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ORIGINAL ARTICLE

Serum levels of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) in predicting the severity of acute pancreatitis

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Abstract

Introduction Acute pancreatitis (AP) is a severe disease associated with significant morbidity and mortality. The overall outcome has improved, but specific treatment(s) remains elusive. The challenge is the early identification and treatment of patients who will develop severe acute pancreatitis. Therefore, the aim of the present study is to investigate plasma levels of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) in the initial phase of predicted severe acute pancreatitis.

Methods Between June 2014 and January 2016, 64 patients with acute pancreatitis and 36 healthy individuals were included to study. Four blood samples, for serum TWEAK measurement, were taken from each individual in each group. The first measurement was taken from the admission blood sample. The subsequent three samples were taken at 12, 24, and 48 h after the hospital admission.

Results Serum TWEAK levels were significantly higher in patients with acute pancreatitis when compared with healthy controls. TWEAK plasma concentrations in severe pancreatitis patients were significantly higher than in mild pancreatitis patients.

Conclusion Serum TWEAK levels increase progressively with the severity of acute pancreatitis and TWEAK might be a novel early marker of severity in acute pancreatitis.

Keywords Acute pancreatitis · Inflammation · Marker · Severity · Tumor necrosis factor-like weak inducer of apoptosis

Introduction

Acute pancreatitis (AP) is a severe disease associated with significant morbidity and mortality.

The overall outcome has improved, but specific treatment(s) remains elusive. The challenge is the early identification and treatment of patients who will develop severe pancreatitis (SP) and infected pancreatic necrosis [1]. Although the incidence of organ failure in the early phase (within the first week) varies among different studies, persistent organ failure (OF) has been shown to be the major determinant of clinical outcomes [2]. Patients who progress to SP have a high mortality rate during their first week of evolution due to multiple organ failure. Those who survive frequently develop extensive necrosis of pancreatic and peripancreatic tissues, and 30–70% of the latter become infected. In these infected patients, multiple organ failure and death can ensue [3, 4].

Parameters enabling an early identification of patients who hold a tendency to develop the severe form of the disease are keenly being pursued to optimize treatment, thus preventing the onset of local complications and systemic organ failure. These severity criteria were initially clinically based.

It is important to stratify the severity of acute pancreatitis. First, early identification of patients with potential severe acute pancreatitis (SAP) may facilitate timely referral to specialists. Second, for specialists, severity-stratification of those patients makes triage and comparison between different studies possible [2].

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Scoring systems incorporating clinical, biochemical, or radiological criteria for severity assessment have been in use for some decades. A number of laboratory markers predicting disease severity have been reported, either based on the degree of the inflammatory reaction, tests that relate to the activation of trypsinogen and other pancreatic proenzymes, tests that measure leakage of certain pancreatic enzymes, or scoring systems (Ranson's, APACHE-II). However, none can accurately predict disease severity within 24 h of onset, and the prediction of pancreatic necrosis at this stage has certainly not been reported [5].

Serum amylase and lipase, the standard tests for acute pancreatitis (AP) diagnosis, are poor predictors of severity.

Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) was first described as a new member in the tumor necrosis factor (TNF) superfamily of ligands by Chicheportich in 1997. It is a 30-kDa cell surface-associated type II transmembrane protein, which can be cleaved to generate a smaller, biologically active form into the extracellular milieu. Currently, a type I transmembrane protein named TWEAK receptor, also known as fibroblast growth factor-inducible 14 (Fn14), which is highly expressed in endothelial cells, has been identified to bind TWEAK with physiological affinity. Recent evidence indicates that TWEAK is a multifunctional cytokine that regulates cell growth, angiogenesis, apoptosis and inflammation via activation of the Fn14 receptor [6]. High levels of TWEAK mRNA have been found in many tissues such as lymph nodes, pancreas, intestine, heart, brain, lung, ovary, vasculature, and skeletal muscle and at low levels in the liver, lung, thymus, kidney, and adipose tissue [7]. Reduced circulating TWEAK (sTWEAK) levels have been found in diseases with increased cardiovascular risk and low-degree chronic inflammation, such as type 2 diabetes, atherosclerosis, or chronic renal failure [7].

The present study aimed to investigate plasma levels of TWEAK in the initial phase of predicted SP, and to assess the ability of this biochemical marker to predict SP.

Patients and methods

Consecutive patients admitted to Emergency Department, Ankara Numune Training and Research Hospital (Ankara, Turkey) with the clinical diagnosis of AP, were included prospectively between June 2014 and January 2016.

Written, informed consent was obtained according to a protocol approved by local institutional ethical committee. Patients with malignant disease, chronic inflammatory disease (including established or suspected chronic pancreatitis), preexisting chronic organ failure (renal failure; creatinine 200 mg/dL or requiring dialysis, heart failure, NYHA class 3, recent myocardial infarction 6 months), unstable coronary syndromes, liver failure (Childs grade B), chronic obstructive airways disease, and immunosuppressive disorders (drugs, hematologic malignancies, HIV) were excluded due to potential variations in the systemic inflammatory responses and their potential influence on treatment decisions.

After informed consent, first measurement was taken from the admission blood sample. The subsequent three samples were taken at 12, 24, and 48 h after the hospital admission.

All blood specimens were immediately processed after they were drawn and stored in aliquots at − 80 °C till quantification of sTWEAK.

Serum sTWEAK levels were determined with a commercially available ELISA kit tested for determination of human serum samples (BenderMedsystems, Wien, Austria) according to the manufacturer's instructions. Briefly, a 1:2 diluted test sample was incubated for 3 h at room temperature in wells pre-coated with an anti-human sTWEAK antibody together with a biotin-conjugated anti-human TWEAK antibody. Streptavidin–HRP (horse reddish peroxidase) binds to the biotin-conjugated anti-human TWEAK. Following incubation, wells were washed three times to remove excess antibody, the substrate solution reactive with HRP was added to the wells and incubated for approximately 10–20 min. A colored product is formed in proportion to the amount of soluble human TWEAK present in the sample. The reaction was terminated by addition of acid and absorbance was measured at 450 nm. A standard curve prepared from seven human TWEAK standard dilutions and subsequently human TWEAK sample concentrations were determined. The absorbance was measured with an automatic ELISA reader (Biochem Immunosystems 100 Cascade Drive, Allentown, PA). Human sTWEAK was detected with this kit at a threshold of 9.7 pg/ml. Intra-assay and inter-assay coefficients of variation were 7.9 and 9.2%, respectively.

All measurements were performed in duplicates.

Routine measurements (CRP, amylase, lipase) were performed using routine laboratory methods.

To establish the study groups, the Atlanta criterion [8] and the Santorini consensus conference [8] were followed. Diagnosing AP, when clinical symptoms are compatible, is linked to serum amylase or lipase levels three times of the normal value; other pathologies may be ruled out with or without imaging techniques. The disease is considered as mild pancreatitis (MP) in the absence of organ dysfunction or local complications, and SP is inferred when local complications and/or a presentation of distant organ dysfunction are present, mainly evaluated by $pO2 \leq 60$ mmHg and/or serum creatinine ≥ 2 mg/dl after rehydration. In any case, dysfunction was transient, and in fact no patient was in an intensive care unit. For patient diagnosis and monitoring, imaging techniques were used in individual cases when necessary, such as simple thorax and abdomen radiology,

abdominal echography, computerized axial tomography (CAT), endoscopic retrograde cholangiopancreatography (ERCP), and magnetic resonance cholangiopancreatography (MRCP). A dynamic CAT scan was performed in all patients with severity criteria (between 7 and 8 points by combining Balthazar criteria with percentage of necrosis) [8].

The data were expressed as mean ± SD and 95% confidence interval (CI). Statistical analysis was carried out using SPSS 11.0 for Windows (SPSS Inc, Chicago, IL). The differences between the groups were estimated using the Chi-square (for gender distribution) and two-tailed unpaired t-test (for normally distributed data). Mann–Whitney-U test was used to evaluate serum TWEAK levels in studied groups. A P value of < 0.05 was considered to be statistically significant.

Results

The study protocol included 64 patients that had AP; all met the eligibility criteria, as well as 36 healthy volunteers.

The overall median age of patients with AP was 50 years (range 29–74 years) in which 22 were male. Of the 64 patients, 43 patients had mild AP and 21 had severe AP. The etiology was gallstones in 52 patients; 36 mild and 16 severe. Alcohol was the cause in eight patients; six mild and two severe. No obvious cause was found in four patients; one mild and three severe. None of the mild cases and three of the severe cases were deceased. The median age of the 36 healthy controls (13 males) were 41 years old (range 25–51 years).

As shown in Table 1, serum TWEAK levels were elevated significantly in patients with AP compared with those in the healthy control group. In addition, in 64 patients with AP, TWEAK levels in acute stage were significantly higher than those in admission stage. Furthermore, this association between the severity of AP and the circulating TWEAK levels was evident.

Mean TWEAK plasma concentrations were meaningfully higher in patients with AP (598.8 ± 87.2 pg/mL) when compared with the healthy control group (437.3 ± 88.7 pg/mL) ($P \leq 0.001$). TWEAK plasma concentrations in SP patients (628.7 ± 86.8 pg/mL) were notably higher than in MP patients (556.2 ± 95.5 pg/mL) ($P \leq 0.004$).

In less than 12 h from onset of pain, the mean plasma concentration of TWEAK in patients with SP was 823.1 ± 151.2 pg/mL (95% CI 748.93–897.07), significantly higher than that of the healthy control group 437.3 ± 88.7 pg/mL ($P \leq 0.001$). The mean level in MP patients (628.8 ± 104.1 pg/mL) (95% CI 571.48–684.52) was also notably higher than that of healthy volunteers ($P \leq 0.004$). Importantly, significantly higher levels of TWEAK were detected in patients with SP within 12 h following onset of pain compared to MP ($P \leq 0.003$).

At 24 h, the mean TWEAK plasma concentration was 1054.1 ± 162.2 pg/mL in patients with SP, still significantly higher than that of the healthy control group ($P \leq 0.001$) and also compared to those with MP 724.3 ± 96.2 pg/mL, ($P \leq 0.005$).

At 48 h, the mean plasma copeptin concentration was 1345.2 ± 225.1 pg/mL in patients with SP, MP 926.6 ± 119.3 pg/mL ($P \leq 0.005$) (Table 1).

Serum level of amylase was 342 (88-1326) U/L in patients with MP and 657 (98-1534) U/L in patients with SP at 12 h, but this difference was not statistically significant. The difference between mean amylase concentrations (mild: 472.4 ± 38.9 U/L; severe: 706.54 ± 109.76 U/L) at 24 h was not statistically significant.

The serum lipase levels at 12 h were 321 (49–701) U/L in patients with MP and 408 (56–803) U/L in patients with SP, and this difference was not statistically significant. The difference between mean lipase concentrations (mild: 4832.2 ± 48.7 U/L; severe: 754.7 ± 111.8 U/L) at 24 h was not statistically significant.

On the other hand, we also evaluated the distinctive ability of C-reactive protein (CRP) because it has been used as a biomarker to predict severity. The admission mean CRP concentrations were higher in severe AP, but this difference was not statistically significant (CRP concentration: mild: 21.15 ± 4.23 mg/L; severe: 49.67 ± 8.76 mg/L).The difference mean CRP concentrations (mild: 172.4 ± 18.7 mg/L; severe: 356.24 ± 89.76 mg/L) at 48 h was not statistically significant. CRP (after 48 h) was found to be reliable in prediction of SP, except admission. There was no statistically

Table 1 The table shows plasma TWEAK levels in pg/mL the three study groups

	At the admission	12 h	24 h	48 h
C	437.3 ± 88.7	437.3 ± 88.7	437.3 ± 88.7	437.3 ± 88.7
MP	556.2 ± 95.5	628.8 ± 104.1*	724.3 ± 96.2*	926.6 ± 119.3*
SP	628.7 ± 86.8	823.1 ± 151.2*	1054.1 ± 162.2*	1345.2 ± 225.1*

C control, *MP* mild pancreatitis, *SP* severe pancreatitis

Data are presented as Mean ± SD

*Statistically significant difference ($p \leq 0.05$)

significant difference between MP and SP groups in the first 24 h.

Discussion

Acute pancreatitis is a potentially life threatening disease with varying severity of presentation. AP is usually a mild and self-limiting disease, but some patients develop a severe form that is associated with high mortality. It is important to stratify the severity of acute pancreatitis. First, early identification of patients with potential SAP may facilitate timely referral to specialists. Second, for specialists, severity-stratification of those patients makes triage and comparison between different studies possible. However, when organ failure is present in the early phase, it may be difficult to determine the final degree of severity because it is unknown whether the patient will prove to have persistent or transient organ failure [2]. Despite the advances in investigational modalities and research techniques, the exact pathogenesis of AP is still unclear. At present, there is no method to accurately predict severity. Many scoring systems have been proposed but all have their drawbacks. The possibility that an affordable, quick, single, and accurate test may exist has led clinicians to investigate numerous (mainly inflammatory mediators) biochemical molecules. Many have been assessed and detected either in serum or urine but for a number of reasons have failed to reach the clinical setting.

The greatest difficulty is the early identification of patients with SP and those likely to benefit from early transfer to specialist units.

Our results show, for the first time, that TWEAK concentrations were significantly higher in AP patients, compared with healthy individuals, and also plasma TWEAK levels showed significant differences between SP and MP in the first day, as a result of which this TWEAK has predictive value as a severity marker on the first day.

Acute pancreatitis results in local and systematic overproduction of inflammatory mediators. It is likely that the pancreatic epithelium itself is at least partly accountable for the augmented chemokine and cytokine production and, therefore, plays a role in the exacerbation of the pancreatic inflammation [9]. Leukocyte chemotaxis in acute pancreatitis is a well-orchestrated process that involves a number of proteins, including pro-inflammatory cytokines, adhesion molecules, matrix metalloproteinases and the large cytokine subfamily of chemotactic cytokines—the chemokines. Numerous chemokines have now been identified as inflammatory mediators with potent leukocyte-activating properties and many of them have been shown to be involved in the patho-physiological process of experimental acute pancreatitis. There are over 50 different chemokines and over 20 different receptors, with overlapping functions. Despite the complexity and apparent redundancy of this system, it is reasonable to believe that specific chemokine receptor antagonists that interfere with leukocyte migration and activation could be useful in acute pancreatitis [10–12]. TWEAK may exert its pro-inflammatory effects by inducing chemokine production and leucocyte migration [6].

TWEAK, a member of the TNF superfamily of cytokines, is a multi-functional cytokine that controls many cellular activities including proliferation, migration, differentiation, apoptosis, angiogenesis, and inflammation [1]. High levels of TWEAK mRNA have been found in many tissues such as lymph nodes, pancreas, intestine, heart, brain, lung, ovary, vasculature, and skeletal muscle and at low levels in the liver, lung, thymus, kidney, and adipose tissue [7].

The TWEAK/Fn14 pathway as an injury-inducible mediator of pleiotropic responses is introduced in a review of the work implicating sustained Fn14 signaling in disease pathogenesis and encompassing the current TWEAK/Fn14-targeting approaches for treatment of human disease [13]. Broad relevance to neurological diseases is supported by a basic TWEAK/Fn14 role in regulating the structure and function of the neurovascular unit, thereby regulating blood–brain barrier (BBB) permeability [13]. Furthermore, BBB damage appears to be an important component of neuropsychiatric systemic lupus erythematosus, and there is an emerging evidence for the role of TWEAK/Fn14 in compromising the BBB in lupus [13]. Also relevant to lupus is the pathogenic role of TWEAK/Fn14 in the renal manifestation of lupus nephritis. Indeed, evidence supporting TWEAK/Fn14-mediated pathological mechanisms in contexts of acute kidney injury and chronic kidney diseases is substantial and clinical targeting of TWEAK is ongoing in lupus nephritis. Also addressed in this Research Topic is the role of TWEAK/Fn14 in the pathological remodeling underlying other inflammatory diseases, namely cardiovascular diseases and obesity-associated Type-2 diabetes, as well as in myocardial remodeling leading to heart failure [13], and a common theme also addressed in these articles is the potential use of soluble TWEAK as a biomarker for cardiovascular diseases. The expression of soluble TWEAK in biological fluids of patients with autoimmune/chronic inflammatory diseases and its potential as a biomarker of these diseases is also more broadly discussed [13].

In our study, we found that the blood level of amylase and lipase rapidly increases within 12 h of onset of disease, and after reaching a peak level, subsequent return of serum amylase and lipase to its normal level does not correlate with resolution of clinical symptoms. Furthermore, the magnitude of the hyperamylasemia does not show significant statistical correlation with disease severity and ultimate prognosis. Like that of amylase, we found that no correlation between lipase activity and disease severity.

In our study, we also found that CRP (after 48 h) was significantly higher in severe AP compared to mild; however, in admission stage CRP did not differ significantly between the two groups. CRP as marker is its delayed peak (48–72 h) and its nonspecific nature as inflammatory marker. Before measurement of CRP, other inflammatory conditions such as cholangitis and pneumonia should be ruled out. Despite the simplicity and easy availability of CRP in clinical practice, many studies have described the limitation of clinical utility of CRP in the early phase of AP, and revealed that usage of CRP alone was potentially failing to detect severe cases of AP at an earlier stage [14]. Our study again demonstrated that CRP showed significantly higher value in severe AP at a later stage; however, it has limitations in prediction of the severity of AP at an earlier stage.

There are a few limitations of the present study. Our study was aimed primarily at identifying the diagnostic importance and the prediction power of serum TWEAK levels in patients with SP during the acute phase. Therefore, longer follow-up (more than 48 h) to identify the significance of TWEAK was not planned and performed in any of the groups. However, measuring serum TWEAK in a longer period might be helpful in better understanding of the diagnostic and even prognostic role of TWEAK in AP, especially in severe form.

Prior to the present study, no published information existed about the role of TWEAK in patients with AP. TWEAK might not serve as a disease-specific marker, but still the augmentation of TWEAK in any clinical condition, as seen in AP in this study, might give us an opportunity to predict disease severity and hence prognosis. Current and future studies in large-scale populations will help us to determine the relevance of TWEAK as a AP biomarker and its potential implementation in clinical practice.

In conclusion, serum TWEAK levels increase progressively with the severity of acute pancreatitis on the first day and TWEAK might be a novel early marker of severity in acute pancreatitis.

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