DOI: 10.7860/JCDR/2023/63566.18357

Original Article

Biochemistry Section

Correlation of Acute Inflammatory Markers with Oxidative Stress Markers in Patients with Chronic Pancreatitis: A Cross-sectional Study

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ABSTRACT

Introduction: Chronic Pancreatitis (CP) is a long-standing inflammation of the pancreas that presents as episodes of acute inflammation or chronic damage, leading to alterations in the organ's normal structure and function. Numerous studies have focused on inflammatory and oxidative stress markers in Acute Pancreatitis (AP), but similar studies in CP are rare.

Aim: The aim of this study was to compare the serum levels of acute inflammatory and oxidative stress markers in CP patients with healthy controls, and to investigate the correlation between acute inflammatory markers and oxidative stress markers.

Materials and Methods: This cross-sectional study was conducted at Jawaharlal Institute of Postgraduate Medical Education Research (JIPMER), Puducherry, India, from January 2018 to December 2021. Forty-five patients diagnosed with CP, based on clinical manifestations, Contrast-Enhanced Computed Tomography (CECT) of the abdomen, and histopathology reports, were enrolled along with 45 healthy controls. Serum biochemical parameters, including inflammatory markers such as high-sensitive C-Reactive Protein (hs-CRP), Interleukin-6 (IL-6), and Tumour Necrosis Factor-alpha (TNF-α), as well as oxidative stress parameters such as Malondialdehyde (MDA), Total Antioxidant Status (TAO), and Total Oxidant Status (TOS), were measured. The Oxidative Status Index (OSI) was calculated. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 21.0.

Results: There were no significant differences in age and gender between the two groups. None of the individuals in the control group had diabetes, and their Body Mass Index (BMI) was within normal limits. Patients exhibited leukocytosis, hypoproteinemia, and hypoalbuminemia. Although all patients presented with pain, none had recent acute pain episodes or elevated serum amylase levels. Compared to healthy individuals, CP patients showed significantly elevated serum concentrations of inflammatory and oxidative stress markers, as well as OSI. Serum antioxidant (TAO) levels were significantly reduced in patients compared to controls. Inflammatory and oxidative stress markers were positively correlated with age, smoking and alcohol intake. There was a positive correlation between acute inflammatory markers and oxidative stress markers in patients with CP.

Conclusion: Serum inflammatory and oxidative stress markers were significantly elevated in patients with CP as compared to controls, indicating key roles in patients with CP.

Keywords: Antioxidants, Fibrosis, Leukocytes, Pancreas, Proinflammatory cytokines

INTRODUCTION

Earlier, the concept was that pancreatitis is caused by self-inflicted injury or infectious agents. A new concept suggests that pancreatitis is a complex inflammatory condition like inflammatory bowel disease [1]. Pancreatitis is broadly classified into AP and CP. The pathogenesis and clinical manifestations in CP are different from AP. AP is defined as episodes of discrete attacks of abdominal pain generally limited to epigastric or right upper quadrant pain and is associated with elevated levels of serum lipase and amylase [2]. In CP, there is intermittent pain with signs of inflammation, cellular fibrosis, and loss of acinar cells (exocrine insufficiency) and islet cells (endocrine insufficiency). Only temporary damage exists in AP, whereas the pancreatic architecture and function are variably lost in CP. The evolutionary change from AP to CP has been reported, with necrosis predominating the early acute events while fibrosis finally sets in [3]. CP includes idiopathic CP, tropical pancreatitis (seen in children and young adults with malnutrition in tropical countries), hereditary pancreatitis (due to gene mutations), and obstructive CP [4].

Numerous studies have shown that patients with AP may progress into the chronic form over time [5-7]. The most significant symptoms in CP are pain, which adversely affects the quality of life. Abdominal pain caused by CP creates internal, deep abdominal discomfort ranging from mild to severe. Pancreatitis is one of the common reasons for gastrointestinal-related hospital admissions [5]. Patients with CP suffer economically due to several hospital visits, excessive sedative medication, and medical and surgical interventions [6].

Several factors are associated with CP, with genetics and lifestyle being the most important [4]. Environmental factors include alcohol, smoking, stress, radiation, and other chemicals and drugs [7-9]. All these factors generate oxidative stress, apoptosis, and mitochondrial DNA damage; however, the exact molecular mechanisms involved in cell death have not been elucidated. Reactive Oxygen Species (ROS) play a major role in the progression of inflammation and further complications [10]. Excess ROS is dangerous and damaging. Pancreatic destruction may affect enzymatic and hormonal production, resulting in pancreatic exocrine and endocrine insufficiency [10]. Identifying and studying all risk factors involved in developing CP is still in its infancy stage [10].

In 2013, our lab conducted a pioneering study using Fine Needle Aspiration Cytology (FNAC) analysis under the guidance of the corresponding author [3]. We reported a strong association of acute pathological inflammation in the gland (pancreas) in a different group of patients with CP, despite any clinical changes. Hence, further analysis was needed to evaluate the acute inflammatory changes in CP [3]. Therefore, the present study was conducted to compare the

levels of inflammatory markers, including IL-6, TNF-α, hs-CRP, as well as oxidative stress parameters such as MDA, TAO, and TOS in the serum of patients with CP and controls.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Surgical Gastroenterology and Department of Biochemistry, JIPMER, Puducherry, India from January 2018 to December 2021. The study was approved by the Institutional Ethical Committee (IEC certificate number- JIP/IEC/2018/021), and written informed consent was obtained from all study subjects.

Inclusion criteria: All patients aged between 18-80 years, diagnosed with CP, were included in the study. The diagnosis of CP was based on the clinical manifestation of intermittent epigastric pain and weight loss with or without exocrine and endocrine insufficiency [2]. A CECT of the abdomen was performed in all patients to confirm the diagnosis, evaluate the characteristics of the pancreas, and rule out complications related to CP. Healthy normal volunteers of the same age group (18-40 years) and gender-matched controls without any known risk factors for diabetes, other associated inflammatory disease conditions, and any clinically relevant conditions or surgical conditions were recruited as controls.

Exclusion criteria: Patients with CP with cancer, patients with other malignant conditions, patients with a prior history of surgery for CP, patients undergoing antioxidant therapy, patients on other chronic, ischemic, and inflammatory disease treatments, patients with uncontrolled psychiatric or neurologic problems, and those who were pregnant or lactating and might affect the antioxidant status were excluded from the study.

Sample size: The sample size was calculated using OpenEpi software, version 3.1, assuming a 95% confidence interval and 80% power. Based on a previous study [11] that reported the mean (SD) oxidative stress among cases as 0.64 (0.35) and among controls as 0.45 (0.13) with 90% power and 95% confidence interval, the calculated sample size was 41. However, in the present study, 45 healthy volunteers and 45 patients with CP were enrolled.

Data collection: Relevant demographic data, clinical and biochemical data, and routine diagnostic tests, such as CECT abdomen, were collected using a standard data collection proforma and evaluated. Anthropometric parameters, including weight and height, were measured, and BMI was calculated using the formula weight (kg) divided by the square of height (m^2). The collected data were recorded in an electronic database [Table/Fig-1] [4,12-15].

Parameters (Manufactrurer)	Method of analysis- ELISA	Cut-off range
hs-CRP (Calbiotech, USA)	450 nm	0.2 to 10 mg/L [4]
IL-6 (Diaclone, France)	620 nm	<2 pg/mL [12]
TNF-α (Diaclone, France)	620 nm	<8 pg/mL [12]
MDA (Immunotag USA)	450 nm	0.2 nmol/mL-60 nmol/mL [13]
TAO (Elabscience, USA)	590 nm	0.049-2.5 mmol/L [14]
TOS (Elabscience, USA)	590 nm	2.5-100 μ mol H_2O_2 Equiv./L [15]

[Table/Fig-1]: Manufacturer's details of ELISA kits used for the estimation of blood biochemical parameters [4,12-15].
hs-CRP: High sensitive C-reactive protein; IL-6: Interleukin-6; TNF-α: Tumour necrosis factor-alpha; MDA: Malondialdehyde; TAO: Total antioxidant status; TOS: Total oxidant status

To determine OSI value, TOS and TAS units were equilised and OSI was calculated using the formula:

$$OSI = \frac{TOS\ (\mu mol H_2O_2 equi/L)}{TAO\ (mmol trolox\ equi/L)} \times *(100)$$

Under strict aseptic conditions, peripheral venous blood samples (5 mL) were collected from all patients upon admission in a vacutainer with a clot activator. The collected blood was centrifuged at 3500 rpm (769 x g) for 15 minutes, and the separated serum was stored at -80°C until further analysis. Routine biochemical parameters were evaluated, and inflammatory and oxidative markers were estimated using commercially available Enzyme Linked Immunosorbent Assay (ELISA) kits according to the manufacturer's instructions [Table/Fig-1]. The suggested cut-off values by Phosat C et al., [4] were used to categorise hs-CRP levels as <1 mg/dL and 10 mg/dL. As there are no standard cut-off points for TNF-α and IL-6, tertile ranges were used [4]. The present study found reference ranges of <4 pg/mL for IL-6 and ≤8 pg/mL for TNF-α based on the manufacturer's data from healthy controls [12].

STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS version 21.0. All results were expressed as mean±Standard Deviation (SD) or median with Interquartile Range (IQR). Differences among the groups were determined using either the Mann-Whitney test, independent t-test, or Pearson Chi-square test. The level of significance was defined by a two-tailed p-value <0.05. Fisher's exact test, Pearson, and Spearman correlations were used to determine the strength of the associations. Receiver-Operating Curves (ROC) were created to determine cut-off values for the best sensitivity and specificity for the biomarkers.

RESULTS

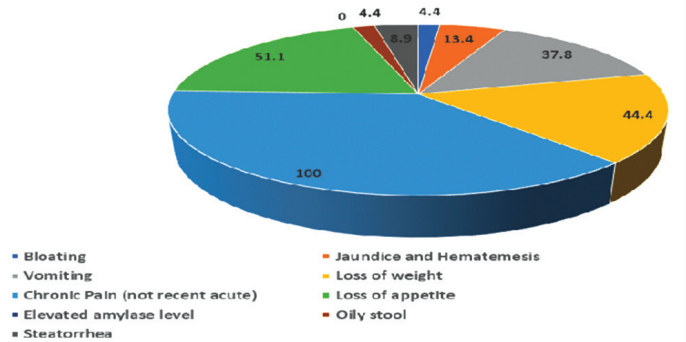
In the present study, there were 32 (71.1%) males and 13 (28.9%) females in the control group, and 34 (75.6%) males and 11 (24.4%) females in the case group, respectively. There were no significant differences in age and gender, but differences were observed in BMI and diabetes mellitus. However, all patients experienced pain, none had a recent acute pain episode or elevated serum amylase levels [Table/Fig-2].

Exocrine insufficiency in the form of steatorrhea was noted in only 4 (8.9%) cases. Loss of appetite and weight loss were noted in 23 (51.1%) and 20 (44.4%) cases, respectively. Out of the 45 patients, 4 (8.9%) exhibited jaundice, and 17 (37.8%) experienced vomiting [Table/Fig-3].

Characteristics	CP n (%)/M±SD	Control n (%)	p-value
Age (years)	39.3±12.9	35.1±10.4	0.1
BMI (kg/m^2)	19.69±3.77	23.01±3.11	<0.001
Gender, n (%)			
Male	34 (75.6)	32 (71.1)	0.63
Female	11 (24.4)	13 (28.9)	
Diabetics, n (%)			
Yes	21 (46.7)	0	<0.001
No	24 (53.3)	45 (100.0)	
Family history of DM	17 (37.8)	14 (31.1)	0.51
Alcohol intake, n (%)			
Nil	22 (48.9)	36 (80.0)	
Chronic alcoholic	23 (51.1)	0(0.0)	
Social drinkers	0	9 (20.0)	
Smoking status, n (%)			
Smokers	20 (44.4)	0	<0.001
Non smokers	25 (55.6)	45(100)	
Diet, n (%) **			
Veg[$]	0 (0.00)	2 (4.4)	0.49
Non veg	45 (100)	43 (95.6)	
Exercise, n (%)			
Never	17 (37.8)	0	<0.001
Everyday	2 (4.4)	15 (33.3)	
Sometimes	5 (11.1)	27 (60.0)	
Rarely	21 (46.7)	3 (6.7)	

History of aetiology, (months)				
Diabetes duration	8.6±129.9	-	-	-
Alcohol-intake duration*	20.6±119.7	-	-	
Smoking duration	9.6±150.3	-	-	
Pain duration	2.9±36.3	-	-	

[Table/Fig-2]: Demographic and baseline characteristics of patients and controls.
All parameters – Chi-square tests except Diet **Fishers-exact test
$Veg-Vegetarians who were vegans and eggetarians
*Duration calculated only for chronic alcoholics and social drinkers were omitted
BMI: Body mass index; SD: Standard deviation



[Table/Fig-3]: Clinical symptoms in patients.

In the present study, the mean glucose level was significantly higher in cases compared to controls. Hypoproteinemia and hypoalbuminemia were reported in 14 (31.1%) and 12 (26.6%) cases, respectively. Though the mean sodium levels were significantly lower in patients compared to controls, sodium level was in normal range. ALP, GGT, potassium, AST, TLC, and LFT were significantly increased in cases compared to controls [Table/Fig-4].

Biochemical parameter	Case (Mean±SD)	Control (Mean±SD)	p-value
Glucose (RBS) (mg/dL)	123.38±57.8	85.4±17.61	<0.001
Urea (mg/dL)	23.47±18.3	21.96±5.95	0.60
Creatinine (mg/dL)	1.018±0.97	0.86±0.17	0.27
Total bilirubin (mg/dL)	1.44± 2.33	0.68±0.35	0.21
Total protein (g/dL)	6.97±0.81	7.3±0.58	0.04
Albumin (g/dL)	3.76±0.86	4.21±0.46	<0.001
Globulin (g/dL)	3.20±0.59	3.07±0.62	0.30
AST (IU/L)	52.24±69.02	26.8±11.9	0.01
ALT (IU/L)	52.96±71.8	30.5±20.52	0.08
ALP (IU/L)	228.71±261.04	82.09±33.38	<0.001
GGT (IU/L)	108.76±166.76	32.87±25.04	<0.001
Hb (g/dL)	12.01±2.44	13.5±1.6	<0.001
Platelets (*10^3 uL)	2.7±1.181	2.57±0.8	0.56
TLC (uL)	10916.7±7625.5	7119.9±2499.03	<0.001
Sodium (mEq/L)	133.5±5.09	138.22±4.2	<0.001
Potassium (mEq/L)	4.30±0.65	4.02±0.34	0.01

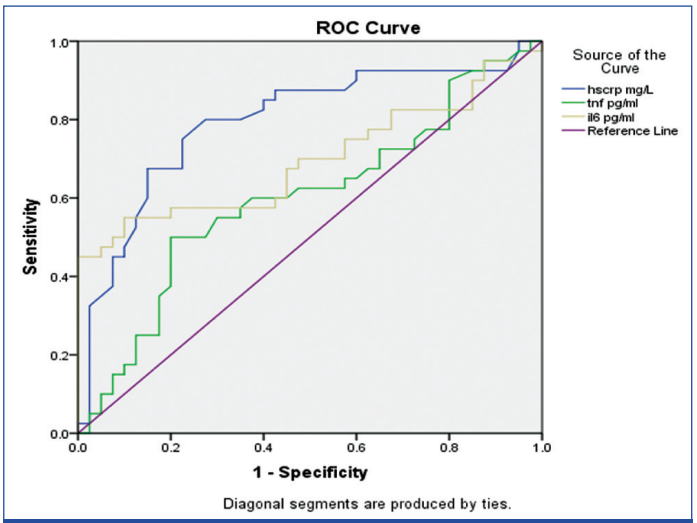
[Table/Fig-4]: Biochemical parameters in patients and controls.
All parameters- Independent t-test except total bilirubin, AST, ALT, ALP, GGT-Mann-Whitney test
RBS: Random blood sugar; AST: Aspartate aminotransaminase; ALT: Alanine aminotransaminase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase; in LFT: Liver function test;
Hb: Haemoglobin; TLC: Total leukocyte count
mg/dL: Milli gram per deciliter; g/dL: Gram per deciliter; IU/L: International unit per litre ul-microlitre; mEq/L: Milli equivalent per litre

The concentration of hs-CRP, IL-6, MDA, TOS, and OSI index in the serum of CP patients was significantly higher compared to controls, while the TAO levels were significantly lower in patients compared to the controls [Table/Fig-5].

The area under the curve for hs-CRP was 0.795 (0.69-0.89), for TNF-α was 0.60 (0.47-0.73), and for IL-6 was 0.70 (0.58-0.82), indicating that they provide fairly accurate results. The ideal cut-off point for hs-CRP was 1.45, with a sensitivity of 92.5% and specificity of 40%. The ideal cut-off points for TNF-α and IL-6 were 10.63 and 3.93, respectively, with a sensitivity of 80% and 82.50% and specificity of 20% and 32.50%, respectively [Table/Fig-6].

Serum levels	CP		Control		p-value
	Mean$±SD	Median (IQR)#	Mean$±SD	Median (IQR)#	
Hs-CRP (mg/L)#	7.38±3.4	8.7 (3.5-10.1)	3.32±3.16	2 (0.85-4.3)	<0.001
TNF-α (pg/mL)#	82.9±109.5	36.63 (12.08-104.99)	57.7±111.8	17.77 (11.68-36.17)	0.12
IL6 (pg/mL)#	26.24±36.04	11.6 (4.66-24.94)	6.25±3.5	5.6 (3.73-8.64)	<0.001
MDA (nmole/mL)$	60.5±35.5	60.83 (23.52-89.36)	11.12±4.8	11.32 (9.00-14.13)	<0.001
TOS (µmol H_2O_2 equi/L)$	251.8±195.53	129 (78.61-445.52)	129.8±130.34	85.02 (51.41-136.13)	<0.001
TAO (mmoltrolox equi/L)$	1.09±0.31	1.03 (0.91-1.3)	1.28±0.27	1.26 (1.1-1.4)	0.017
OSI index (*10AU)$	2.45±1.96	1.75 (0.73-4.28)	1.02±0.95	0.66 (0.42-1.25)	<0.001

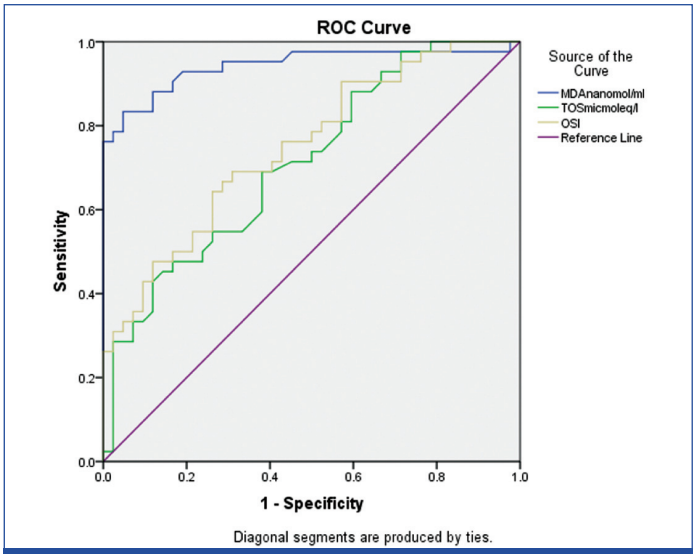
[Table/Fig-5]: Comparison of inflammatory markers and oxidative stress markers between cases and controls.
Hs-CRP, IL-6, and TNF-α- Mann-whitney test; MDA, TOS, TAO and OSI-Independent T-test
Hs-CRP: High-sensitive C-reactive protein; IL-6: Interleukin-6; TNF-α: Tumour necrosis factor-alpha; and MDA: Malondialdehyde; TAO: Total antioxidant status; TOS: Total oxidant status; OSI: Oxidative status index; mg/L: Milli gram per litre; pg/mL: Pico gram per milli liter; nmol/mL: Nanomole per milli litre; µmol eq/l: Micromole H_2O_2 equivalent per litre; mmol/l: Millimole per litre; AU: Arbitrary unit;
$: Mean±SD; #: Median (IQR)



[Table/Fig-6]: ROC curve for the inflammatory markers.
The area under the curve for Hs-crp {0.795 (0.69-0.89)}, TNF-α {0.60 (0.47-0.73)}, IL-6 {0.70 (0.58-0.82)}. For Hs-crp test, the ideal cut off point is 1.45 {sensitivity-92.5% and specificity 40%}, TNF-α {sensitivity-80% and specificity 20%}, and IL-6 {sensitivity- 82.50%, specificity- 32.50%} the ideal cut off point is 10.63 and 3.93, respectively

In the present study, the area under the curve for MDA was 0.944 (0.88-0.99), indicating excellent accuracy in predicting the result. TOS had an area under the curve of 0.70 (0.58-0.81), TAO had an area under the curve of 0.65 (0.54-0.77), and OSI index had an area under the curve of 0.75 (0.65-0.85), indicating fairly accurate results [Table/Fig-7].

IL-6 and MDA had a weak positive correlation with age. IL-6 had a moderate negative correlation with BMI, while MDA had a moderate positive correlation with BMI (p-value <0.001). TAO had a weak negative correlation with alcohol intake duration, and OSI had a weak positive correlation with alcohol intake duration (p-value=0.03 and 0.02, respectively). MDA had a moderate positive correlation with alcohol intake duration (p-value <0.001). Hs-CRP, TOS, and OSI had weak positive correlations with smoking duration. IL-6 and MDA had a moderate positive correlation with smoking duration (p-value ≤0.001). IL-6, TOS, and OSI had weak positive correlations



[Table/Fig-7]: ROC curve for the oxidative stress markers.
The area under the curve for MDA is {0.944 (0.89-0.99)}, TOS {0.70 (0.58-0.81)}, TAO {0.65 (0.54-0.77)} and OSI Index {0.75 (0.65-0.85)}. For MDA test, the ideal cut-off point is 11.65 {sensitivity-97.50% and specificity 55.00%}, TAO cut-off point is 0.94 {sensitivity-91.10% and specificity 28.90%}, and TOS and OSI*10AU {sensitivity- 90.00%, specificity- 30.00%} the ideal cut off point is 58.34 and 0.46, respectively

with diabetes duration (p-value ≤0.001). Hs-CRP and MDA had a moderate positive correlation with diabetes (p-value ≤0.001) [Table/Fig-8].

Hs-CRP had a weak positive correlation with MDA and a moderate positive correlation with TOS and OSI (p-value <0.001). IL-6 had a weak positive correlation with MDA (p-value=0.02). IL-6 had a weak positive correlation with TOS and OSI (p-value <0.001) [Table/Fig-9].

smokers in the present study. Additionally, 40% of the patients with CP had a history of both alcohol and smoking. Recurrent exposure to alcohol and smoking can lead to frequent episodes of acute pancreatitis, eventually progressing to CP [18,19]. While alcoholism is often associated with CP, it should be noted that only 10% of heavy alcohol drinkers actually develop pancreatitis [20]. Although none of the female participants in our study were alcoholic, a previous study conducted in 2001 [22] suggests that alcoholic pancreatitis is more common in men than in women.

Unlike acute pancreatitis, where various scoring systems are used to predict disease severity [23], there is no widely accepted scoring system for CP. However, several studies have shown that elevated levels of TLC, hs-CRP, IL-6, and TNF-α, which are markers of acute inflammation, can serve as valuable prognostic indicators of severity and systemic complications in acute pancreatitis [24-28]. While CRP is commonly used as an inflammatory marker in clinical practice [11], IL-6 has been identified as the most important predictor of AP severity [29]. It is important to note that these markers are not typically considered markers of chronic inflammation. However, there is evidence suggesting that IL-6 and TNF-α play crucial roles in the pathogenesis of CP. Consistent with other studies [30,31], our findings showed that patients with CP had significantly higher TLC, TNF-α, and IL-6 levels compared to healthy controls.

In the present study, 5 out of 45 (11%) TNF-α measurements were not within the detectable range, which may have contributed to the lack of significant difference in TNF-α production compared to healthy individuals. Similar issues have been raised in earlier reports [31-34]. This could be attributed to the short production and high hepatic clearance of TNF-α. When neutrophils are highly

Parameters	Age (years)		BMI (Kg/m^2)		Alcohol intake (Months)		Smoking habbit (Months)		Diabetes (Months)	
	r- value	p-value	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value
Hs-CRP (mg/L)	0.07	0.5	-0.07	0.49	0.10	0.34	0.21*	0.04	0.37**	<0.001
TNF-α (pg/mL)	0.03	0.83	-0.13	0.26	-0.10	0.37	-0.04	0.74	0.12	0.31
IL6 (pg/mL)	0.24*	0.03	-0.34**	<0.001	0.16	0.13	0.36**	0.001	0.22*	0.04
MDA (nmol/mL)	0.22*	0.04	-0.42**	<0.001	0.34**	<0.001	0.52**	<0.001	0.50**	<0.001
TAO (mmol/L)	-0.11	0.32	0.02	0.87	-0.25*	0.03	-0.21	0.05	-0.1	0.35
TOS (μmoleq/L)	0.12	0.27	-0.14	0.18	0.19	0.08	0.24*	0.03	0.26*	0.01
OSI	0.15	0.16	-0.15	0.15	0.24*	0.02	0.28**	0.01	0.28**	0.01

[Table/Fig-8]: Correlation analysis of inflammatory markers and clinical parameters.
**Weak correlation by spearman correlation analysis; **Moderate correlation by spearman correlation analysis
BMI: Body mass index; Hs-CRP: High-sensitive C-reactive protein; IL-6: Interleukin-6; TNF-α: Tumour necrosis factor-alpha; MDA: Malondialdehyde; TAO: Total antioxidant status; TOS: Total oxidant status; OSI: Oxidative status index; mg/L: Milli gram per litre; pg/mL: Pico gram per milli liter; nmol/mL: Nanomole per milli litre; mmol/L: Millimol per litre; μmoleq/L: Micromole equivalent per litre; AU: Arbitrary unit

Parameters	MDA (nmol/mL)	p-value	TAO (mmol/L)	p-value	TOS (μmol eq/L)	p-value	OSI (AU)	p-value
Hs-CRP (mg/L)	0.34**	<0.001	-0.17	0.11	0.42**	<0.001	0.42**	<0.001
TNF-α (pg/mL)	0.14	0.22	0.03	0.81	0.17	0.14	0.14	0.21
IL6 (pg/mL)	0.25*	0.02	-0.05	0.63	0.30**	<0.001	0.3**	<0.001

[Table/Fig-9]: Correlation* analysis of inflammatory markers and oxidative stress parameters.
*Spearman correlation analysis
Hs-CRP: High-sensitive C-reactive protein; IL-6: Interleukin-6; TNF-α: Tumour necrosis factor-alpha; MDA: Malondialdehyde; TAO: Total antioxidant status; TOS: Total oxidant status; OSI: Oxidative status index
mg/L: Milli gram per litre; pg/mL: Pico gram per milli liter; nmol/mL: Nanomole per milli litre; mmol/L: Millimole per litre; μmol eq/L: Micromole H_2O_2 equivalent per litre; AU: Arbitrary unit

DISCUSSION

In the present study, 95% of patients experienced pain in the upper abdomen, specifically in the upper right or left quadrant and the epigastric area, which is consistent with previous research [16]. Other common symptoms observed were jaundice, vomiting, and loss of appetite and weight. Abdominal pain is often the initial symptom in CP, and some patients may eventually develop diabetes, which is a common complication of advanced CP [17].

The TLC and LFT were significantly increased in patients. This could be attributed to the underlying etiology of chronic alcoholism, biliary obstruction, and obstructive jaundice. More than 50% of the patients had a history of alcohol consumption, and 44.4% were

activated, they generate more elastase, which can inactivate TNF-α and make it undetectable after being released into circulation. A study by Xie MJ et al., examining tissue expressions of different cytokines in rats with CP revealed the crucial role of TNF-α and IL-6 expression in pancreatitis [35]. On the other hand, some studies have concluded that there is no relationship between TNF-α and CP [34]. However, TNF-α is reported to be a potent inducer of IL-6 from leukocytes [36].

Patients with diabetes have been reported to have decreased plasma Total Antioxidant Capacity (TAC) levels but higher plasma MDA levels [37]. Our present results showed that oxidative stress significantly increased the levels of MDA, which is consistent with a

study conducted on patients with diabetes [38]. Increased levels of oxidative stress markers such as TOS, Oxidative Stress Index (OSI), and MDA have also been widely reported in patients with acute pancreatitis [11,39,40], and a similar pattern was observed in our patients with CP.

The duration of alcoholism, smoking, and the presence of diabetes were found to significantly correlate with the levels of inflammatory and oxidative markers in our study of patients with CP, indicating the involvement of multiple risk factors in the development of CP. In reality, CP is most commonly caused by a combination of risk factors [41].

According to the necrosis-fibrosis sequence theory, CP is caused by episodes of severe acute pancreatitis [42], disproving the concept that AP rarely progresses to the chronic form [43]. Our study also supports the idea that acute inflammation is a major feature in patients with CP. Our observations in this laboratory experiment were consistent with clinical scenarios in patients with CP, where we observed acute inflammatory infiltrates and abscesses in pathological pancreas specimens [3]. Based on our study findings, further research may clarify whether recurrent acute pancreatitis is the reason for the recurrent pain in patients with CP. Our study suggests a significant acute inflammatory process in patients with CP, which may be a contributing factor to the etiology and progression of the disease.

Limitation(s)

Due to technical difficulties and high costs, further confirmation of acute inflammatory markers through protein expression in a relatively larger sample size was not conducted. The cost-effectiveness, ease of application, and reproducibility of laboratory parameters are fundamental challenges in daily clinical practice.

CONCLUSION(S)

The present study observed an increased inflammatory process and oxidative stress in patients with CP compared to controls. The significant elevation of acute inflammatory markers such as TLC, CRP, IL-6, and TNF-α, as well as the positive correlation between acute inflammatory markers and oxidative stress markers in patients with CP, suggest the presence of a significant acute inflammatory process in these individuals. Oxidative damage, typically associated with acute inflammation, is also observed in patients with CP. All these findings from the present study indicate that recurrent episodes of acute inflammation occur in patients with CP. This acute inflammation may play a major role in the development and complications of the disease in CP patients.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Feb 22, 2023
- Manual Googling: May 17, 2023
- iThenticate Software: Jun 20, 2023 (14%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: This work was supported by the Jawaharlal Institute of Post-graduate Medical Education Research (JIPMER) intramural fund under Grant (JIP/Res/Intramural/phs2/2020-21).
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Feb 17, 2023**
Date of Peer Review: **Apr 22, 2023**
Date of Acceptance: **Jun 23, 2023**
Date of Publishing: **Aug 01, 2023**