Level of serum soluble Tim-3 expression in early-phase acute pancreatitis

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ABSTRACT

Background/Aims: *T-cell immunoglobulin and mucin domain 3 (Tim-3) assumedly play a crucial immunomodulatory role in inflammatory response. Data on the potential role of soluble Tim-3 (sTim-3) in acute pancreatitis (AP) are scarce. We conducted a prospective clinical study to characterize its role in the early-phase AP.*

Methods: *In total, 44 patients with AP (16 mild and 28 none-mild) who presented within 24 hours on admission and 20 healthy volunteers (NC) were included in our study. Serum interleukin (IL)-6, IL-10, tumor necrosis factor (TNF)-α, and sTim-3 levels were detected using enzyme-linked immunosorbent assay (ELISA).*

Results: *Levels of the pro-inflammatory cytokines IL-6 and TNF-α and the anti-inflammatory cytokine IL-10 in the none-mild and mild groups were significantly elevated compared with those of the NC group. The sTim-3 levels of the none-mild and mild group were significantly increased compared with the NC. The sTim-3 level positively correlated with the IL-6 and TNF-α but showed no obvious correlations with the IL-10 level. The sTim-3 level positively correlated with the APACHE II score.*

Conclusion: *The results indicate that sTim-3 participates in the early progression of AP by positively regulating the pro-inflammatory cytokines and that the measurement of serum sTim-3 is an early marker for predicting AP.*

Keywords: *Acute pancreatitis, soluble Tim-3, inflammatory cytokine*

INTRODUCTION

Acute pancreatitis (AP) is an acute inflammation of the pancreas; it is clinically characterized by abrupt onset of deep epigastric pain and biochemically by an increase in serum amylase or lipase. Approximately 70%-80% of patients with AP have mild disease; however, in 20%-30% of patients, it runs as a severe course (1) with a 15%-20% mortality rate (2). The pathogenesis of AP is still an enigma to clinicians and basic research scientists.

Abnormal inflammatory responses play an important role in the development of AP. The initial injury in AP is characteristically sterile, deregulates the immunological reaction in systemic inflammatory response syndrome (SIRS), dominates the pathological process, and leads to circulatory disturbances and multiple organ dysfunction (3). The relationship between AP and SIRS has been widely investigated. Multiple organ dysfunction (MOD) and infection are the main causes of mortality in severe AP (SAP). Therefore, ameliorating the immune reaction of AP has been the focus of investigation in recent years (4). Because the occurrence and development of AP involves a variety of inflammatory cells and cytokines, immune system alterations may be complex during the course of AP (5). In view of the complex immune system changes, individualized immunomodulation therapy needs to be developed according to the different stages of AP (6). The clinical course of AP is divided into three stages. The initial stage is associated with the first peak of mortality, which is characterized by SIRS and MOD; hence, it is particularly important to regulate the early immune reaction (7).

T-cell immunoglobulin and mucin domain protein 3 (Tim-3) is a member protein of the Tim family, which was shown to play an important role in CD4+ Th1, T helper 17, CD8+ T1, dendritic and natural killer cells as well as macrophages/monocytes (8,9). Studies have confirmed that Tim-3 is a negative regulator of some chronic diseases, such as human immunodeficiency virus (HIV), hepatitis B virus (10), atherosclerosis (11), and diabetes (12). It was found that Tim-3 mRNA could be alternatively spliced to generate two mRNA molecules. Longer mRNA molecules

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directed the synthesis of Tim-3, such as full-length Tim-3, and the shorter ones, which should be soluble (sTim-3) without the region encoding the mucin domain and transmembrane domain, were supposed to direct the synthesis of a splice variant of Tim-3 (13). Recently, F. Ren et al revealed that sTim-3 was involved in the development of sepsis (14). Chiba M et al found serum sTim-3 might be associated with the disease severity of systemic sclerosis (15).

Few studies on the potential role of soluble Tim-3 (sTim-3) in AP are available. Here, we investigated sTim-3 expression in patients with AP and evaluated the correlations of sTim-3 with interleukin (IL)-6, IL-10, and tumor necrosis factor-alpha (TNF-α) to initially investigate its role in the early phase of AP.

MATERIALS AND METHODS

Study design and population

This prospective clinical study was performed between November 2016 and 2017. Patients in this study were enrolled from the department of gastroenterology and intensive care unit. Overall, 44 patients with AP who presented within 24 hours on admission (onset time within 48 hours) and 20 healthy volunteers (healthy examination patients) were reviewed. Diagnostic criteria were based on two or more of the following characteristics: (1) abdominal pain consistent with AP; (2) serum amylase and/or lipase 3 times higher than the normal upper limit; and/or (3) computed tomography (CT) findings of AP. According to the Atlanta classification, AP was classified into three degrees of severity: mild AP, moderately SAP, and SAP (16). For better pairing analysis in the study, patients with AP were divided into the mild and none-mild groups (moderate SAP and SAP).

The exclusion criteria were age <18 years, AP or pregnancy induced by trauma, diagnosis of chronic pancreatitis, history of tumor or immune-related disease, and refusal to participate in the study.

Data collection

Clinical data collected and analyzed from patients included gender, age, pancreatitis etiology, and acute physiology and chronic health evaluation (APACHE) II score (age, acute physiology score, and chronic health points). Peripheral venous blood samples were collected within 24 hours of diagnosis. All samples were collected in a container containing ethylene diaminetetraacetic acid (EDTA).

sTim-3 detection

Human sTim3 enzyme-linked immunosorbent assay (ELISA) kits (eBioscience; US) were used to detect sTim-3 levels according to the manufacturer's instructions. Briefly, the standard was reconstituted into different concentrations using distilled water, the standard concentration was considered the horizontal axis, and optical density (OD) values were the vertical axis. Regressed data were used to create a standard curve using computer software. OD was detected at the wavelength of 450 nm using an xMark microplate reader, and the concentrations of sTim-3 were calculated according to the standard curve.

Inflammatory cytokines detection

Serum IL-6, IL-10, and TNF-α levels were determined using a commercial ELISA kit (eBioscience; US) according to the manufacturer's instructions, respectively.

Statistical analysis

All data were analyzed using the Statistical Package for Social Sciences (SPSS), version 13.0, software (SPSS Inc.; Chicago, IL, USA) for Windows. Data were expressed as mean±standard deviation for quantitative variables. All continuous variables were analyzed using the Mann-Whitney U-test for two-group comparison and the Kruskal-Wallis test for multiple comparisons. Categorical variables were compared using the Fisher's exact probability. Correlations were assessed using the Spearman's rank correlation coefficient. $p<0.05$ was considered significant.

RESULTS

Clinical characteristics

Table 1 shows the clinical characteristics of the population. A total of 16 patients with mild AP (mild group) and 28 patients with moderate SAP and SAP (none-mild group) were enrolled in this study. Twenty healthy volunteers were enrolled as normal controls (NC group). The average ages of the mild group, none-mild group, and NC were 49.9, 51.6, and 45.5 years, respectively. There were no significant differences in age, gender, and etiology between the groups. The APACHE II score was significantly higher in the none-mild group than that in the mild group.

Serum IL-6, IL-10, and TNF-α levels

The levels of the pro-inflammatory cytokines IL-6 and TNF-α of the none-mild and mild groups were significantly higher than those of the NC group ($p<0.001$, Figure 1). Among the AP subgroups, the IL-6 (208.7±57.7

Table 1. Clinical characteristic of patients with AP and NC

Patient characteristics	Mild, n=16	None-mild, n=28	NC, n=20	p
Ages (years), mean±SD	49.9±10.2	51.6±8.9	45.5±7.1	0.065
Gender (male/female), n	7/9	15/13	9/11	0.767
Etiology, n				0.667
Cholelithaiasis	6	13		
Alcohol	3	4		
Hyperlipidemia	5	10		
Other	2	1		
APACHE II score, mean±SD	4.8±1.4	11.2±2.4	-	<0.001

AP: acute pancreatitis; SD: standard deviation; APACHE, acute physiology and chronic health evaluation; NC: normal controls; Mild: mild acute pancreatitis; None-mild: moderately severe acute pancreatitis and severe acute pancreatitis

pg/mL) and TNF-α (139.9±30.3 pg/mL) levels of the none-mild group were significantly higher than those of the mild group (80.5±14.2 pg/mL, 68.5±22.6 pg/mL) (*P*<0.001). The anti-inflammatory cytokine IL-10 levels of the none-mild and mild groups were significantly higher than those of the NC group (p<0.001, Figure 1). Among the AP subgroups, the IL-10 level of the none-mild group (22.8±5.8 pg/mL) was significantly lower than that of the mild group (59.9±21.4 pg/mL; p<0.001).

Serum sTim-3 levels

The sTim-3 levels of the none-mild and mild groups were significantly higher than those of the NC group (p<0.001, Figure 1). Among the AP subgroups, the sTim-3 level of the none-mild group (917.6±276.6 pg/mL) was significantly higher than that of the mild group (492.8±95.5 pg/mL; p<0.001).

Correlations of the serum sTim-3 with IL-6, IL-10, and TNF-α levels and the APACHE II score

In the correlation analysis, the sTim-3 level was positively correlated with the IL-6 and TNF-α levels, whereas the sTim-3 level was not significantly correlated with the IL-10 level; the sTim-3 level was positively correlated with the APACHE II score (r=0.545, p<0.001) (Figure 2).

DISCUSSION

The pathological changes of AP vary from acute edema and cellular infiltration to acinar cells necrosis, necrotic blood vessels hemorrhage, and intra- and extra-pancreatic fat necrosis and form a parenchymal inflammatory response to SIRS. The initial protease cascade does not necessarily determine the severity of AP (17). In contrast, more and more evidence has suggested that innate immune is crucially related to the pathogenesis and disease severity of AP (18). F. Ren et al found that sTim-3 was involved in the development of sepsis and that Tim-3 had an immunosuppressive effect on the monocytes during sepsis; the emergence of sTim-3 disturbed Tim-3 homeostasis (14).

To our knowledge, this is the first study to evaluate serum sTIM-3 level in patients with AP. Here, the serum sTIM-3 level was elevated in the early phase of AP. We hypothesized that sTim-3 is involved in the process of AP and regulates pro- and anti-inflammatory cytokine expression. To confirm this hypothesis, the correlations of sTim-3 with the pro-inflammatory cytokines IL-6 and TNF-α and anti-inflammatory cytokine IL-10 were analyzed. IL-6 is a very important pro-inflammatory cytokine involved in inflammatory and immune responses (19). The upregulation of IL-6 indicated that IL-6 serves as a valuable early marker for AP. TNF-α is a polyphonic cytokine and acts as a central regulator of inflammation (20) and is mainly secreted by monocytes and macrophages but also released by pancreatic acinar cells after an inflammatory trigger (21). Several studies have shown that TNF-α plays an important role in the inflammatory response of disease progression and pathogenesis of AP (22). With the release of pro-inflammatory mediators, anti-inflammatory cytokines are subsequently produced, resulting in compensatory anti-inflammatory response syndrome (CARS). Multiple activated immune cells, such as monocytes/macrophages, regulatory T cells, and Th1 cells produce IL-10 (23). The results indicated positive correlations of sTim-3 with IL-6 and TNF-α levels but did not show any obvious correlation with IL-10. These results conform to the previous hypothesis. Our study indicates that an excessive inflammatory reaction occurs in the early stage of AP, and inhibiting sTim-3 activation may have a therapeutic effect on excessive inflammatory responses.

Early detection of the tendency toward SAP and early intervention are important in the treatment and reduction of mortality in AP. The APACHE II score is a very good predictor of severity (24). In the correlation analysis, the serum sTim-3 level was positively correlated with the APACHE II score (r=0.545, p<0.001). This indicates that serum sTim-3 is as an early marker for predicting of AP.

There are several limitations to our study. First, the population was relatively small. Second, it is necessary to monitor immune cells and inflammatory cytokines to develop

individualized immunomodulatory therapies in different stages of AP; therefore, a further dynamic study of serum sTim-3 in AP is needed. Third, the precise mechanism by which sTim-3 is released into the circulation has not yet been clarified.

In conclusion, our results indicate that sTim-3 participates in the early progression of AP by positively regulating pro-inflammatory cytokines, and inhibiting sTim-3 activation might have a therapeutic effect on the excessive inflammatory response. Furthermore, the measurement of serum sTim-3 may be an early marker for predicting AP.

Ethics Committee Approval: Ethics committee approval was received for this study from the Affiliated Changzhou No.2 People's Hospital with Nanjing Medical University Ethics Committee (Decision Date: March 1, 2016; Approval No.: 2017-101).

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

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