The relationship between serum histon levels and the severity of acute pancreatitis

Elmas Biberci Keskin[1], Ali Tüzün İnce[1], Bilge Sümbül Gültepe[1,2], İbrahim Hakkı Köker[1], Hakan Şentürk[1]

[1]Department of Gastroenterology, Bezmialem Vakıf University School of Medicine, İstanbul, Turkey
[2]Department of Microbiology, Bezmialem Vakıf University School of Medicine, İstanbul, Turkey

Cite this article as: Biberci Keskin E, İnce AT, Sümbül Gültepe B, Köker İH, Şentürk H. The relationship between serum histon levels and the severity of acute pancreatitis. Turk J Gastroenterol 2019; 30(9): 807-10.

ABSTRACT

Background/Aims: Despite various scoring systems and imaging methods, it is hard to predict the severity and the course of acute pancreatitis (AP), thereby necessitating better and more reliable markers. Since inflammation plays a key role in the pathogenesis of AP, we sought to determine whether histone, which is a novel inflammatory marker, may play a role in the prediction of severity and prognosis.

Materials and Methods: A total of 88 consecutive adult patients (>18 years) with a first AP episode were prospectively enrolled in the study. Severe AP was defined as having a revised Atlanta score >3 in the first 48 h after admission. Circulating histone 3 and 4 levels were measured using the enzyme-linked immunosorbent assay method.

Results: Eighty-eight consecutive adult patients with a first episode of AP were divided into two groups according to severity, in which 56 (63.6%) were assigned to the mild AP group and 32 (36.4%) to the severe AP group. White blood cell, hemoglobin, creatinine, and aspartate aminotransferase levels were significantly higher in the severe AP group. However, there was no difference in serum histone levels between the groups, and there was no correlation between revised Atlanta score and serum histone levels either.

Conclusion: Serum histone levels did not significantly differ between the severe and mild AP groups. Therefore, these markers may not provide additional benefit for determining the severity of AP.

Keywords: Acute pancreatitis, histone, pancreatic complications

INTRODUCTION

Acute pancreatitis (AP) is an inflammatory disease that is classified into three categories: mild, moderate, and severe. The mild form has a self-limiting course without accompanying organ failure or systemic complications, the moderate form involves transient organ failure (<48 h) or limited local complications (i.e., pancreatic necrosis and fluid collection), and the severe form is described as having persistent organ failure (>48 h) accompanied by further local and systemic complications (1). Despite advances over the last decades, AP still has a high risk of in-hospital mortality, particularly in severe cases, due to the lack of a specific treatment regimen (2).

Nucleosome, a subunit of nuclear chromatin, contains a central core protein formed by histone and DNA (3). This nucleosome is demonstrated to be released in extensive cell damage or death. An increase in nuclear proteins during the course of different conditions, such as sepsis, has been reported. The mechanism by which nucleosomes are released to the circulation is linked with neutrophil-related cell death at infection and inflammation sites (4).

The inflammatory cascade in AP is thought to have its origin by activation of pancreatic proteases that cause acinar cell apoptosis and necrosis. Pancreatic acinar cell damage or death releases nucleosome components that contain DNA and histones into the circulation (5). These nucleosomes (DNA and histone) exhibit proinflammatory activity and trigger the accumulation of immune cells into the pancreas, leading to the generation of cytokines and other soluble mediators of inflammation, which are held responsible for the development of remote organ injury (6). A recently published study demonstrated that these circulating histones that are released into the systemic circulation during AP because of pancreatic cell damage may be useful biomarkers for diagnosis, prediction of in-hospital course, and guiding therapy (7). Furthermore, serum histone levels may be related to prognosis and mortality. Therefore, the aim of the present study was to investigate the relationship between serum histone levels and the severity, prognosis, and in-hospital mortality of patients with AP.

MATERIAL AND METHODS

The study protocol was approved by the local ethics committee of Bezmialem Vakif University. Written in-

Corresponding Author: **Elmas Biberci Keskin; elmasbiberci@yahoo.com**
Received: **August 11, 2018** Accepted: **December 13, 2018**

DOI: **10.5152/tjg.2019.18592**

formed consent for the procedure was obtained from all patients. A total of 88 consecutive adult patients with AP who were admitted to our emergency internal medicine department from September 2017 to March 2018 were prospectively enrolled in the study. The age of the patients were >18 years. The diagnosis of AP was established if a patient had the presence of two out of the three following criteria: (1) constant upper abdominal pain, (2) increased serum amylase and/or lipase levels at least three times above the normal value, and (3) characteristic findings from abdominal imaging (transabdominal ultrasound (US) and/or contrast-enhanced computed tomography (CT)). Alcohol-induced AP was defined as daily consumption of alcohol >30 g for men, 20 g for women, or 50 g/day 1 month prior to hospitalization after exclusion of gallstones in at least two of the following examinations: US, contrast-enhanced CT scan, and magnetic resonance imaging (MRI) in accordance with the guidelines for alcohol consumption as published by the Danish Medical Health Authorities (8). Gallstone-induced AP was defined as serum aspartate aminotransferase (AST) level >150 U/l in combination with the presence of gallstones or sludge identified via US, MRI, or endoscopic retrograde cholangiopancreatography. The severity of AP was determined according to both revised Atlanta criteria (moderate and severe AP were analyzed together because of the low number of patients with severe AP) and Japanese Severity Score (1,9). Primary end-point was defined as in-hospital mortality or recurrent pancreatitis.

Patients with pancreatic calcifications, cysts, or other signs of chronic pancreatic defects; history of chronic liver or renal failure, congestive heart failure, coronary artery disease, or acute infection (within the previous 14 days); the presence of any chronic inflammatory and autoimmune diseases; and known malignancy were excluded from the study.

Blood samples for histone were obtained from the antecubital peripheral vein and collected into tubes containing calcium-EDTA within 24 h of admission. Serum was separated by centrifugation at 3000g for 20 min and stored at −80°C until later analysis. Serum histone levels were determined by enzyme-linked immunosorbent assay method (Bester, Wuhan, China).

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences for Windows software, version 21 (IBM Corp.; Armonk, NY, USA) for statistical analysis. Kolmogorov-Smirnov test was used for the normality of distribution of data. Continuous variables were expressed as mean±standard deviation, whereas categorical variables were presented as numbers and percentages. Student's t and nonparametric Mann-Whitney U tests were used to determine the differences between values for normally and non-normally distributed variables, respectively. Categorical variables were reported as percentages and were analyzed by either chi-square test or Fisher's exact test, as appropriate. Pearson rank tests were used to indicate the correlation of the severity of AP and histone levels. A two-tailed $p \leq 0.05$ was considered as significant.

RESULTS

Eighty-eight consecutive adult patients (>18 years) with AP were divided into two groups according to the severity of AP, in which 56 (63.6%) of them were assigned to the mild AP group and 32 (36.4%) to the non-mild AP group. Gallstones were the cause of AP in 52 (59.1%) patients, and other causes (e.g., alcohol, cancer, and hypertriglyceridemia) were found in the remaining 36 (40.9%) patients. No patient underwent an open surgical treatment.

Table 1. Baseline clinical characteristics, laboratory values and mortality of the study patients.

	Mild pancreatitis (n=56)	Severe pancreatitis (n=32)	p
Age, years	50.4±13.8	53.7±16.1	0.31
Female n, %	33(59.8)	17(53.1)	0.59
Etiology, n, %			
Gallstone	37(66.1)	15(46.9)	0.07
Others	19(33.9)	17(53.1)	
Organ failure n, %	-	2(6.2)	
Glucose (mg/dL)	139.2±64.8	172.7±106.1	0.07
WBC count (10^3/L)	10.597±3894	15013±7610	0.001
Hgb, g/dL	13.3±1.5	14.7±1.6	0.03
PLT count (10^3/L)	243±71	225±73	0.38
Cr, mg/L	0,9±0,3	1,2±0,6	<0.01
CRP, mg/L	2.6±3.9	5.9±8.5	0.10
ALT, U/L	262.9± 40.1	130.1±24.4	0.09
AST, U/L	111±19.1	239±40.2	0.02
GGT, U/L	250.7±33.7	251±44.7	0.72
Amylase, U/L	1064.2±143.3	1615±277.1	0.08
Lypase, U/L	3062.9±608.1	4149.9±825.4	0.28
Histone 3	0.33±0.24	0.31±0.19	0.65
Histone 4	0.59±0.41	0.71±1.01	0.46
In-hospital mortality	0	2 (6.2%)	0.14
30-day mortality	0	2 (6.2%)	0.14

The study patients' demographic and clinical characteristics are shown in Table 1. Leukocyte, hemoglobin, and AST were significantly higher in the severe AP group than in the mild AP group (p=0.31). However, the groups did not differ significantly with regard to gender and age. In addition, there was no difference in serum histone levels between the groups, and there was also no correlation between revised Atlanta score and serum histone levels.

Two (2.2%) patients in the severe AP group died during hospitalization due to multiorgan failure, and no mortality was detected within the first month following discharge. Serum histone levels were not higher in patients who suffered primary end-point (p=0.52). In addition, there was no correlation between revised Atlanta score and serum histone levels either (Table 2).

When Japanese Severity Score was used to define severity, there was still no difference between serum histone levels and the severity of AP (Table 3).

DISCUSSION

In the present study, there was no relationship between the severity of AP and serum histone levels. In addition, these biochemical markers did not predict death or recurrent AP.

Table 2. Correlation analysis of serum histone levels with Revised Atlanta and Japanese Severity Scores.

		Correlation co-efficient	p
Histone 3	Revised Atlanta Score	r: -0.102	0.34
	Japanese Severity Score	r: -0.096	0.37
Histone 4	Revised Atlanta Score	r:-0.111	0.33
	Japanese Severity Score	r: -0.078	0.47

Prediction of severity plays an important role in the management of AP. Severe AP occurs in approximately 15%-20% of patients (10). The early recognition of such patients is crucial to prevent morbidity and mortality. The 50% mortality rate of acute severe pancreatitis may well be decreased to 8% with a timely recognition and initiation of appropriate therapy (11).

It is difficult for physicians to predict which patients with AP will develop severe symptoms. Although several scoring systems have been developed to predict the severity of AP, these have limitations and provide little additional information in the assessment of these patients (12). Imaging techniques cannot reliably establish severity in the early period, given that necrosis is usually absent upon admission and may only occur after 48 h (13). Since scoring systems and imaging methods could not provide sufficient information to determine the severity of AP, there is a need for other parameters. Since the inflammatory process is the basic pathophysiology in AP, several inflammatory markers have been investigated so far. All inflammatory mediators, such as tumor necrosis factor-α, growth-related oncogene-α/cytokine-induced neutrophil chemoattractant, intercellular adhesion molecule-1, interleukin (IL)-1, IL-6, IL-8, IL-10, platelet-activating factor, monocyte chemoattractant protein-1, complement component C5a, substance P, hydrogen sulfide, and neutral endopeptidase, were investigated (14,15). However, these mediators are not practically available or lack accuracy for severity predictions in patients with AP.

Histones are components of nucleosomes that are released into the circulation from damaged cells and can promote inflammation. Several studies that investigated the plasma concentrations of histone in experimental animal models and patients have demonstrated that it plays a key role in the development of AP (16,17). In a study that

Table 3. Serum Histon levels based on etiology and Japanese Severity Score.

		MILD (n=47, 53.4%)	SEVERE (n=41, 46.6%)	p
Mean Japanese Severity Score		0.8±0.4	3.7±0.5	<0.01
Histone 3	Entire group	0.06±0.19	0.04±0.05	0.37
	Biliary etiology (n=52, 59.1%)	0.08±0.22	0.02±0.03	0.22
	Non-biliary etiology (n=36, 40.9%)	0.01±0.02	0.05±063	0.55
Histone 4				
	Entire group	0.10±0.13	0.09±0.12	0.76
	Biliary etiology (n=52, 59.1%)	0.10±0.14	0.11±0.10	0.89
	Non-biliary etiology (n=36, 40.9%)	0.09±0.10	0.08±0.14	0.71

investigated the role of histone for predicting the severity of AP, circulating histone concentrations were found to be significantly higher in mice with severe AP than in those with mild AP. It also reported an early increase in histone concentration which was within the first 24 h of disease onset (7). Another study investigating the correlation between on-admission serum histone levels and the development of severe AP found that on-admission nucleosome levels were significantly higher in severe AP than in mild or moderate AP (17).

In our study, serum histone levels did not differ between severe and mild pancreatitis. The most common etiology for AP in our study patients was gallstones which contrasted previous published studies in which alcohol consumption constituted the majority of the etiology. The degree and the pathophysiology of inflammation may vary according to the etiology, and this might have affected our results.

Our study had several limitations. First, the sample size was relatively small and without any control group. Second, a single serum histone measurement may not reflect the long-term course.

Serum histone levels did not differ significantly between severe AP and mild AP. Therefore, these markers may not provide additional benefit for determining the severity of AP.

Ethics Committee Approval: Ethics committee approval was received from the Ethics Committee of Bezmialem Vakif University School of Medicine.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - E.B.K.; Design - İ.H.K.; Supervision - H.Ş.; Materials - B.S.G.; Data Collection and/or Processing - H.Ş.; Analysis and/or Interpretation - M.B.; Writing Manuscript - E.B.K.; Critical Review - A.T.İ.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Banks PA, Bollen TL, Dervenis C, et al. Pancreatitis-Classification Working Group: Classification of acute pancreatitis - 2012: revision of the Atlanta classification and definitions by international consensus. Gut 2013; 62: 102-11. [CrossRef]
2. Goldacre MJ, Roberts SE. Hospital admission for acute pancreatitis in an English population, 1963 - 98: database study of incidence and mortality. BMJ 2004; 328: 1466-9. [CrossRef]
3. Oudet P, Gross-Bellard M, Chambon P. Electron microscopic and biochemical evidence that chromatin structure is a repeating unit. Cell 1975; 4: 281-300. [CrossRef]
4. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. Science 2004; 303: 1532-5. [CrossRef]
5. Nieminen A, Maksimow M, Mentula P, et al. Circulating cytokines in predicting development of severe acute pancreatitis. Crit Care 2014; 18: R104. [CrossRef]
6. Hoque R, Malik AF, Gorelick F, et al. Sterile inflammatory response in acute pancreatitis. Pancreas 2012; 41: 353-7. [CrossRef]
7. Szatmary P, Liu T, Abrams ST, et al. Systemic histone release disrupts plasmalemma and contributes to necrosis in acute pancreatitis. Pancreatology 2017; 17: 884-92. [CrossRef]
8. Danish Medical Health Authorities. Alkohol. Sundhedsstyrelsen, Danish. Available from: URL: http://www.sst.dk/.
9. Ueda T, Takeyama Y, Yasuda T, et al. Utility of the new Japanese severity score and indications for special therapies in acute pancreatitis. J Gastroenterol 2009; 44: 453-9. [CrossRef]
10. Banks PA, Freeman ML. Practice guidelines in acute pancreatitis. Am J Gastroenterol 2006; 101: 2379-400. [CrossRef]
11. Werner J, Feuerbach S, Uhl W, Büchler MW. Management of acute pancreatitis: From surgery to interventional intensive care. Gut 2005; 54: 426-36. [CrossRef]
12. Tenner S. Initial management of acute pancreatitis: critical decisions during the first 72 hours. Am J Gastroenterol 2004; 99: 2489-94. [CrossRef]
13. Balthazar EJ, Robinson DL, Megibow AJ, Ranson JH. Acute pancreatitis: value of CT in establishing prognosis. Radiology 1990; 174: 331-6. [CrossRef]
14. Bhatia M, Moochhala S. Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. J Pathol 2004; 202: 145-56. [CrossRef]
15. Bhatia M, Wong FL, Cao Y, et al. Pathophysiology of acute pancreatitis. Pancreatology 2005; 5: 132-44. [CrossRef]
16. Ou X, Cheng Z, Liu T, Tang Z, Huang W, Szatmary P, et al. Circulating histone levels reflect disease severity in animal models of acute pancreatitis. Pancreas 2015; 44: 1089e95. [CrossRef]
17. Penttila AK, Rouhiainen A, Kylanpaa L, Mustonen H, Puolakkainen P, Rauvala H, et al. Circulating nucleosomes as predictive markers of severe acute pancreatitis. J Intensive Care 2016; 4: 14. [CrossRef]