International Journal of Medical and Health Research
ISSN: 2454-9142
Received: 06-07-2019; Accepted: 10-08-2019
www.medicalsciencejournal.com
Volume 5; Issue 10; October 2019; Page No. 23-26

Prognostic value of Interleukins (IL)-6 and Tumor Necrosis Factor (TNF)-α as predictors of systemic complications in Acute Pancreatitis: A Clinico-observational study

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Abstract
Background: Early assessment of severity in acute pancreatitis (AP) is a key measure to provide rational and effective management.
Aim: of this study is to determine the prognostic value of interleukins (IL)-6 and tumor necrosis factor (TNF)-α as predictors of systemic complications in AP.
Materials and Methods: total 210 patients with confirmed AP were enrolled in the study. The severity of AP was defined according to Atlanta criteria. Measurements of interleukins and TNF-α were performed on the 1st day and on 3rd day of admission.
Results: on day 1st & day 3rd serum levels of IL-6 found highly significantly different ($p < 0.000$) between the severe group and the mild group and TNF-α was found higher ($p < 0.05$) in the severe group than in mild cases on day 1st only. On day 1, IL-6 had a higher sensitivity (92%), specificity (86.7%) and accuracy (88%) compared with TNF-α showed low level of sensitivity and diagnostic accuracy on both the days.
Conclusion: our study confirmed that by determining the serum concentration of IL-6 on the 1st day and on the 3nd day of admission represents a valuable diagnostic tool in the assessment of severity and course of disease in patients with acute pancreatitis.

Keywords: Necrosis, Interleukins, predictors

Introduction
According to National Health Service (NHS), UK, less than 1 in every 100,000 people develops acute pancreatitis each year. It is slightly more common in men than in women. Nearly 60%–80% of all cases of AP in developed countries are attributable to either gallstone disease or alcohol abuse [1, 2].

The overall mortality of AP is about 10–15% but reaches up to 30%–40% in patients with severe disease [3, 4]. Sepsis related multiorgan failure and infected pancreatic necrosis account for about 40–50% of all mortality in acute pancreatitis [5, 6]. Mortality in AP occurs in two peaks. Nearly 50% of deaths occur early within the first week due to massive inflammatory responses leading to multiorgan failure. Septic complications related to infected pancreatic necrosis leading to multiorgan failure are the prime cause of death, late in the disease. The course and severity of AP can fluctuate rapidly and unpredictably [7, 8, 9].

Despite intense research over centuries, the exact pathogenesis of AP remains elusive. Although many theories have been proposed, none of them appear to be complete. Some of the propositions include abnormal biliopancreatic duct common pathway theory, pancreatic auto digestion theory, gallstone migration theory, enzyme activation theory, kinin and complement activation theory, microcirculation disturbance theory, and pancreatic acinar cell apoptosis and necrosis theory, all of which are still controversial.[2] They can only explain certain aspects of pathogenesis or suit disease due to specific etiologies.

Several multifactorial scoring systems and routine clinical and biochemical parameters measured on admission and during the first 48 hours of hospitalization are used to estimate severity and promptly provide a rational and effective management. Still, insufficiently is known of the relationship between the clinical course of AP in humans and the dynamic of the major cytokines, in the presence or absence of pancreatic necrosis and distant organ complications. The purpose of our study was to determine the potential clinical value of interleukins (IL-6) and tumor necrosis factor (TNF-α), as biochemical markers for predicting development of systemic complications in patients with AP.

Materials and methods
Study Subjects
The present Clinico-observational study was carried in 210 patients of acute pancreatitis with mild and severe form as diagnosed by clinicians in SMS, Hospital, Jaipur.

Ethical approval and Informed consent

The study protocol was reviewed by the Ethical Committee of SMS Medical College and Hospital and was granted ethical clearance. After explaining the purpose and details of the study, a written informed consent was obtained from the participants. It was emphasized that strict confidentiality

would be maintained at all times and that the participants or guardians could withdraw at any time without being penalized. After informed consent, patients were interviewed using a structural questionnaire to ascertain demographic and medical history and undergo a physical examination including name, age, sex, height, weight, BMI, pulse, BP, respiratory rate, past history, personal history, family history, drug history and occupation history. After completing the history and physical examination patients were underwent blood investigations.

Inclusion Criteria

1. An 18–60 year old patients of either sex, diagnosed with acute pancreatitis. The diagnosis of acute pancreatitis was established by the criteria set by the Atlanta guidelines[9], namely, any two of the following three criteria to be fulfilled:
   a. Clinical features suggestive of acute pancreatitis
   b. Serum amylase or lipase levels elevated to more than three times the upper limit of normal
   c. Ultrasonography (USG) or computed tomography showing features of acute pancreatitis.
2. Onset of pain to be within 24 h before admission to the hospital
3. Patients predicted to develop SAP by the following criteria on admission: Patients fulfilling the diagnostic criteria for a systemic inflammatory response syndrome (SIRS), defined by the presence of two or more of the following:
   - Rectal temperature >38°C (100.4F) or <36°C (96.8F)
   - Heart rate >90 beats/min
   - Respiratory rate >20/min or PaCO2 <32 mmHg
   - White blood cell count>12,000/mm3, <4000/mm3, or >10% bands.

The diagnosis made on the basis of consistent clinical picture combined with three fold increase in the levels of serum amylase or lipase and consistent morphological findings obtained by USG or/and CT scan within 72 hrs of admission.

Exclusion Criteria

1. Patients with known immune deficient status
2. Primary hyper triglyceridemia
3. On long-term cyclooxygenase inhibitors (more than 3 months)
4. Severe cardiac disease
5. Preexisting hepatic disorders (total bilirubin >1.5 times the upper limit of normal)
6. Psychiatric disorders
7. Preexisting renal compromise (serum creatinine >2.0mg/dl)
8. Received parenteral nutrition within 2 weeks of the study Patients with high amylase or lipase due to trauma, surgery, post ERCP, pancreatic tumor, uremia, diabetic ketoacidosis.

Patient screening and selection

At initial screening, the diagnosis of acute pancreatitis and presence of SIRS was confirmed on clinical, biochemical (serum lipase, renal function tests, liver function tests, serum electrolytes, complete hemogram, arterial blood gas analysis), and radiological investigations (USG, contrast-enhanced computed tomography abdomen). After verifying the absence of any exclusion criteria, total 210 patients were divided into 2 groups (Group A was a mild disease group and Group B was a severe disease group) According to the Atlanta criteria10 – the definition of severe acute pancreatitis is associated with organ failure and/or local complications such as necrosis, abscess or pseudocyst.

Severe pancreatitis is further characterized by 3 or more Ranson criteria or 8 or more APACHE (Acute Physiology and Chronic Health Evaluation) points. Organ failure is defined as shock, pulmonary insufficiency, renal failure or gastrointestinal bleeding more than 500 ml/24 hours. Systemic complications, such as disseminated intravascular coagulation or severe metabolic disturbance may also be seen.

Mild acute pancreatitis is associated with minimal organ dysfunction and an uneventful recovery, and it lacks the described features of severe acute pancreatitis and lacks the local complications. Patients with mild acute pancreatitis respond to appropriate fluid administration with prompt normalization of physical signs and laboratory values.

Laboratory protocol

Venous blood sample was taken from all subjects from the anti cubital vein under all aseptic precautions. Serum samples for IL-6 and TNF-α were collected on admission (day 1) and on the morning of day 3 after admission. All the blood serum samples were frozen immediately after collection and stored at -70 C until analysis. Serum levels of IL-6 and TNF-α were determined with ELISA method. The minimum detectable values of IL-6 and TNF-α was 5 pg/mL and 1 pg/mL, respectively.

Statistical analysis

The recorded data was compiled and entered in a spreadsheet computer program (Microsoft Excel 2010) and then exported to data editor page of SPSS version 20 (SPSS Inc., Chicago, Illinois, USA).

Descriptive statistics included computation of percentages, means and standard deviations were calculated. Analysis was performed with Mann-Whitney rank sum test. The correlations between serum markers were analysed by Pearson and Spearman's correlation. For all tests, confidence interval and p-value were set at 95% and ≤ 0.05 respectively. Cut-off values were chosen as values that achieved the highest sensitivity and specificity, as well as positive (PPV) and negative predictive values (NPVs).

Results

Table 1: Portrayed demographic and clinical characteristics of the study participants as out of total 210 patients 129 (61.5%) males and 81(38.5) female with a mean age of 48.0±1.9 were studied. 151(71.9) patients were diagnosed as mild acute pancreatitis and 59 (28.1) patients were the severe cases. The aetiologies were alcoholic 100 (47.6), biliary 77 (36.6) and idiopathic 33 (15.7) respectively.

Table 2: revealed that on day 1st& day 3rd serum levels of IL-6 found highly significantly different ($p < 0.000$) between the severe group and the mild group and TNF-α was found higher ($p < 0.05$) in the severe group than in mild cases on day 1st only.

Table 3: showed that there was good correlation found between IL-6 &TNF-α ($p<0.001$), IL-6 & CRP ($p= 0.003$ & 0.002) on both the days but not between TNF-α and CRP

(p= 0.070 & 0.074)
Table 5: On day 1, IL-6 had a higher sensitivity (92%), specificity (86.7%) and accuracy (88%) compared with TNF-α showed low level of sensitivity and diagnostic accuracy on both the days.

Table 1: Demographic and clinical characteristics of the study participants

	Mild (n = 151)	Severe (n = 59)	Total (n = 210)
Male: Female	78:49	51:32	129:81
Age (yr.)	46.9±1.2	49.5±2.3	48.0±1.9
Etiology			
Alcoholic	81 (62.7)	19 (23.4)	100 (47.6)
Biliary	28 (21.7)	49 (60.5)	77 (36.6)
Idiopathic	20 (15.6)	13 (16.1)	33 (15.7)

Table 2: Mean serum concentration level of biomarkers at day1 & day3

Variables		Day 1 Mean ± SD	Day 3 Mean ± SD
IL-6	Mild	31.05 ± 10.4	6.2 ± 2.2
	Severe	121.1 ± 59.6	57.9 ± 8.1
	p-value	0.000**	0.000**
TNF-α	Mild	1.5 ± 0.7	1.3 ± 0.6
	Severe	5.3± 1.9	4.1± 2.5
	p-value	0.042*	0.07

Test applied: Mann-Whitney rank sum test. ** indicate highly significant ($p < 0.000$), * indicate significant ($p < 0.05$)

Table 3: Correlation between serums TNF-α and IL-6 on days 1 and 3

Variables	Day 1	Day 3
IL-6/TNF-α	0.847	0.741
	(p< 0.001)	(p< 0.001)

IL-6 = interleukin-6; TNF-α = tumor necrosis factor- α

Table 4: Sensitivity, specificity, and accuracy of biomarkers in predicting severity of acute pancreatitis

	Day 1	Day 3
Sensitivity (%)		
IL-6	92.0	80.0
TNF-α	57.1	50.0
Specificity (%)		
IL-6	86.7	96.0
TNF-α	79.5	68.9
Accuracy (%)		
IL-6	88.0	80.0
TNF-α	75.8	63.3

Cutoff values: IL-32 pg/mL; TNF-α = 2 pg/mL

Discussion

Our data confirmed the findings that IL-6 and TNF-α are significantly higher in severe acute pancreatitis than in the mild disease, from day 1 to day 3. The results suggest that in the early stage of acute attack, the inflammatory cytokines may play an important role in the pathogenesis of acute pancreatitis [10, 11]. The pathogenic role of TNF-α in acute pancreatitis is still incompletely defined, although raised serum TNF-α levels have been measured in a few preliminary studies [12, 13]. There are many reasons for expecting that TNF-α is involved in the pathogenesis of pancreatitis.

First, high concentrations of TNF-α cause intravascular thrombosis and disseminated haemorrhagic necrosis of tissue, also found in acute necrotizing pancreatitis [14, 15]. This cytotoxic effect of TNF-α is partly mediated by the activated phospholipases, arachidonic acid metabolites, proteolytic enzymes and oxygen free radicals, which all may play important roles in the pathogenesis of the disease [16].

Secondly, TNF-α helps to mediate many systemic septic responses, such as fever, hypotension, shock, catabolic hormone release and multiple organ injury [17], which are all hallmarks of severe acute pancreatitis

The serum TNF- α levels measured in the present investigation were surprisingly low and showed that it had significant difference between severe and mild groups in the first day. In predicting the severity of acute pancreatitis, TNF-α was not as good as IL-6 in the early stage (days 1 and 3) of the acute pancreatitis. Banks *et al.*[11] reported that TNF- α was higher in the severe pancreatitis group but not significantly different from the mild disease group. Exley *et al* [12]. Showed the association between TNF-α and biliary pancreatitis was stronger.

Conclusion

In conclusion, we confirmed that by determining the serum concentration of IL-6 on the first day and on the 3nd day of admission represent a valuable diagnostic tool in the assessment of severity and course of disease in patients with acute pancreatitis. Routine use of pro-inflammatory cytokines as predicting factors of severity of acute pancreatitis is still not feasible in most hospitals, due to high costs and inaccessibility of analytic methods.

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