinsic stimulants (7-9). In experimental animal models, it has been shown that wide apoptotic cell death is related to a clinically mild presentation of acute pancreatitis. In these models, in subjects with clinically severe acute pancreatitis, there were wide series of cellular necrosis but limited apoptosis. Therefore, they concluded that induction of the apoptotic cascade in the acinar cells is related to clinically mild acute pancreatitis. In experimental pancreatitis series, induction of apoptosis is shown to be related with the degree of improvement, while inhibition is related with the severity of the clinical presentation (10-12).

Cytokeratin 18 (CK18) is a cytoskeletal protein. It is located in the pancreas, liver, biliary system, and small intestine (13). It is released during the hepatic and pancreatic apoptosis as a substrate of caspases. CK18 and CK8 are released together by the exocrine acinar and endocrine islet cells. In experimental studies, it was shown that increased apoptosis was associated with increased CK18 release (14,15). Caspases use CK18 as substrate, and fragmented CK18 exists, but is only related with apoptosis (16). This fragmented CK18 is referred to as MD30 antigen.

In this study, we aimed to show the relation between serum CK18 (fragmented CK18 - MD30 antigen) and the clinical presentation of acute pancreatitis in humans. We hypothesized that if apoptosis is important in clinically mild disease and if CK18 is the marker of apoptosis, then we could use CK18 as a clinically predictive factor of clinical presentation in acute pancreatitis. We hypothesized that in clinically mild disease, because apoptosis is a major cell death pathway, the serum CK18 - serological marker of apoptosis - levels should be lower.

MATERIALS AND METHODS

This prospective study was done in Gaziantep University School of Medicine, Department of Gastroenterology, after obtaining the approval of Gaziantep University's ethics committee. After obtaining the informed consent of the patients, a total of 58 acute pancreatitis patients were enrolled in the study. The exclusion criteria were acute onset of chronic pancreatitis, post endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis, chronic viral hepatitis, alcoholic hepatitis and other causes of chronic hepatitis, diabetes mellitus, and malignancy history.

Table 1. Ranson's Criteria

0 hour	
Age	>55 years
White blood cell count	>16000/mm^3
Blood glucose	>200 mg/dl
Lactate dehydrogenase (LDH)	>350 U/L
Aspartate aminotransferase (AST)	>250 U/L
48th hour	
Hematocrit	Fall by >10%
Blood urea nitrogen	Increase by >5 mg/dl despite fluid
Serum Ca^{++}	<8 mg/dl
pO2	<60 mmHg
Base deficit	>4 mEq/L
Fluid sequestration	>6 L

Adapted from Ranson JH et al. Surg Gynecol Obstet 1974; 139: 69-81.

The demographic parameters, hospital stay, accompanying diseases, comorbidities, and organ failures during the hospital stay were recorded prospectively.

When the patients were hospitalized, total blood count, biochemistry analysis including liver function tests, amylase, serum electrolytes, and blood glucose levels were obtained. The tests were repeated 48 hours after the admission in order to define the Ranson criteria (Table 1). All patients were evaluated according to Ranson criteria, and were divided into two groups. The patients with a Ranson score <6 were accepted as the mild pancreatitis (MP) group, and patients with a score of ≥6 were accepted as the severe pancreatitis (SP) group.

During this first admission, blood samples from the peripheral vein were obtained in order to measure serum CK18 levels. The samples were centrifuged at 5000 rpm for 10 minutes and the serums were separated (Eppendorf Centrifuge 5810, Eppendorf AG Hamburg, Germany). These serum samples were stored at -80°C. Hemolyzed serum samples were excluded from the study. Four patients were excluded from the study due to hemolyzed serum samples. After collecting all the samples, serum CK18 was measured with ELISA method (ELX 800 Universal Microplate Reader. Bio-Tek Instruments Inc, VT, USA) by using the M30-ELISA kit (Peviva AB, M30 Apoptosense Elisa Ref 10010, Strömkarlsvagen, Bromma, Sweden), which is produced for detecting the fragmented CK18 by caspases (CK18Asp396-NE:M30 neo-epitope). Serum CK18 levels were measured after all the patients were managed in the standard manner and clinical follow-up was terminated.

The Statistical Package for the Social Sciences (SPSS) for Windows 11.5 software program was used for statistical analysis. Values are given in mean ± standard deviation. For comparison of two independent groups, Student t test and Mann-Whitney U tests were used, and for more than two independent factors, ANOVA test was used. Chi-square test was used for calculating the relations between continuous variables and relation analysis. A value of $p<0.05$ was considered as statistically significant.

RESULTS

In this study, we had 58 patients with acute pancreatitis. Four hemolytic serum samples were excluded from the study. Thirty-two of remaining 54 patients were male (59.3%) and 22 were female (40.7%). The mean age of the patients was 59.6±20, with a range of 20-90 years. Mean CK18 levels between males and females were not statistically different (240.6±68.6 vs. 225.5±72.8 U/L respectively; $p>0.05$).

When the etiological factors were evaluated, it was found that 35 of 54 patients (64.8%) had biliary and the remaining 19 patients (35.2%) had non-biliary etiology. Mean CK18 levels were not statistically different between the different etiological causes as biliary or non-biliary (234.4±74.9 vs. 226.6±64.1 U/L respectively; $p>0.05$).

Thirty-six patients (66.7%) were classified as MP group (Ranson score <6) and 18 patients (33.3%) as SP group (Ranson score ≥6). In the MP group, 15 patients (41.7%) were male and 21 (58.3%) were female. The mean age was 55.4±19.9. In the SP group, 7 patients (38.9%) were male and the mean age was 66.9±18.3. There were no statistically significant differences in gender or age between groups. Demographic and clinical variables are shown in Table 2. Mean Ranson score of all patients was 3.7±2.3. The score was 2.2±0.9 in the MP group and 6.7±0.7 in the SP group. Mean CK18 levels were 271.2±45.5 in the MP group and 152.6±38.2 IU/L in the SP group. This difference was statistically significant ($p<0.001$). There was a negative correlation between the Ranson score and the serum CK18 levels ($p<0.001$; $r= -0.724$).

The mean hospital stay was 4.3±1.9 days in the MP group and 11.6±5.8 days in the SP group. This

Table 2. The demographic and clinical variables according to groups

		MP Group (n=36)	SV Group (n=18)	p
Gender	M	21 (58.3%)	7 (38.9%)	NS
	F	15 (41.7%)	11 (61.1%)	NS
Age		55.4±19.9	66.9±18.3	NS
Continuing abdominal pain (after 48 hours of hospitalization)		7 (19.4%)	14 (77.8%)	<0.001
Continuing fever (after 48 hours of hospitalization)		6 (16.7%)	11 (61.1%)	=0.001

difference was also significant (p<0.001). There was a negative correlation between the hospital stay and the serum CK18 levels; thus, serum CK18 levels were higher in shorter hospital stay patients. This correlation was also significant (p<0.001; r= -0.591).

Patients were evaluated at the 48th hour of their hospital stay according to their continuing abdominal pain and fever. There were 7 (19.4%) patients in the MP group and 14 (77.8%) in the SP group with continuing pain (p<0.001). Six (16.7%) patients in the MP group and 11 (61.1%) in the SP group had continuing fever (p=0.001) (Table 2). In the pain-free group, the serum CK18 levels were higher than in the sustained pain group (249.9±62.8 vs. 202.9±74.6 IU/L, respectively; p=0.01). Serum CK18 levels were also higher in patients without fever than the others (247.1±62.5 vs. 198.6±78.1 U/L, respectively; p=0.007).

Eleven of 54 patients (20.3%) were managed in the intensive care unit (ICU). All these patients were in the SP group. Mean serum CK18 levels of patients managed in the ICU was 146.9±31.4 U/L and of patients managed outside the ICU was 253.4±60.8 U/L, and this difference was significant (p<0.001).

DISCUSSION

Acute pancreatitis is not an uncommon disease and can carry severe morbidity and also mortality. The clinical presentation of acute pancreatitis differs from mild disease to severe pancreatic necrosis. Thus, in clinical practice, patients may have mild abdominal pain or multiorgan deficiencies. The events that regulate the severity of acute pancreatitis are, for the most part, unknown. It is generally believed that the earliest events in acute pancreatitis occur within acinar cells and result in acinar cell injury. The acinar cell response to injury may, itself, be an important determinant of disease severity.

In experimental animal studies, it has been shown that apoptosis in acinar cells had a protective effect for pancreatic necrosis. Histopathologic severity of pancreatitis was reduced with the induction of apoptosis (10,11). On the other hand, inhibition of apoptosis worsened the clinical and pathologic presentation of acute pancreatitis (13). According to these studies, it is postulated that induced apoptosis in acinar cells more than the necrotic pathway may improve the severity of acute pancreatitis.

Bhatia et al. (17) showed in experimental models that mild pancreatitis is related to extensive apoptotic acinar cell death. They showed extensive acinar cell necrosis rather than apoptosis in clinically severe pancreatitis. They thus hypothesized that apoptosis could be a favorable response to acinar cells and that favoring induction of apoptotic, as opposed to necrotic, acinar cell death might reduce the severity of an attack of acute pancreatitis.

Mareninova et al. (18) studied the relation between cell death pathway and the disease severity. In their experimental study, they showed that apoptosis induced with caspases protects the pancreas from necrosis and severe disease. In other studies, it was also shown that the severity of experimental pancreatitis directly correlates with the extent of necrosis and inversely with that of apoptosis (19).

In this study, we aimed to show the correlation between serum CK18 levels (which is a marker of apoptosis) and the clinical presentation of acute pancreatitis in clinical practice. This is the first human study on this topic.

In different studies, the CK18 levels were shown to be correlated with disease severity and pancreatic injury in acute pancreatitis (14,20). Thus, some authors postulated that increased expression of CK18 might have a protective effect on the pancreatic injury (21).

In our study, we measured serum CK18 (fragmented CK18-MD30 antigen) levels on the first day of the hospital stay of acute pancreatitis patients in order to define if CK18 has a role in the clinical presentation of acute pancreatitis and if it can be used as a predictive serological marker of disease severity. We found a negative correlation between the disease activity score (Ranson score) and the serum CK18 levels (p<0.001; r= -0.724). It also had a negative correlation with hospital stay (p<0.001; r= -0.591). Serum CK18 levels were also lower in patients who were managed in the ICU (p<0.001).

To our knowledge, there has been no human study done previously evaluating the serum CK18 levels and the disease severity in acute pancreatitis patients. Our results showed that there is a negative correlation between disease severity and serum CK18 levels. CK18 levels were lower in mild disease than in severe onset of disease. It can thus be thought that CK18 might be a serological predictive marker for acute pancreatitis for disease acti-

vity. As a result, the serum CK18 level of a patient with acute pancreatitis in the first admission may be useful in estimating the disease severity. A high CK18 level may be related with mild disease and lower levels may be related with severe disease. Of course, the weak point of this study is the small number of patients in groups to define a cut-off value of CK18. Furthermore, absence of a healthy control group may be a handicap for this study. Nevertheless, the aim of this study was to show the correlation between the serum CK18 levels and the clinical presentation of the disease. Other studies with larger patient groups and with a control group may be able to demonstrate whether CK18 may be used as a serological predictive marker for acute pancreatitis. However, as mentioned before, this is the first human study on this topic and it may guide further studies.

In conclusion, apoptosis/necrosis ratio in acute pancreatitis has a major role in the clinical presentation. Apoptosis is shown to have a protective effect on pancreatic injury. Thus, in mild disease the apoptosis pathway, and in severe disease the necrosis pathway, is dominant. We aimed to study the relation between the disease activity and the apoptotic serological marker serum CK18 levels. It was shown that the serum CK18 levels were higher in mild than in severe disease. Thus, in future, serum CK18 might be used as a predictive factor for estimating the disease severity in acute pancreatitis.

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