

Original Article

Association between serum interleukin-35 levels and severity of acute pancreatitis

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Abstract: Inflammatory cytokines have been reported to be associated with pathogenesis of acute pancreatitis. The aim of this study was to measure the serum IL-35 levels in patients with acute pancreatitis and analyze the relationship between IL-35 levels and the disease severity. Thirty-two patients with acute pancreatitis and 32 healthy control subjects were included into the study. The serum levels of IL-35 were measured by enzyme-linked immunosorbent assay upon admission and the following seven days. The relationships with severity and etiology during the clinical course were analyzed. Serum IL-35 levels in patients with acute pancreatitis at the time of admission (5.25 ± 0.37 ng/mL) were significantly higher than those in healthy controls (1.93 ± 0.16 ng/mL, $P < 0.001$). Moreover, serum IL-35 levels in patients with severe attacks (7.15 ± 0.48 ng/mL) were significantly higher than those with moderately severe attacks (5.14 ± 0.49 ng/mL, $P = 0.01$) and mild attacks (3.69 ± 0.53 ng/mL, $P < 0.001$). However, there was no significant difference of serum IL-35 levels among patients with acute pancreatitis due of alcohol, gallstone and idiopathy. In addition, the peak serum concentrations of IL-35 were on day 1 after admission. Our results demonstrate that increased serum IL-35 levels may be related to the inflammatory response in patients with acute pancreatitis, suggesting that IL-35 may be used for a potential biomarker of acute pancreatitis.

Keywords: Acute pancreatitis, serum interleukin-35, biomarker

Introduction

Acute pancreatitis (AP) is a severe disease with high morbidity and mortality. The most common causes of AP are alcoholism and biliary stones [1]. Despite advances in treatment, the mortality rate of severe acute pancreatitis (SAP) remains as high as 10%-30% [2]. Early diagnosis and prognostic evaluation are extremely important and may reduce the morbidity and mortality associated with AP [1, 2]. Although the exact mechanisms that trigger the inflammatory and necrotizing process are not well understood, it is generally accepted that pro-inflammatory cytokines play important roles in either pathogenesis or systemic complications of AP [3]. The serum levels of pro-inflammatory cytokines, such as interleukin-1 (IL-1), IL-6, IL-8, IL-18 and tumor necrosis factor (TNF) have been reported to be significantly higher in AP, which recruit neutrophils, monocytes and lymphocytes into the pancreas,

resulting in a systemic inflammatory response [4, 5].

Anti-inflammatory cytokines, however, do not match the increase of pro-inflammatory cytokines, if the anti-inflammatory response is not sufficiently strong, an excessive inflammatory response can lead to early organ dysfunction and SAP [3]. Therefore, the outcome of this disease depends on the balance between the pro-inflammatory and anti-inflammatory responses. IL-10, a critical anti-inflammatory cytokines, have been widely investigated in AP. In acute pancreatitis patients, serum IL-10 levels were significantly higher in patients with mild disease than in those with severe disease, its concentration peaked on the first day but progressively decreased [6-8]. The IL-10/IL-6 ratio was significantly lower in patients with severe acute pancreatitis [9]. Moreover, IL-10 has been shown to reduce the severity of experimental acute pancreatitis in rats [10, 11].

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Table 1. Demographic characteristics of the patients with acute pancreatitis and the healthy control group

	Acute pancreatitis group (n=32)	Control group (n=32)	P value
Age, years	48.4±17.7	46.2±19.3	0.879
Male/Female	19/13	20/12	0.798
Smoking	15.6% (n=5)	6.25% (n=2)	0.230
Hypertension	12.5% (n=4)	6.25% (n=2)	0.391
Hyperlipidemia	12.5% (n=4)	3.12% (n=1)	0.162
BMI (kg/m ²)	28.1±3.8	27.5±4.2	0.744

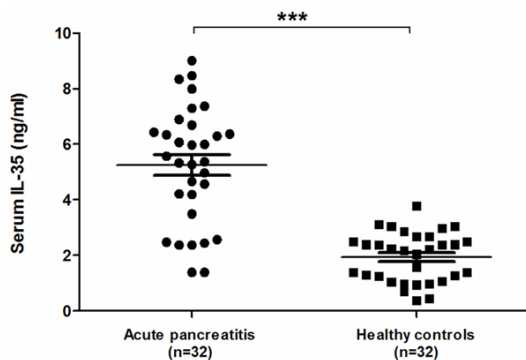


Figure 1. Serum IL-35 concentrations at the time of admission in 32 patients with acute pancreatitis and 32 healthy controls. Data are expressed as mean ±SEM. ****P*<0.001.

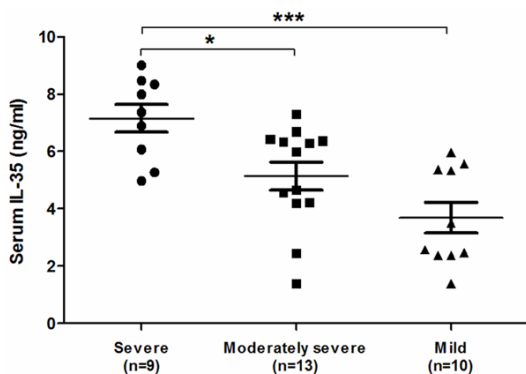


Figure 2. Relationship between severity indexes and serum IL-35 levels. Data are expressed as mean ±SEM. **P*<0.05, ****P*<0.001.

IL-35, first defined in 2007, is a recently characterized potent anti-inflammatory cytokine that is predominantly produced by Foxp3⁺ regulatory T cells (Tregs) [12]. IL-35 is a heterodimer cytokine comprised of the subunit Epstein-Barr virus-induced gene 3 (EBI3) and p35 [12]. IL-35 suppresses the inflammatory response through the expansion of regulatory T cells and suppression of Th17 cell development [13, 14].

IL-35 also directly suppresses the proliferation of CD4⁺CD25⁻ effector cells. Additional evidences demonstrated that IL-35 can efficiently reduce the progression of inflammatory diseases and autoimmune diseases. IL-35 can limit airway inflammation and IgE production in a dust mite allergen-specific mouse models [15]. In several humans diseases, IL-35 has also been reported to be involved in the pathogenesis of the inflammatory process, such as chronic hepatitis B virus infection, asthma and COPD [16, 17].

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However, to the best of our knowledge, the plasma concentration of IL-35 has not yet been systematically evaluated in patients with AP. In this study, we measured the plasma concentrations of IL-35 from patients with AP at the time of admission and healthy control subjects by ELISA. We also assessed the relationship between IL-35 levels and the severity, etiology of the disease.

Materials and methods

Patients

The study was approved by the Institutional Review Board of our hospital and was performed in accordance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all of the patients and controls before the research according to the committee's regulations. Between January, 2014 and January, 2015, Thirty-two patients with acute pancreatitis in our Intensive Care Unit at the time of admission (within the initial 72 h after the onset of disease) and 32 healthy volunteers were included into the study. Time interval between onset and admission was 32±4 hours (0-24 h in 22 patients, 24-48 h in 8 patients, and 48-72 h in 2 patients). The diagnosis and evaluation of the severity of acute pancreatitis were established on the basis of acute abdominal pain, at least 3-fold elevated levels of serum amylase, and computed tomography (CT). All patients had no previous history of acute pancreatitis and were hospitalized with palliative management. The severity of AP was categorized according to the revised Atlanta classification system [18]. There were 9 severe cases, 13 moderately severe cases and 10 mild cases. Etiology of acute pancreatitis

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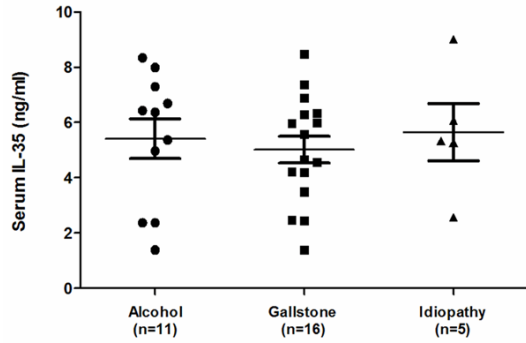


Figure 3. Relationship between etiology and serum IL-35 levels. Data are expressed as mean \pm SEM.

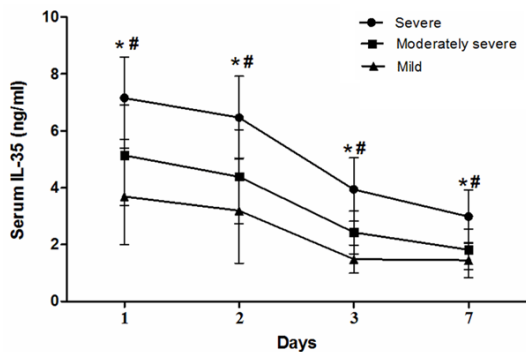


Figure 4. Serum concentrations of IL-35 in patients with severe, moderately severe and mild pancreatitis on hospital days 1, 2, 3 and 7. Data are expressed as mean \pm SEM. * P <0.05, compared with moderately severe pancreatitis; # P <0.05, compared with mild pancreatitis.

was presumed to be alcohol in 11 patients, gallstone in 16 patients and idiopathy in 5 patients.

Measurement of plasma IL-35

Serum samples were collected upon admission to the hospital and on the mornings of days 2, 3 and 7 after admission. All the blood serum samples were frozen immediately after collection and stored at -80°C until analysis. Serum IL-35 concentrations were quantified by using a commercial human IL-35 heterodimer ELISA kit (Biolegend, San Diego, CA) according to the manufacturer's protocol. All samples were assayed in duplicate. The mean concentration was determined for each sample. The sensitivity of the assay is 0.13 ± 0.01 ng/ml.

Statistical analysis

Statistical analysis was performed using SPSS version 16.0 statistical software (SPSS, Chi-

cago, IL, USA). The results are expressed as mean \pm SEM. Chi-square and Mann-Whitney U test was used to evaluate differences between two groups. The Kruskal-Wallis test was used to evaluate differences among more than two groups. Correlations were evaluated with the Spearman rank test. P value <0.05 was considered statistically significant.

Results

Serum IL-35 levels in patients with acute pancreatitis

A total of 32 patients with AP and 32 healthy controls were evaluated for serum levels of IL-35. The demographic features of the patients included into the study are summarized in **Table 1**. There were no significant differences between AP and control group with respect to age, gender, smoking status, hypertension, hyperlipidemia and BMI (P >0.05). As shown in **Figure 1**, the mean value of serum IL-35 levels in patients with acute pancreatitis at the time of admission were (5.25 ± 0.37) ng/mL and were significantly higher than those in healthy controls (1.93 ± 0.16 ng/mL, P <0.001).

Relationship between severity on admission and serum IL-35 levels

As shown in **Figure 2**, the mean value of serum IL-35 levels in patients with severe attacks (7.15 ± 0.48 ng/mL) were significantly higher than those with moderately severe attacks (5.14 ± 0.49 ng/mL, $P=0.01$) and mild attacks (3.69 ± 0.53 ng/mL, P <0.001). However, there was no significant difference between the moderately severe cases and mild cases ($P=0.059$).

Relationship between etiology and serum IL-35 levels

As shown in **Figure 3**, the mean values of serum IL-35 levels in patients with acute pancreatitis due to alcohol, gallstone and idiopathy were (5.42 ± 0.72) , (5.02 ± 0.49) and (5.65 ± 1.03) ng/mL, respectively. There was no significant difference among these groups.

Time course of serum IL-35 levels

As shown in **Figure 4**, IL-35 peaked on day one after admission and then progressively decreased in the following days. Serum levels of IL-35 were significantly higher in patients with severe pancreatitis than in those with

moderately severe and mild pancreatitis on the first day of admission and on the 2nd, 3rd and 7th days.

Discussion

IL-35 works as an inflammation inhibitor in several autoimmune diseases, such as collagen induced arthritis, asthma and COPD [16, 19, 20]. However, the role of IL-35 in acute pancreatitis disease has yet to be understood, and there has been no report so far.

It is generally accepted that activated leukocytes and pro-inflammatory cytokines play an important role in the pathogenesis of acute pancreatitis. Most of previous studies have shown that the levels of proinflammatory cytokines, including IL-1, IL-6, IL-8, IL-18 and TNF- α are higher in severe forms of acute pancreatitis. Proinflammatory cytokines are associated with systemic inflammatory response syndrome (SIRS) and multiple organ failure syndrome in acute pancreatitis [4]. Anti-inflammatory cytokines including IL-10, IL-1 receptor antagonist, and soluble IL-2 receptor are also reported to be significantly higher in patients with severe acute pancreatitis [6, 21]. Similarly, in this study, we found that serum IL-35 levels are significantly elevated in patients with acute pancreatitis compared with healthy controls. Moreover, we have first demonstrated that serum IL-35 levels are significantly correlated with the severity indexes of acute pancreatitis. These results suggest that serum IL-35 levels may reflect severity and organ dysfunction in acute pancreatitis and that IL-35 may be closely related to T cell response in this disease.

In the present study, we also monitored the serial serum levels of IL-35 in patients with acute pancreatitis within one week of admission. The results showed that IL-35 peaked on day one after admission and then progressively decreased in the following days. The results are in accordance with previous studies and suggest that in the early stage of acute attack, a compensatory anti-inflammatory response usually occurs in parallel with pro-inflammatory response. Therefore, earlier assessment of the both pro- and anti-inflammatory mediators is important in the prediction of acute pancreatitis.

So far, a variety of single serum parameters, such as C-reactive protein (CRP), creatinine and

calcium have been reported to be useful indicators of the severity of acute pancreatitis. Among these biochemical markers, the simplest and most widely available test is CRP. Serum CRP levels above 12~15 mg/dL correlate with severe disease [22]. However, CRP measurements involve a delay of 48 hours or longer before prediction. The sensitivity of CRP on day 1 after admission is not as good as it is on day 2 (56% vs. 83%) [23]. The recognition of early involvement of inflammatory cytokines in acute pancreatitis has generated significant research interest in the utility of cytokine levels to serve as early prognostic indicators. However, no cytokine has proved to be useful enough to be incorporated into routine clinical use. A recent study performed by Nieminen et al. [4] determined serum levels of 47 cytokines in patients with acute pancreatitis, they found that 14 of 47 cytokines were significantly higher in the severe acute pancreatitis group than in the patients with mild or moderately severe acute pancreatitis, suggesting that combining cytokines with other on-cytokine-related prognostic markers might improve the predictive power for severe acute pancreatitis.

In conclusion, this is the first study showing that patients with acute pancreatitis have higher serum IL-35 levels than healthy subjects and serum IL-35 levels reflect the severity of acute pancreatitis. However, further clinical and experimental studies are needed to evaluate diagnostic and prognostic value of serum IL-35 levels in acute pancreatitis.

Disclosure of conflict of interest

None.

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