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ORIGINAL ARTICLE

Investigation of Cytokines, Biochemical Parameters and Oxidative Stress Levels in Serum of Patients with Acute Pancreatitis

Akut Pankreatitli Hastaların Serumlarında Sitokinler, Biyokimyasal Parametreler ve Oksidatif Stres Düzeylerinin Araştırılması

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ABSTRACT

Objective: This study aimed to find new serum biochemistry parameters, especially for the early identification of severe Acute Pancreatitis (AP). In the study, serum cytokine levels (TNF-A, IL-1, IL-6, IL-10, IL-21), biochemical parameters (Aquaporin-1, Hepcidine, Iron, Zinc, Copper, Nitric Oxide, C-Reactive protein) and oxidative stress parameters analysis were performed.
Method: A total of 46 patients with AP and 46 healthy controls were included in this study. Serum cytokine levels, biochemical, and oxidative stress parameters by the ELISA method and CRP by the immunoturbidimetric method were measured.
Results: When patients with AP and control groups were compared, all studied parameters were found statistically significant ($p<0.05$). However, IL-1 was insignificant ($p>0.05$). Therefore, the Mann-Whitney U test, which is a non-parametric test, was deemed appropriate to determine whether there was a significant difference between the patient and control groups.
Conclusions: Investigating the role of cytokines, oxidative stress and other biochemical parameters in the pathogenesis and course of the disease may contribute to a better understanding of the disease process and its therapeutic value. It showed a significant increase in oxidative stress parameters and biochemical parameters such as aquaporin-1, hepcidin, lipase, and amylase, which may help in the diagnosis of AP.

Keywords: Acute pancreatitis, cytokine, oxidative stress, some other biochemical parameters

ÖZ

Amaç: Bu çalışma, özellikle şiddetli AP'nin erken teşhisi için yeni serum biyokimya parametreleri bulmayı amaçladı. Çalışmada serum sitokin seviyeleri (TNF-A, IL-1, IL-6, IL-10, IL-21), biyokimyasal parametreler (Aquaporin-1, Hepsidin, Demir, Çinko, Bakır, Nitrik Oksit, C-Reaktif protein) ve oksidatif stres parametreleri analizi yapıldı.
Yöntem: Bu çalışmaya AP'li toplam 46 hasta ve 46 sağlıklı kontrol dahil edildi. ELISA yöntemi ile serum sitokin düzeyleri, biyokimyasal ve oksidatif stres parametreleri, immünotürbidimetrik yöntemle de CRP ölçüldü.
Bulgular: AP'li hastalar ve kontrol grubu karşılaştırıldığında çalışılan tüm parametreler istatistiksel olarak anlamlı bulundu ($p<0.05$). Ancak IL-1'in önemsiz olduğu bulundu ($p>0.05$). Bu nedenle hasta ve kontrol grupları arasında anlamlı fark olup olmadığını belirlemek için parametrik olmayan bir test olan Mann-Whitney U testi uygun bulunmuştur.
Sonuç: Sitokinlerin, oksidatif stresin ve diğer biyokimyasal parametrelerin hastalığın patogenezindeki ve seyrindeki rolünün araştırılması, hastalık sürecinin ve tedavi edici değerinin daha iyi anlaşılmasına katkı sağlayabilir. AP tanısında yardımcı olabilecek aquaporin-1, hepsidin, lipaz ve amilaz gibi oksidatif stres parametrelerinde ve biyokimyasal parametrelerde önemli bir artış gösterdi.

Anahtar Kelimeler: Akut pankreatit, sitokin, oksidatif stres, diğer bazı biyokimyasal parametreler

Introduction

Acute pancreatitis (AP) is an inflammatory disease of the exocrine pancreas with highly variable severity, ranging from self-limited disease to severe progressive disease with organ dysfunction and death (1,2). There is no intervention to modify the progression or severity of pancreatitis. What distinguishes pancreatitis from other diseases of the gastrointestinal tract is its tendency to amplify the localized process by inducing a general systemic inflammatory response. TNF-a is produced by macrophages and is associated with acute and chronic inflammation and autoimmune diseases (3). A study conducted in mice with TNF-a deficiency has shown that TNF-a plays an important role in the regulation of embryo development. Interleukin-1 (IL-1) regulates the differentiation of lymphoid cells in autoinflammatory, autoimmune, infectious and degenerative diseases and potentiates the effect of neutrophils and macrophages (4). IL-6 is a pleiotropic cytokine with complex roles in inflammation and metabolic disease. Within the pancreatic islet, IL-6 stimulates secretion by a-cells of the prosurvival incretin hormone glucagon-like peptide 1 and acts directly on β-cells to stimulate insulin secretion in vitro (5,6). It allows to prevent diabetes and delay the progression to insulin dependence. IL-6 can be produced by and act on multiple tissues in the body (6), therapeutic pirfenidone treatment increases IL-10 secretion from macrophages before changes in histology and modulates the immune

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phenotype of inflammatory cells with reduced levels of inflammatory cytokines (7).

C-reactive protein (CRP) is an acute phase protein that reflects a measure of the acute phase response, a good indicator of inflammation, its secretion mainly dependent on IL-6. Persistently elevated hepcidin (HEPC) levels caused by IL-6 block the iron (Fe) transporter ferroportin in macrophages, hepatocytes, and intestinal epithelial cells, leading to Fe deficiency and anemia of chronic inflammation (8,9). In lymphocytes, IL-6 stimulates B-cell differentiation into immunoglobulin-producing cells. IL-10 is a cytokine with anti-inflammatory properties and important immunoregulatory functions, and an essential regulator of the immune system. It is a potent suppressor of antigen presenting cells and lymphocytes (9).

IL-21 is mainly produced by natural killer, T cells, and CD4+ T cells. All CD4+ T helper subsets can produce varying amounts of IL-21 depending on the stimulation context and cytokine environment, promoting inflammation and the immune response (10).

Nitric oxide (NO), Proinflammatory mediator bradykinin (BK), induces NO production in vascular endothelial cells, BK has been shown to elicit Ca2+ signals. It is a signaling molecule that plays a key role in the pathogenesis of inflammation (11).

The hepatic peptide hormone HEPC is the main regulator of Fe absorption and tissue distribution. Fe levels are controlled by the liver peptide hormone HEPC. Defects in HEPC regulation contribute to the pathogenesis of many Fe disorders (12,13).

Zinc (Zn) is a key element in numerous proteins and plays an important role in cell functions in defense against free radicals and repair of DNA damage. AP is a chronic inflammation of the pancreas that results in progressive fibrosis of the pancreas, ultimately resulting in malnutrition-associated pancreatic exocrine insufficiency (PEI) (14,15). Adversely affect the development and function of T and B cells, phagocytosis, intracellular killing and cytokine production (15).

Oxidative stress occurs in response to oxidative damage produced by the body's antioxidant and scavenging activities by a harmful stimulant. AP plays a role in the etiopathogenesis of many diseases such as cancer (16,17).

Copper (Cu) produced by the portal circulation is mainly absorbed by the liver, excess Cu can trigger oxidative damage, reactive oxygen stress generation, and damage biological molecules (18).

Aquaporins (AQPs) are a family of water-permeable transmembrane proteins. In mammals, they are broken down into classical aquaporins that are permeable to water glycerol and urea (19,20). AQPs assure crucialphysiological functions in both the exocrine and endocrine pancreas. Indeed, they are involved in pancreatic juice and insulin secretion. The possible role of AQPs in the development of inflammatory processes is highlighted (21).

In our study, TNF-a, IL-1, IL-6, IL-10, IL-21, NO, CRP, biochemical parameters: AQP-1, HEPC, Fe, Zn, Cu and oxidative stress levels were measured in patients with AP. By looking at these parameters, it is possible to contribute to the parameters that may cause the etiology of the disease and, as a result, to the studies on treatment.

Material and Methods

Patients and healthy control groups

A total of 46 patients with AP and 46 healthy controls were included in this study. Patients, diagnosed with AP in the gastroenterology department of the Harran University Hospital Internal Diseases Department, participated in our study. Patients admitted to the hospital with abdominal pain in the first 2 days of diagnosis of AP and patients who did not have any additional chronic diseases were included. 64 U/L (min:25, max:294), lipase average value is 1404.69±1900.99 U/L (min:11, max:6000). The study included 38 men and 54 women. The total sample of the patient group was 46, and the mean age for both genders was (43±19). For the control group, we recruited control groups from 46 volunteers. We recruited 19 men and 27 women in the healthy control group without any current disease history or pathological condition, and the mean age of the control group was 46 ± 13 years for both sexes. When we collected the blood, we transferred it to the gel (biochemistry tubes) tube. We were careful in obtaining blood samples from the patient and control groups to minimize hemolysis as hemolysis can greatly affect the validity of test results. The blood was centrifuged at 4000 rpm for 10 minutes and the serum was separated. We then collected the supernatant (plasma) without sediment and then stored it in the freezer.

In our study, the blood samples of the patients were centrifuged at 4000 rpm and the serum samples were stored in a deep freezer at -80°C. The samples taken were studied with the principle of ELISA to analyze the levels of TNF-a, IL-1, IL-6, IL-10, IL-21, AQP-1 and HEPC. Measurement of the total antioxidant status (TAS) were made using brand commercial kits (Rel Assay Diagnostic, Gaziantep) on a microplate reader system (Varioskan Lux; Thermo Scientific). Briefly, free radical reactions were initiated by the Fenton reaction and monitored by absorbance of the dianisidyl radicals. This reaction was measured spectrophotometrically at 660nm. Using this method, the antioxidative effect was measured as the relative amount of free dianisidyl radicals. The precision of this test has high accuracy (<3% error rate). The data were expressed in mmol Trolox equivalent/L. Measurement of the total oxidative status (TOS) were made using brand commercial kits (Rel Assay Diagnostic, Gaziantep) on a microplate reader system (Varioskan Lux; Thermo Scientific) according to the method of Erel. Briefly, oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation

reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically (at 530nm), is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide (H_2O_2), and the results are expressed as µmol H2O2 equivalent/L.Then to calculate the oxidative stress index (OSI), the resulting TAS units were converted to mmol/L, and the OSI value was calculated according to the following formula: OSI (arbitrary unit) = TOS (mmol H_2O_2 equivalent/L)/TAS (mmol Trolox equivalent/L) (22,23).

Principle of NO

NO in the samples rapidly decomposes into nitrate and nitrite. Spectrophotometric quantification of nitrite using Griess reagent is sensitive but does not measure nitrate. Reduction of nitrate to nitrite is performed using the NADH-dependent enzyme nitrate reductase, followed by spectrophotometric analysis measurement of total nitrite at 545nm with Griess reagent (21).

Principle of Fe, Zn, Cu and CRP

In our study, the Fe Atellica device was used. It is measured spectrophotometrically by combining with ferrosine to release a colored chromophore that absorbs at 571/658 nm. Zn changes the red-orange color of 5-Br-PAPS to light pink under alkaline conditions. The change in absorbance at 548 nm is proportional to the total Zn level in the sample. Cu in the samples changes the red-orange color of DiBr-PAESA to purple under acidic conditions. The change in absorbance at 572 nm is proportional to the total Cu concentration in the sample. Consolidation occurs when the serum containing CRP is mixed with the latex reagent, turbidity is measured at 571 nm.

Statistical analysis

The conformity of the data to the normal distribution in the patient and control groups was tested with Shaphiro Wilk. Student's t test was used to compare the normally distributed features in the parameters while the non-normally distributed groups were compared using the Mann Whitney U test. The Sperman correlation coefficient was examined for the relationship between the parameters. Mean ± standard deviation values for numerical variables are given as descriptive statistics. SPSS Windows version 24.0 package program was used for statistical analysis and $p<0.05$ was considered statistically significant.

Results

Since all the p-values were less than 0,05, the participants in the patient group had statistically signicantly higher concentrations of AQP-1, HEPC, TOS, and Cu (31.18 ± 10.76 U/L; 107.12 ± 36.15 ng/ml; 16.77 ± 4.02 µmol H2O2 equiv/L; 127.31±28.32µg/dl) compared to the control group (5.51 ± 2.80 U/L; 32.65 ± 7.50 ng/ml; 11.49 ± 2.47 µmol H2O2 equiv/L; 88.77±22.07µg/dl), respectively. On the other hand, the participants in the patient group had statistically signicantly lower concentrations of TAS, Fe, and Zn (1.14 ± 0.14 mmol Trolox equiv/L; 33.79±16.32 µg/dl; 73.75±15.36µg/dl) compared to the control group (1.46± 0.18 mmol Trolox equiv/L; 79.10 ± 32.52 µg/dl; 88.57±14.66 µg/dl), respectively.

Since all the p-values were less than 0,05, the participants in the patient group had statistically signicantly higher concentrations of TNF-a , IL-1, IL-6, IL-10, IL-21, CRP, and NO (86.59 ± 32.45 ng/L; 37.73 ± 14.09 pg/L; 131.51± 21.43 ng/L; 189.66 ± 103.44 pg/L; 190.13 ± 152.96 ng/L; 3.16±1.91 mg/dl; 58.32±9.83 µmol\L) compared to the control group (35.15 ± 10.04 ng/L; 21.43 ± 7.27 pg/L; 50.58 ± 20.82 ng/L; 87.07 ± 14.15 pg/L; 64.37 ± 22.63 ng/L; 0.61±0.56mg/dl; 28.79±2.71 µmol\L), respectively.

The correlation coefficient (r) values were calculated among the ten variables; TNF-alpha, IL-10, IL-1, IL-6, IL-21, AQP-1, HEP-C, TAS, TOS, OSI. Fe, Zn, Cu, CRP, and NO were used in the study. In the correlation analysis for the control group, TNF-alpha levels increase with increasing of NO levels (r=0.394, p=0.031). A statistically significant negative correlation was shown between IL-10 levels and IL-1 levels (r=-0.476, p=0.008). A significant negative correlation was detected between IL-6 levels and the levels of AQP-1 (r=-0.368, p=0.045). IL-21 levels were negatively correlated with Cu levels (r=-0.388, p=0.034). A moderate negative significant correlation was shown between TAS levels and TOS levels (r=-0.646, p=0.000). A high negative significant correlation was seen between TOS levels and OIS levels (r=-0.723, p=0.000).

For the patient group, TNF-alpha levels increased with increasing levels of IL-10, IL-21 and HEP-C (r=0.504, p=0.001; r=0.363, p=0.017; r=0.537, p=0.000), respectively. IL-10 levels increased with increasing the levels of IL-21 and HEP-C (r=0.427, p=0.004; r=0.393, p=0.009), respectively. A significant positive correlation was obtained between IL-1 levels and Fe levels (r=0.305, p=0.047). IL-6 levels were positively correlated with levels of IL-21 and HEP-C (r=0.368, p=0.015; r=0.310, p=0.043), respectively. AQP-1 levels were positively correlated with the levels of Fe and Zn (r=0.413, p=0.006; r=0.302, p=0.049), respectively. A significant positive correlation was detected between TAS levels and OSI levels (r=0.437, p=0.003). A significant high positive correlation was found between TOS levels and OSI levels (r=0.855, p=0.000). The Fe levels are positively correlated with the levels of Zn (r=0.317, p=0.038). A significant moderate positive correlation was provided between Zn levels and CRP levels (r=0.560, p=0.000). A significant positive correlation was computed between CRP levels and NO levels (r=0.436, p=0.003).

Table 1: Descriptive statistics of AQP-1, HEPC, TAS, TOS, OSI, Fe, Zn, and Cu

Parameters	Control group (n=46)			Patient group (n=46)			**p-value**
AQP-1 (U/L)	10.58	10.52	5.51 2.80	1.26	47.64	31.18 10.76	*p*<0.01
HEPC (ng/ml)	14.85	42.63	32.65 7.50	55.22	250.78	107.12 36.15	*p*<0.01
TAS (mmol Trolox equiv/L)	10.06	10.74	1.46 0.18	0.72	10.45	1.14 0.14	*p*<0.01
TOS (μmolH_2O_2 equiv/L)	90.11	22.15	11.49 2.47	70.6	25.6	16.77 4.02	*p*<0.01
OSI (Arbitrary units)	0.55	10.56	0.79 0.21	0.67	20.33	1.49 0.41	*p*<0.01
Fe (μg/ dl)	32	184	79.10 32.52	30	66	33.7916.32	*p*<0.01
Zn (μg/dl)	60.4	116.2	88.5714.66	51.4	18.9	73.75	*p*<0.01
Cu (μg/dl)	63	130.9	88.77	81.5	245.1	127.31	*p*<0.01

Table 2: Descriptive statistics of TNF-a, IL-1, IL-6, IL-10, IL-21, CRP, and NO

Parameters	Control group (n=46)			Patient group (n=46)			**p-value**
TNF-a (ng/L)	12.98	60.85	35.15 10.04	48.07	172.33	86.59 32.45	*p*<0.01
IL-1 (pg/L)	4.09	35.17	21.43 7.27	20.44	84.16	37.73 14.09	*p*<0.01
IL-6 (ng/L)	24.53	91.16	50.58 20.82	80.11	174.95	131.51 21.43	*p*<0.01
IL-10 (pg/L)	45.72	115.72	87.07 14.15	91.83	679.06	189.66 103.44	*p*<0.01
IL-21 (ng/L)	10.26	17.30	64.37 22.63	0.26	940.63	190.13 152.96	*p*<0.01
CRP (mg/dl)	0.05	20.17	0.61	0.10	60.84	3.16	*p*<0.01
NO (μmol\L)	23.16	34.27	28.792.71	39.12	75.27	58.32	*p*<0.01

Table 3. Correlation matrix of the variables for control group.

	IL-10	IL-1	IL-6	IL-21	AQP-1	HEPC	TAS	TOS	OSI	Fe	Zn	Cu	CRP	NO
TNF-a	0.067 (0.727)	0.060 (0.753)	-0.220 (0.243)	0.042 (0.824)	0.091 (0.631)	0.160 (0.399)	-0.292 (0.117)	0.088 (0.643)	0.296 (0.112)	-0.057 (0.763)	0.002 (0.993)	0.006 (0.973)	0.217 (0.250)	**0.394*** (0.031)
	IL-10	**-0.476**** (0.008)	-0.141 (0.457)	0.064 (0.737)	0.299 (0.109)	-0.322 (0.082)	0.206 (0.274)	-0.180 (0.340)	-0.239 (0.204)	-0.031 (0.871)	-0.123 (0.518)	0.047 (0.806)	0.281 (0.132)	-0.044 (0.817)
		IL-1	0.283 (0.129)	0.003 (0.986)	-0.036 (0.851)	0.322 (0.082)	-0.185 (0.328)	0.191 (0.311)	0.191 (0.312)	0.186 (0.324)	0.034 (0.859)	-0.033 (0.863)	-0.167 (0.378)	0.037 (0.845)
			IL-6	-0.233 (0.216)	**-0.368*** (0.045)	0.145 (0.445)	0.181 (0.338)	0.106 (0.576)	-0.023 (0.903)	-0.092 (0.627)	-0.082 (0.665)	0.226 (0.229)	0.141 (0.458)	-0.242 (0.197)
				IL-21	0.233 (0.214)	-0.071 (0.708)	-0.297 (0.111)	-0.006 (0.973)	0.270 (0.149)	0.064 (0.736)	-0.035 (0.854)	**-0.388*** (0.034)	0.024 (0.899)	0.347 (0.061)
					AQP-1	-0.153 (0.420)	-0.147 (0.439)	0.010 (0.960)	0.071 (0.709)	0.235 (0.211)	0.045 (0.813)	-0.103 (0.588)	0.195 (0.301)	0.356 (0.053)
						HEPC	-0.133 (0.485)	0.012 (0.952)	0.143 (0.452)	0.359 (0.051)	-0.135 (0.478)	-0.019 (0.921)	0.170 (0.370)	0.107 (0.573)
							TAS	-0.017 (0.928)	**-0.646**** (0.000)	-0.200 (0.290)	-0.086 (0.650)	0.237 (0.208)	-0.043 (0.822)	-0.263 (0.160)
								TOS	**0.723**** (0.000)	0.124 (0.513)	-0.036 (0.852)	-0.147 (0.439)	0.077 (0.685)	0.108 (0.569)
									OSI	0.222 (0.237)	-0.039 (0.836)	-0.222 (0.238)	0.106 (0.576)	0.255 (0.173)
										Fe	-0.152 (0.422)	-0.063 (0.741)	0.009 (0.962)	0.161 (0.396)
											Zn	0.127 (0.502)	-0.092 (0.630)	-0.117 (0.539)
												Cu	0.218 (0.248)	-0.102 (0.593)
													CRP	0.056 (0.770)

p-values (in parentheses) are presented bolded if *p* ** and *p* *

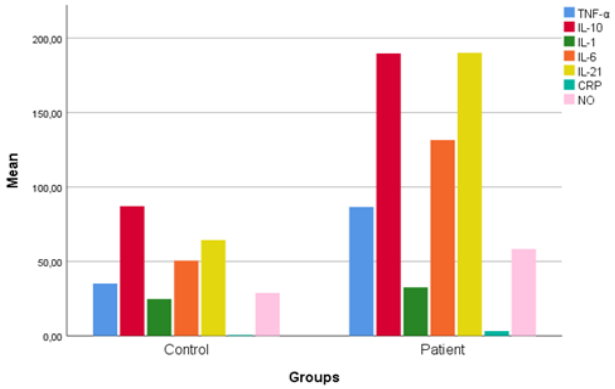

Figure 1: Comparison of TNF-a, IL-10, IL-1, IL-6, IL-21, CRP, and NO

Discussion

AP is one of the most common diseases of the gastrointestinal tract and its prognosis mainly depends on the development of organ failure and peri pancreatic necrosis infection (1,2). It has led to the identification of new molecular therapeutic targets such as TNF-a and interleukin-6, both of which are important activators of the inflammatory response in AP (24).

Severe acute pancreatitis is associated with high morbidity and mortality. Early severity classification remains a formidable problem that must be tackled to improve outcomes. We aim to find new plasma cytokines for the early diagnosis of severe AP according to the revised Atlanta criteria. Acute pancreatitis is now divided into two distinct subtypes, necrotizing pancreatitis and interstitial edematous pancreatitis, based on the presence or absence of necrosis, respectively. Since the cytokine profiles of the patients, especially TNF- and IL-6, are distinctive for severe AP, these parameters were analyzed.

TNF-a is a central regulator of inflammation, a proinflammatory cytokine secreted by monocytes and macrophages (25). Kylänpää et al. showed that TNF-a directly damaged the cells of multiple organs in AP and caused ischemia, hemorrhage, necrosis, inflammation and edema (25). Serum TNF-a levels were not considered to be a good indicator of disease severity because the liver can rapidly clear TNF-a before it enters the general circulation. Gasiorowska et al. have shown that TNF-a levels also increased in patients with chronic pancreatitis (26). In our study, TNF-a levels in AP patients significantly increased when compared to the control group (Table 2, $p<0.05$ Figure 1). TNF-a, a key regulator of proinflammatory cytokines, is thought to play an important role in the pathogenesis of AP (26).

CRP belongs to the pentraxin protein family of hepatic origin and serves as the main component of any inflammatory reaction (27). CRP is secreted in response to IL-6 and TNF-a proinflammatory cytokines. It plays a role in innate immunity by facilitating the phagocytosis of damaged and foreign cells (28). IL-6 is animportant proinflammatory cytokine involved in inflammation and immune responses. In vitro, Beringer et al. showed increased secretion of IL-6 from human pancreatic periacinar myofibroblast cells in the presence of several inflammatory mediators TNF-a, IL-17, IL-1β and growth factors, which served as an early marker (28). In our study, CRP levels increased significantly when compared to the control group (Table 2, $p<0.05$; Figure 1). Additionally, it was observed that TNF-a and IL-6 significantly increased compared to the control group (Table 2, $p<0.05$, Figure 1). After having been synthesized in a local lesion in the initial stage of inflammation, IL-6 moves through the bloodstream to the liver, where it then secretes a broad range of acute phase proteins such as CRP (28). On the other hand, IL-6 reduces the production of fibronectin, albumin and transferrin. It can be suggested that treatments for IL-6 should be used to prevent organ damage (29).

IL-1 is an important cytokine for autoinflammatory, autoimmune, infectious and degenerative diseases. IL-1 induction plays a role in the pathogenesis of acute pancreatitis (30). Resaher revealed that IL-1β could induce trypsin activation and reduce cellular viability of pancreatic acinar cells and release cytokines IL-1 from inflamed pancreatic tissue with the development of distant organ dysfunction (29).

In our study, IL-1 levels were found significantly higher when compared to the control group (Table 2, $p<0.05$, Figure 1). IL-1 promotes the recruitment of inflammatory cells at the site of inflammation by inducing the expression of adhesion molecules on endothelial cells and the release of chemokines by stromal cells (29).

IL-10 is an essential regulator of the immune system because of its anti-inflammatory properties and its role in re-establishing the immune system (30). Zhou et al. showed that IL-10 attenuated the inflammatory response and reduced TNF-a secretion in acute pancreatitis. It has been confirmed that IL-10 attenuates the severity of inflammation in AP by reducing serum amylase and TNF-a secretion as well as pancreatic pathological score (31).

IL-10 levels were found significantly higher when compared to the control group (Table 2, $p<0.05$, Figure 1). IL-10 appears to be a potent negative feedback regulator by influencing the control and resolution of inflammation through autocrine and paracrine mechanisms. Because of these effects, IL-10 may play an important role in the diagnosis and treatment of the disease (32).

IL-21 is involved in the differentiation and proliferation of β-cells and thus in the formation and maturation of antibodies. Linnebacher et al. associated an increased septic risk in AP patients with IL-21-related polymorphisms (32). IL-21 levels in AP patients were found significantly higher compared to the control group (Table 2, $p<0.05$, Figure 1). As IL-21 is a potent antitumor agent, making it a promising candidate for the development of therapeutic tools, an increased risk of septic shock in AP patients with high levels of IL-21 may be considered (11,33).

The ability of NO to limit endothelial activation and inhibit leukocyte adhesion is the anti-inflammatory properties of AP. It has been observed that NO synthase inhibitors increase ultrastructural degenerative changes in pancreatic acinar cells in the course of acute pancreatitis, demonstrating the protective role of endogenous NO in this disease (34,35). In our study, we observed that NO levels were significantly higher compared to the control group (Table 1, $p<0.05$, Figure 1) NO is produced at high levels during human inflammatory reactions. NO is proinflammatory at low concentrations by inducing vasodilation and recruitment of neutrophils, while at high concentrations it downregulates adhesion molecules, suppresses activation, and induces apoptosis of inflammation (34). The fact that the level of damage and the increase in NO in pancreatic cells are in parallel may be an important marker for diagnosis, and amino acids such as lysine and arginine may be effective for treatment by affecting NO levels. While Emerald et al. showed that increased NO levels were protective from the disease (35), Andican et al. showed the opposite (34).

The hepatic peptide hormone HEPC is the main regulator of Fe absorption and tissue distribution (13). HEPC reduces Fe entry into plasma from absorbing duodenal cells and Fe recycling macrophages by blocking Fe production and degrading Fe producing ferroportin. Wang et al. found decreased serum Fe levels in patients with AP (detected by a secretin test) compared to controls (14). Fe regulates HEPC homeostasis, HEPC production is suppressed in case of deficiency, and HEPC increases during infection. Therefore, Fe levels are found significantly low because HEPC impairs Fe absorption. Xu et al. found increased Fe levels, but did not find a significant difference (13). In another study, Julián-Serrano et al. reported low Fe levels, but they could not find a significant difference (36). Further studies on Fe and HEPC in patients with AP may be a better guide for understanding the subject.

AQP-1 is a glycoprotein responsible for rapid passive water transport across the biological membrane. In acute or chronic pancreatitis, which are considered inflammatory syndromes, patients are implicated in many pancreatic diseases, including pancreatitis, cystic fibrosis, and cancer. Arsenijevic et al. showed decreased levels of AQP1 in a rat model of acute pancreatitis and a mouse model exhibiting exocrine pancreatic insufficiency (20). Pancreatitis can cause multi-organ failure, including lung and colon, showing altered expression of AQPs (37). In a study that invastigateing the role of AQP1 in the pathophysiology of pancreatitis, it was found that AQP1 expression decreased in both ductal and acinar cells in a cerulein-induced pancreatitis model (38). This suggests that low AQP1 levels may contribute to exocrine insufficiency. In our study, AQP1 levels were significantly higher in serum when compared to the control group (Table 1, $p<0.05$). In the course of AP, AQP1 cells in the ducts and acini were damaged as a result of acute inflammation. AQP1 levels were high in patients with AP. It can also be thought that AQP1 plays a role in the passage of water to the pancreas, because fluid therapy plays the most important role in patients with AP. It can be predicted that treatments that increase the levels of AQP1 in the pancreas may facilitate fluid passage and improve the course of the disease (20,39).

Zn plays a crucial role in the immune system. It is necessary to facilitate the coordination of immune activation during responses to infection. Recent studies have reported that Zn deficiency increases organ damage, systemic inflammation and mortality in sepsis. Muneoka et al. have shown that Zn has a number of effector mechanisms that may play a role in the development of acute and chronic pancreatitis (15).

Since AP severity is mediated by inflammatory cells, Zn deficiency may also worsen the disease by stimulating the inflammatory response (15). In our study, Zn levels were found significantly lower compared to the control group (Table 2, $p<0.05$; Figure 1). Zn can trigger ROS production through mitochondrial and extra mitochondrial pathways. A few studies in isolated mitochondria show that the cation interferes with the activity of the electron transport chain, inhibiting cellular respiration (17). An abnormal Zn metabolism is accompanied by severe oxidative stress due to increased free radical production. As a result, it can be expected that Zn supplements may improve the prognosis in AP patients by increasing antioxidant levels.

Cu is necessary for the human body to maintain the daily stability of organs and metabolic processes. It plays a role in glucose metabolism and synthesis and release of proteins and enzymes (19). Lener et al. found that high Cu levels appear to be associated with pancreatic cancer. They report that high Cu levels may result in higher ROS levels, which affects the risk of development and progression of pancreatic cancer (40). In our study, Cu levels were found significantly higher compared to the control group (Table 2, $p<0.05$; Figure 1). Cu can trigger the generation of ROS and consequent damage to biological molecules. The inflammatory response is closely linked to oxidative stress. Further studies on Cu in AP will elucidate the possible mechanisms (19,40).

AP is a complex inflammatory disease caused by more than one etiology, the pathogenesis of which has not been fully elucidated. Oxidative stress is important for regulation of signaling pathways associated with inflammation, recruitment of inflammatory cells, release of inflammatory factors, and other processes, and plays a key role in the emergence and development of AP. In recent years, antioxidant therapy, which suppresses oxidative stress by scavenging reactive oxygen species, has become the research topic of AP. However, conventional antioxidant drugs have problems such as poor drug stability and low delivery efficiency that limit their clinical translation and application. Nanomaterials bring a whole

new opportunity for antioxidant treatment of AP. Antioxidant drugs including small size, good stability, high permeability and long retention effect can be used not only as effective carriers but also directly as antioxidants. After discussing the relationship between oxidative stress and AP first, we focused on demonstrating its effects on oxidative stress-related indicators in pathological conditions. This provides references for follow-up research and encourage clinical practice (41) .

Oxidative stress occurs in response to oxidative damage when the body's antioxidant and scavenging activities cannot cope with active oxidants produced by a harmful stimulant (42). Robles et al. have shown that oxidative stress plays a critical role in the pathogenesis and various complications of pancreatitis (43). In our study, the TOS was found significantly higher compared to the control group. On the other hand, T-AOC were found significantly lower compared to the control group. (Table 2, $p<0.05$ Figure 1). It has revealed the mechanism by which oxidative stress can cause chronic inflammation, which in turn mediates many chronic diseases, including cancer, diabetes, and cardiovascular, neurological, and pulmonary diseases (43).

TNF-a is an inflammatory cytokine. On the other hand, IL-10 is an anti-inflammatory cytokine. As shown in Table-3 there is a statistically significant positive correlation between TNF-a and IL-10 ($r=0.504$, $p<0.01$). IL-1 is a cytokine that increases in inflammation and Fe is a decreasing element in inflammation, so there is a positive correlation between IL-1 and Fe ($r=0.305$, $p<0.05$). HEPC is an acute phase reactant synthesized in the liver that increases in inflammation, and there is a positive correlation between TNF-a and ($r=0.537$, $p<0.01$). IL-21 and IL-10 are anti-inflammatory cytokines and there is a positive correlation between IL-10 and IL-21 ($r=0.427$, $p<0.01$). IL-10 is a cytokine secreted to suppress inflammation, and there is a positive correlationbetween between IL-10 and HEPC ($r=0.537$, $p<0.01$). AQP-1 is a cell membrane protein involved in the transport of water molecules. In AP disease, blood levels increase as inflammatory events destroy the cell membrane. The level of Fe decreases in inflammatory events. In the study, there is a positive correlation between AQP-1 and Fe, ($r=0.413$, $p<0.01$). IL-1 is a cytokine that increases in inflammation. Fe is an element that decreases in case of inflammation. In our study, there is a positive correlation between IL-1 and Fe ($r=0.305$, $p<0.05$). NO is a molecule secreted to prevent damage to organs by the effect of vasodilation in inflammatory events. CRP is one of the main proteins involved in inflammation. There is a strong/moderate statistically significant positive correlation between CRP and NO, ($r=0.436$, $p<0.01$).

IL-6 is an inflammatory cytokine. HEPC is an inflammatory acute phase reactant. There is a statistically significant negative correlation between IL-6 and HEPC ($r=-0.310$, $p<0.05$). Zn is an antioxidant element and its deficiency increases the tendency to inflammatory conditions. AQP-1 levels are increased in inflammatory conditions. There is a negative correlation between AQP-1 and Zn ($r=-0.302$, $p<0.05$). Zn is an antioxidant element, and its levels may increase in inflammatory conditions. Fe, on the other hand, decreases due to the increase in HEPC levels in inflammatory conditions. There is a negative correlation between Fe and Zn, ($r=-0.387$, $p<0.05$). CRP is an acute phase protein that increases in inflammation. Zn deficiency causes inflammatory reactions. There is a strong statistically significant positive correlation between Zn and CRP, ($r=0.560$, $p<0.01$).

Conclusion

In the pathogenesis of oxidative stress acute pancreatitis, the generation of ROS directly oxidizes various biomolecules, exacerbating the oxidative load through a respiratory burst due to the recruitment of ROS-producing inflammatory cells in the pancreas, thereby causing further damage. TNF-a, IL-1β and IL-6 are proinflammatory cytokines. AP has a multifactorial and complex etiology. It is an important factor in the pathogenesis of AP. At the same time, the levels of these cytokines can be a guide for diagnosis and treatment in patients with Acute Pancreatitis. The predicted and concentrations of inflammatory cytokines released from the pancreas may provide new clues for the elucidation of the roles of pancreatitis in the pathophysiological processes and the development of new treatment protocols. As a result of our study in AP patients, oxidative stress levels increase while antioxidant defense levels decrease. For this reason, we can hope that treatments that reduce oxidative stress levels or increase antioxidant levels can improve the course of the disease.

Ethical statement: The study protocol was approved by the Harran University, Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (protocol number: 2019/03; March 11, 2019). Our study was planned in accordance with the criteria set in the Helsinki Declaration. Informed consent forms were obtained from both AP patients participating in the study and healthy controls.

Conflict of Interest: The authors declare that there is no conflict of interest.

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References

1.Walkowska J, Zielinska N, Karauda P, Tubbs RS, Kurtys K, Olewnik Ł. The Pancreas and Known Factors of Acute Pancreatitis. J Clin Med 2022;11:5565.

2.Peery AF, Crockett SD, Murphy CC, et al. Burden and Cost of Gastrointestinal, Liver, and Pancreatic Diseases in the United States: Update 2018. Gastroenterology 2019;156:254-272.e11.

3.Rasineni K, Srinivasan MP, Balamurugan AN, et al. Recent advances in understanding the complexity of alcohol-induced pancreatic dysfunction and pancreatitis development. Biomolecules 2020;doi: 10.3390/biom10050669.

4.Adjuto-Saccone M, Soubeyran P, Garcia J, et al. TNF-a induces endothelial-mesenchymal transition promoting stromal development of pancreatic adenocarcinoma. Cell Death Dis 2021;doi: 10.1038/S41419-021-03920-4.

5.Jiménez-Maldonado A, Montero S, Lemus M, et al. Moderate and high intensity chronic exercise reduces plasma tumor necrosis factor alpha and increases the langerhans islet area in healthy rats. J Musculoskelet Neuronal Interact 2019;19:354–61. https://pubmed.ncbi.nlm.nih.gov/31475943/ (accessed November 5, 2022).

6.van Duijneveldt G, Griffin MDW, Putoczki TL. Emerging roles for the IL-6 family of cytokines in pancreatic cancer. Clin Sci 2020;134:2091–115.

7.Bava EP, George J, Tarique M, et al. Pirfenidone increases IL-10 and improves acute pancreatitis in multiple clinically relevant murine models. JCI Insight 2022;doi: 10.1172/JCI.INSIGHT.141108.

8.Linnemann AK, Blumer J, Marasco MR, et al. Interleukin 6 protects pancreatic β cells from apoptosis by stimulation of autophagy. FASEB J 2017;31:4140–52.

9.Komolafe O, Pereira SP, Davidson BR, Gurusamy KS. Serum C-reactive protein, procalcitonin, and lactate dehydrogenase for the diagnosis of pancreatic necrosis. Cochrane Database Syst Rev 2017;doi: 10.1002/14651858.CD012645.

10.Zheng Z, Huang G, Gao T, et al. Epigenetic Changes Associated With Interleukin-10. Front Immunol 2020;11:1105.

11.McMichael EL, Jaime-Ramirez AC, Guenterberg KD, et al. IL-21 enhances natural killer cell response to cetuximab-coated pancreatic tumor cells. Clin Cancer Res 2017;23:489–502.

12.Jakubowska MA, Ferdek PE, Gerasimenko O V., Gerasimenko J V., Petersen OH. Nitric oxide signals are interlinked with calcium signals in normal pancreatic stellate cells upon oxidative stress and inflammation. Open Biol 2016;doi: 10.1098/rsob.160149.

13.Xu Y, Alfaro-Magallanes VM, Babitt JL. Physiological and pathophysiological mechanisms of hepcidin regulation: clinical implications for iron disorders. Br J Haematol 2021;193:882–93.

14.Wang CY, Canali S, Bayer A, Dev S, Agarwal A, Babitt JL. Iron, erythropoietin, and inflammation regulate hepcidin in Bmp2-deficient mice, but serum iron fails to induce hepcidin in Bmp6-deficient mice. Am J Hematol 2019;94:240–8.

15.Wang M, Phadke M, Packard D, Yadav D, Gorelick F. Zinc: Roles in pancreatic physiology and disease. Pancreatology 2020;20:1413–20.

16.Vujasinovic M, Hedström A, Maisonneuve P, et al. Zinc deficiency in patients with chronic pancreatitis. World J Gastroenterol 2019;25:600–7.

17.Kozłowska A, Wojtacha P, Majewski M, Równiak M. The cytokine alterations/abnormalities and oxidative damage in the pancreas during hypertension development. Pflugers Arch 2019;471:1331–40.

18.Mei Q xiang, Hu J hui, Huang Z hua, et al. Pretreatment with chitosan oligosaccharides attenuate experimental severe acute pancreatitis via inhibiting oxidative stress and modulating intestinal homeostasis. Acta Pharmacol Sin 2021;42:942–53.

19.Zeng L, Ai C, Zhang J, Zheng J. Essential element Cu and non-essential element Hg exposures have different toxicological effects in the liver of large yellow croaker. Mar Pollut Bull 2019;139:6–13.

20.Arsenijevic T, Perret J, Van Laethem JL, Delporte C. Aquaporins Involvement in Pancreas Physiology and in Pancreatic Diseases. Int J Mol Sci 2019;doi: 10.3390/IJMS20205052.

21.Granger DL, Taintor RR, Boockvar KS, Hibbs JB. Measurement of nitrate and nitrite in biological samples using nitrate reductase and Griess reaction. Methods Enzymol 1996;268:142–51.

22.Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004;37:277–85.

23.Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005;38:1103–11.

24.Song R, Yu D, Park J. Changes in gene expression of tumor necrosis factor alpha and interleukin 6 in a canine model of caerulein-induced pancreatitis. Can J Vet Res 2016;80:236.

25.Kylänpää L, Rakonczay Z, O'Reilly DA. The clinical course of acute pancreatitis and the inflammatory mediators that drive it. Int J Inflam 2012;doi: 10.1155/2012/360685.

26.Gasiorowska A, Talar-Wojnarowska R, Kaczka A, Borkowska A, Czupryniak L, Małecka-Panas E. Subclinical Inflammation and Endothelial Dysfunction in Patients with Chronic Pancreatitis and Newly Diagnosed Pancreatic Cancer. Dig Dis Sci 2016;61:1121–9.

27.Keany KM, Fosgate GT, Perry SM, Stroup ST, Steiner JM. Serum concentrations of canine pancreatic lipase immunoreactivity and C-reactive protein for monitoring disease progression in dogs with acute pancreatitis. J Vet Intern Med 2021;35:2187–95.

28.Beringer A, Thiam N, Molle J, Bartosch B, Miossec P. Synergistic effect of interleukin-17 and tumour necrosis factor-a on inflammatory response in hepatocytes through interleukin-6-dependent and independent pathways. Clin Exp Immunol 2018;193:221–33.

29.Takahashi K, Nishida A, Shioya M, et al. Interleukin (IL)-1β Is a Strong Inducer of IL-36γ Expression in Human Colonic Myofibroblasts. PLoS One 2015;doi: 10.1371/JOURNAL.PONE.0138423.

30.Lin WR, Lim SN, Yen TH, Alison MR. The Influence of Bone Marrow-Secreted IL-10 in a Mouse Model of Cerulein-Induced Pancreatic Fibrosis. Biomed Res Int 2016;doi: 10.1155/2016/4601532.

31.Zhou X, Liu Z, Jang F, Xiang C, Li Y, He Y. Autocrine Sonic hedgehog attenuates inflammation in cerulein-induced acute pancreatitis in mice via upregulation of IL-10. PLoS One 2012;doi: 10.1371/JOURNAL.PONE.0044121.

32.Linnebacher A, Mayer P, Marnet N, et al. Interleukin 21 Receptor/Ligand Interaction Is Linked to Disease Progression in Pancreatic Cancer. Cells 2019;doi: 10.3390/CELLS8091104.

33.Sutherland APR, Belle T Van, Wurster AL, et al. Interleukin-21 is required for the development of type 1 diabetes in NOD mice. Diabetes 2009;58:1144–55.

34.Andican G, Gelisgen R, Unal E, et al. Oxidative stress and nitric oxide in rats with alcohol-induced acute pancreatitis. World J Gastroenterol 2005;11:2340–5.

35.Emerald BS, Mohsin S, D'souza C, et al. Diabetes Mellitus Alters the Immuno-Expression of Neuronal Nitric Oxide Synthase in the Rat Pancreas. Int J Mol Sci 2022;doi: 10.3390/IJMS23094974.

36.Julián-Serrano S, Yuan F, Wheeler W, et al. Hepcidin-regulating iron metabolism genes and pancreatic ductal adenocarcinoma: a pathway analysis of genome-wide association studies. Am J Clin Nutr 2021;114:1408–17.

37.Garg PK, Singh VP. Organ Failure Due to Systemic Injury in Acute Pancreatitis. Gastroenterology 2019;156:2008–23.

38.Venglovecz V, Pallagi P, Kemény L V., et al. The Importance of Aquaporin 1 in Pancreatitis and Its Relation to the CFTR Cl- Channel. Front Physiol 2018;doi: 10.3389/FPHYS.2018.00854.

39.Gao Z, Xu J, Sun D, et al. Traditional Chinese medicine, Qing Ying Tang, ameliorates the severity of acute lung injury induced by severe acute pancreatitis in rats via the upregulation of aquaporin-1. Exp Ther Med 2014;8:1819–24.

40.Lener MR, Scott RJ, Wiechowska-Kozłowska A, et al. Serum Concentrations of Selenium and Copper in Patients Diagnosed with Pancreatic Cancer. Cancer Res Treat 2016;48:1056–64.

41.Zheng X;, Zhao J;, Llorent-Martínez EJ, et al. Research Progress of Antioxidant Nanomaterials for Acute Pancreatitis. Mol 2022, Vol 27, Page 7238 2022;27:7238.

42.Esrefoglu M. Experimental and clinical evidence of antioxidant therapy in acute pancreatitis. World J Gastroenterol 2012;18:5533–41.

43.L R, ND V, H I. Role of Oxidative Stress in the Pathogenesis of Pancreatitis: Effect of Antioxidant Therapy. Pancreat Disord Ther 2013;doi: 10.4172/2165-7092.1000112.