Original Article

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Biochemistry Section

# Interleukin-6 Concentration in Early Assessment of Severity and Prediction of Outcome among Patients with Acute Pancreatitis: A Prospective Cohort Study

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## ABSTRACT

**Introduction:** Various inflammatory markers, such as C-reactive Protein (CRP), Interleukin (IL), and Tumour Necrosis Factor (TNF), are used as indicators of illness severity in acute pancreatitis. Concentrations of inflammatory mediators are correlated to the severity of the disease. If detected before the occurrence of multiple organ dysfunction, early initiation of aggressive therapy might prevent its development. IL-6, released by macrophages in response to tissue injury, peaks 24 hours after the onset of inflammation and can be used to predict early disease severity. The Bedside Index of Severity in Acute Pancreatitis (BISAP) scoring system was used to assess the severity of acute pancreatitis.

**Aim:** To study the association of IL-6 levels with the BISAP in predicting the outcome.

**Materials and Methods:** A prospective cohort study was conducted in the Department of General Surgery at Government Medical College, Kottayam, Kerala, India. The duration of the study was 12 months, from January 1, 2019 to December 31, 2019. The study was done on patients of either gender, aged between 18 and 60 years, with clinical features suggestive of acute pancreatitis. After obtaining informed consent, a 4 mL venous blood sample was taken, and serum amylase and serum lipase levels were estimated. If serum amylase/lipase levels were three times above the normal range, a diagnosis of acute pancreatitis was made according to revised Atlanta criteria. Meanwhile, the BISAP score was calculated. After reaching a sample size of 77 for the present study, IL-6 estimation was performed using an Enzyme-linked Immunoassay (ELISA) kit. At the end of the study, IL-6 concentration was compared with the BISAP score to assess severe acute pancreatitis. The data entered in Microsoft Excel were analysed using the Chi-square test and Fisher's-exact test.

**Results:** The mean age of the study participants was 30 years, with 37 patients (48%) between the ages of 20-40 years and 40 patients (52%) between the ages of 40-60 years. Out of the 77 study subjects, 71 were males and six were females. There was a significant association between Intensive Care Unit (ICU) admission with persistent organ failure, which indicates severe acute pancreatitis.

**Conclusion:** In the present study, no significant association was found between IL-6 and the BISAP score in the early assessment and prediction of the severity of acute pancreatitis.

**Keywords:** Enzyme-linked immunosorbent assay, Inflammation, Inflammatory markers, Pancreas

## INTRODUCTION

Acute pancreatitis is an acute inflammatory process of the pancreas, with varying degrees of involvement of peripancreatic tissues. The key enzyme in the activation of pancreatic zymogens is trypsin, which further leads to the release of cytokines including IL-1, IL-6, IL-8, TNF-α, and platelet-activating factor. Consequently, the hepatic synthesis of acute phase reaction proteins such as CRP is induced. Leukocyte migration and activation are the major determining factors for both local and systemic complications [1,2]. According to the revised Atlanta classification, acute pancreatitis has been classified into mild acute pancreatitis (absence of organ failure and local or systemic complications), moderately severe pancreatitis (no organ failure or transient organ failure less than 48 hours with or without local complications), and severe acute pancreatitis (persistent organ failure more than 48 hours that may involve one or multiple organs) [3].

Severe acute pancreatitis develops in 20% of patients with acute pancreatitis, occurring in two phases. The first phase involves extensive pancreatic inflammation and/or necrosis, followed by Systemic Inflammatory Response Syndrome (SIRS), which can lead to Multiple Organ Dysfunction Syndrome (MODS) within the first week. Approximately 50% of deaths occur within the first week of the disease due to MODS. Infected pancreatic necrosis or fluid collection typically occurs in the second week. Proinflammatory cytokines can cause respiratory, renal, and hepatic failure. The second phase, also known as the late phase, begins 14 days after the onset of the disease and is characterised by infection of the gland, necrosis, and systemic complications that contribute to increased mortality [4,5].

No single laboratory or clinical sign is pathognomonic for acute pancreatitis [4]. The acinar cells of the pancreas secrete amylase and lipase, which are common laboratory biomarkers used to establish the diagnosis of acute pancreatitis [4]. Alanine Aminotransferase (ALT), Trypsinogen Activation Peptide (TAP), and trypsinogen-2 are also useful diagnostic markers for acute pancreatitis. IL-6 has various proinflammatory actions through which it is involved in the development of acute phase response in various tissues [5].

In patients with acute pancreatitis, isolated peripheral blood monocytes secrete increased amounts of IL-6, which is strongly associated with systemic complications, MODS, and death in these patients. It serves as a mediator for the synthesis of acute phase proteins, including CRP. Serum concentrations of IL-6 peak 24 hours after the onset of inflammation, making it useful for prediction disease severity [6,7]. The BISAP scoring system is used for the early prediction of the severity and prognosis of acute pancreatitis [8].

The BISAP score can be obtained within 24 hours of the patient's admission to the hospital. It is considered a simple, prompt, rapid, and economical scoring system in clinical practice [8]. Acute pancreatitis is a disease of variable severity, with some patients experiencing a mild,

self-limiting attack (80%), while others manifest with severe, highly morbid, and often lethal conditions (20%). Management is carried out by classifying the disease to recognise, anticipate, and treat its complications [4]. The BISAP score is a newly developed prognostic scoring system that includes data frequently evaluated at the time of admission and is accurate in predicting the patient's outcome. However, the BISAP score has the disadvantage of not easily distinguishing between transient and persistent organ failure [9].

The IL-6, which is released by macrophages in response to tissue injury, is a mediator responsible for the synthesis of acute-phase proteins, including CRP. Its serum concentrations peak just 24 hours after the onset of inflammation, making it a useful predictor of early disease severity. Additionally, when compared to other severity indices such as Acute Physiology and Chronic Health Evaluation (APACHE) II, Glasgow Coma Scale (GCS), and Computed Tomography (CT) severity index, IL-6 is more specific and does not pose any harm like radiation hazards [8,10,11].

Considering that there is no single ideal method for assessing the severity of pancreatitis, and taking into account the disadvantages of multifactorial scoring systems, there is a need for newer and promising markers. IL-6 appears to be the most promising parameter for use in clinical routine. The present study aimed to evaluate the usefulness of IL-6 concentration and the BISAP score in predicting outcomes, specifically ICU admission, for early assessment of severe acute pancreatitis.

## MATERIALS AND METHODS

This prospective cohort study was conducted in the Department of General Surgery at Government Medical College, Kottayam, Kerala, India. The duration of the study was 12 months, from January 1, 2019 to December 31, 2019. The study received approval from the Institutional Research and Ethics Committee (IEC/IRB order Ref No170/2018).

**Inclusion criteria:** Patients of either sex, aged between 18 years and 60 years, who presented with symptoms suggestive of acute pancreatitis and had a history of abdominal pain onset within 24 hours before admission to the surgery department were included in the study.

**Exclusion criteria:** Patients with chronic pancreatitis, primary hypertriglyceridaemia, those on long-term cyclooxygenase inhibitors for more than three months, and those with cardiac, liver, or renal diseases were excluded from the study.

**Sample size calculation:** The sample size was calculated using the formula based on a study conducted by Khanna AK et al., [9]:

$$N = \frac{Z^2 \times \text{Sensitivity} \times (1\text{-Sensitivity})}{d^2}$$

Z= 1.96, Sensitivity of BISAP score=74, D=10

By applying the formula, Sample size obtained was 77.

### Study Procedure

Informed consent was obtained, and 4 mL of venous blood was collected under aseptic precautions. Serum amylase and serum lipase levels were estimated from the blood sample. A diagnosis of acute pancreatitis was made according to the Revised Atlanta classification [3] if serum amylase/lipase levels were three times above the normal range. The blood sample was then centrifuged, and the serum was separated. A 2 mL serum sample from each patient included in the study was stored in an Eppendorf vial at -80°C for IL-6 analysis. Simultaneously, the BISAP score was calculated. Daily evaluations were performed until the patient's discharge. IL-6 estimation was conducted using the diaclone IL-6 ELISA kit, which has a sensitivity of 2 pg/mL. Based on the highest sensitivity and specificity values obtained from the study conducted by Khanna AK et al., a cut-off value of ≥50 pg/mL was selected for IL-6 to assess the severity of pancreatitis [9].

The BISAP score was calculated using the following criteria [12,13]:

- Blood Urea Nitrogen (BUN) > 25 mg/dL
- Impaired mental status (GCS score <15)
- Systemic Inflammatory Response Syndrome (SIRS)- It is defined as two or more of the following: 1) temperature <36°C or >38°C; 2) respiratory rate >20 breaths/min or $PaCO_2$ <32 mmHg; 3) pulse >90 beats/min; 4) White Blood Cells (WBC) < 4,000 or >12,000 cells/$mm^3$ or >10% immature bands.
- Age >60 years
- Pleural effusion detected on imaging

One point is added for each variable to give a score ranging from 0 to 5. A score of 0-2 points indicates lower mortality (<2%), while a score of 3-5 points indicates higher mortality (>15%). In the present study, ICU admission with persistent organ failure was considered an indicator of outcome in severe acute pancreatitis [3]. Organ failure included shock (systolic blood pressure <90 mmHg), pulmonary insufficiency (arterial $PO_2$ <60 mmHg at room air or the need for mechanical ventilation), or renal failure (serum creatinine level >2 mg/dL after rehydration or haemodialysis) [9].

## STATISTICAL ANALYSIS

The statistical data were entered into Microsoft Excel, and the statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software version 16.0. The efficacy of IL-6 concentrations compared with the BISAP score for the early assessment of severe acute pancreatitis was determined using the Chi-square test and Fisher's-exact test.

## RESULTS

Among the study subjects, 37 (48%) were between the age group of 20-40 years, and 40 (52%) were between the age group of 41-60 years. The distribution of study subjects based on age group showed 92 males and 8 females. Based on IL-6 concentration, 25 (32.46%) had a value of <50 pg/mL, while 52 (67.53%) had a value of >/= 50 pg/mL. The observed mortality rate among the study subjects was 15.58% [Table/Fig-1].

| Distribution of the study subjects based on the age group | |
|---|---|
| **Age group (in years)** | **n (%)** |
| 20-40 | 37 (48.05) |
| 41-60 | 40 (51.95) |
| Total | 77 |
| **Distribution of study sample based on gender** | |
| Male | 71 (92.21) |
| Female | 6 (7.79) |
| **Distribution of the study subjects based on the IL-6 concentration** | |
| <50 pg/mL | ≥50 pg/mL |
| 25 (32.46) | 52 (67.53) |
| **Distribution of study subjects based on mortality** | |
| **Mortality** | **n (%)** |
| Yes | 12 (15.58) |
| No | 65 (84.42) |
| Total | 77 |

**[Table/Fig-1]:** Demographic distribution of the study subjects.

In the age group of 20-40 years, a high BISAP score was found in 2 (5.4%) cases, while in the age group of 41-60 years, a high BISAP score was found in 3 (7.5%) cases. Among males, a high BISAP score was found in 4 (5.6%) cases, while among females, a high BISAP score was found in 16.7% of cases. Among those who required ICU admission, 1 (16.1%) had a high BISAP score, while among those who did not require ICU admission, none had

a high BISAP score [Table/Fig-2]. In the age group of 20-40 years, 23 (62.2%) had an IL-6 level of >50 pg/mL, while in the age group of 41-60 years, 29 (72.5%) had an IL-6 level of >50 pg/mL. Among males, an IL-6 level of >50 pg/mL was found in 50 (70.4%) cases, while among females, an IL-6 level of >50 pg/mL was found in 2 (33.3%) cases [Table/Fig-3].

| Parameters | BISAP score | | Total |
|---|---|---|---|
| | 0-2 (n=72) | 3-5 (n=5) | |
| | n (%) | n (%) | n (%) |
| **Age group (in years)** | | | |
| 20-40 | 35 (94.6) | 2 (5.4) | 37 (100) |
| 41-60 | 37 (92.5) | 3 (7.5) | 40 (100) |
| **Gender** | | | |
| Male | 67 (94.4) | 4 (5.6) | 71 (100) |
| Female | 5 (83.3) | 1 (16.7) | 6 (100) |

**[Table/Fig-2]:** Distribution of the study subjects based on age group, gender and Bedside Index of Severity in Acute Pancreatitis (BISAP) score.

| Age group (in years) | IL-6 | | Total |
|---|---|---|---|
| | <50 pg/mL | ≥50 pg/mL | |
| | n (%) | n (%) | n (%) |
| 20-40 | 14 (37.8) | 23 (62.2) | 37 (100) |
| 41-60 | 11 (27.5) | 29 (72.5) | 40 (100) |
| Total | 25 (32.5) | 52 (67.5) | 77 (100) |
| **Gender** | | | |
| Male | 21 (29.6) | 50 (70.4) | 71 (100) |
| Female | 4 (66.7) | 2 (33.3) | 6 (100) |

**[Table/Fig-3]:** Distribution of the study subjects based on age group, gender and Interleukin-6 (IL-6).

Among those who required ICU admission, 5 (16.1%) had a high BISAP score, while among those who did not require ICU admission, none had a high BISAP score. This difference was not statistically significant, with a p-value of 0.232. Among those who required ICU admission (31 out of 77 patients), all of them (100%) had an IL-6 level of >50 pg/mL, while among those who did not require ICU admission (46 out of 77 patients), only 45.7% (21 out of 46) had an IL-6 level of >50 pg/mL. This difference was statistically significant, with a Chi-square value of 35.254 and a p-value of 0.0001 [Table/Fig-4].

| ICU admission with persistent organ failure | IL-6 level | | p-value | BISAP score | | p-value |
|---|---|---|---|---|---|---|
| | ≤50 pg/mL n (%) | >50 pg/mL n (%) | | 0-2 n (%) | 3-5 n (%) | |
| Yes | 0 | 31 (100) | 0.0001 | 26 (83.9) | 5 (16.1) | 0.232 |
| No | 25 (54.3) | 21 (45.7) | | 46 (100) | 0 | |
| Total | 25 | 52 | 77 | 72 | 5 | 77 |

**[Table/Fig-4]:** Distribution of the study subjects based on Intensive Care Unit (ICU) admission with persistent organ failure, IL-6 and BISAP score.
BISAP: Bedside index of severity in acute pancreatitis

The association between IL-6 and the BISAP score was determined using Fisher's-exact test (p-value 0.343), which did not show any statistically significant findings in the present study [Table/Fig-5].

| BISAP score | IL-6 level | | p-value |
|---|---|---|---|
| | < or= 50 pg/mL | >50 pg/mL | |
| 3-5 | 1 | 4 | 0.343 |
| 0-2 | 24 | 48 | |
| Total | 25 | 52 | |

**[Table/Fig-5]:** Association of IL-6 and BISAP score.
IL-6: Interleukin-6; BISAP: Bedside index of severity in acute pancreatitis

## DISCUSSION

The present study focused on the IL-6 concentration as a biomarker for the early assessment of severe acute pancreatitis. The findings of the current study confirmed that IL-6 concentration can be used as a reliable indicator for early stratification of the severity of acute pancreatitis within 24 hours of admission. This is consistent with the study by Rao SA and Kunte AR, which stated that measuring IL-6 within 48 hours of onset is a reliable biomarker for predicting the progression to severe pancreatitis [10].

The BISAP score can be easily obtained within 24 hours of the patient's admission to the hospital. It is a simple, prompt, rapid, and economical scoring system in clinical practice. In the present study, 93.5% of the study subjects had a low BISAP score of 0-2, indicating mild to moderate pancreatitis. Only 6.5% had a high BISAP score of 3-5, indicating severe acute pancreatitis. In contrast, Khanna AK et al., in their study, found that 36 (50%) of the study subjects had a low BISAP score of 0-2, while 36 (50%) had a high BISAP score of 3-5. They also concluded that the BISAP score has the disadvantage of not easily distinguishing transient from persistent organ failure [9].

In the present study, among the 31 patients who required ICU admission, 16.1% (5 out of 31) had a high BISAP score. Among those who did not require ICU admission (46 out of 77 patients), none had a high BISAP score. However, this difference was not statistically significant, with a p-value of 0.232. A study conducted by Khanna AK et al., on 72 patients reported a sensitivity, specificity, positive predictive value, and negative predictive value of the BISAP score for detecting severe acute pancreatitis as 74.2%, 68.3%, 63.4%, and 77.8%, respectively [9]. In current study conducted on 77 patients to test the efficacy of IL-6 score, among those in the age group of 20-40 years, 62.2% had an IL-6 level of >50 pg/mL, while among those in the age group of 41-60 years, 72.5% had an IL-6 level of >50 pg/mL. However, this difference showed no statistical significance (p-value 0.333). According to a study conducted by Khanna AK et al., on 72 patients, IL-6 was measured in 60 patients. Among them, 53.3% had an IL-6 value <50 pg/mL, and 46.7% had a value of ≥50 pg/mL. The sensitivity, specificity, positive predictive value, and negative predictive value of IL-6 concentration for detecting severe acute pancreatitis were 93.1%, 96.8%, 96.4%, and 93.8%, respectively, in that study [9].

Among the patients who required ICU admission (31 out of 77 patients), all of them (100%) had an IL-6 level of >50 pg/mL, whereas among those who did not require ICU admission (46 out of 77 patients), only 45.7% (21 out of 46) had an IL-6 level of >50 pg/mL. This difference was statistically significant, with a Chi-square value of 35.254 and a p-value of 0.001. Therefore, IL-6 concentration was found to be a better screening tool than the usual scoring system in predicting acute severe pancreatitis. The present study's finding was supported by a study done by Li J et al., who found that IL-6 was superior to CRP in predicting pancreatic necrosis and mortality in individuals with acute pancreatitis, demonstrating the diagnostic utility of IL-6 similar to the findings of the present study [12].

A study by Aoun E et al., showed that IL-6 and IL-8 can predict severe acute pancreatitis with a satisfactory degree of accuracy [7]. Similarly, a study conducted by Kolber W et al., demonstrated the diagnostic use of IL-6 as an independent biomarker to predict the onset of severe acute pancreatitis, critical organ failure, the requirement for intensive care, and death from acute pancreatitis. These findings align with the results found in the present study [14].

According to Nieminen A et al., IL-6 and Hepatocyte Growth Factor (HGF) levels were the only two out of 14 cytokines that were elevated in severe acute pancreatitis and were independent predictive indicators of severity [15]. When comparing IL-6 concentration and the BISAP score in relation to the outcome of severe acute pancreatitis, which is defined as the presence of persistent organ failure and the need for ICU admission, IL-6 concentration was found to have diagnostic value in predicting the outcome in acute pancreatitis.

### Limitation(s)

The present study does not provide information on the demographic diversity of the sample, thus limiting the generalisability of the findings.

## CONCLUSION(S)

When comparing the IL-6 concentration and BISAP score in relation to the outcome of acute severe pancreatitis, specifically the need for ICU admission, IL-6 concentration was found to be superior to the BISAP score. The present study statistically demonstrates that higher IL-6 concentrations (≥50 pg/mL) within 24 hours of the onset of acute pancreatitis have higher sensitivity and are a better tool compared to the BISAP score for early assessment of severe acute pancreatitis. It is important to note that there is currently no single ideal method for assessing the severity of the disease. Therefore, further studies with larger and more diverse patient cohorts are needed, as well as exploration of additional potential markers for the early assessment of severe acute pancreatitis. Individual preferences and available Institutional facilities also influence the method chosen for prognostic assessment of acute pancreatitis.

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