Japanese Journal of Gastroenterology and Hepatology

Research Article

Immunological Pathways in the Development of Postoperative Hyperamylasemia and Postoperative Pancreatitis

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Received: 10 May 2020

Accepted: 04 June 2020

Published: 10 June 2020

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1. Abstract

1.1. Introduction: Postoperative pancreatitis complications can be separated into two main groups based on literature reviews and clinical trials. Some authors suggest that postoperative pancreatitis is a severe complication that should be treated conservatively and operatively if needed. Other authors defend an opposite opinion that only postoperative hyperamylasemia exists, which is a transitory condition with no risk of complications.

1.2. Methods: At the University Hospital for Active Treatment Alexandrovska, Clinic of General and Liver-Pancreatic Surgery, a cohort of patients with increased amylase concentrations in the postoperative period were studied and followed with immunological tests for interleukin (IL)-6 and IL-10. The working hypotheses were the following: i) IL-6 and IL-10 are directly relevant in the development of postoperative pancreatitis; ii) the concentrations of IL-6 and IL-10 are prognostic factors in the early postoperative period and mark the boundary at which patients will develop postoperative pancreatitis; iii) the postoperative period is accompanied by transient postoperative hyperamylasemia and absence of complications.

1.3. Results: The results show that, with a 95% confidence interval (CI) for IL-6 and with a 99% CI for IL-10, a statistically significant difference is found in the concentration of IL-6 and IL-10 in patients who developed postoperative pancreatitis versus those with transient hyperamylasemia. Discussion/Conclusion: The present study shows a direct link between the immunological markers, IL-6 and IL-10, and the course of both postoperative pancreatitis and transient hyperamylasemia, making these two markers valuable in elucidating the differences in the development of postoperative pancreatitis and transient hyperamylasemia in the early postoperative period.

2. Keywords: Postoperative pancreatitis; Interleukin 6; Interleukin 10

3. Introduction

Postoperative pancreatitis is a severe complication in the postoperative period. Although postoperative pancreatitis develops in a pathway quite similar to acute pancreatitis, there are a number of specifics in its etiology and evolution, making postoperative pancreatitis a matter of clinical interest and providing it with individual characteristics. The cases of postoperative inflammation of the pancreatic gland constitute 10% of all cases of acute pancreatitis. Different aspects dependent on immunological cell diversity of the pancreas, pro- and an- ti-inflammatory cytokines, and biochemical reactions may cause postoperative inflammation of the pancreatic gland. In the early postoperative period, another clinical event can be observed-postoperative hyperamylasemia. This is a transitory condition, associated with higher levels of serum amylase, which develops with no risk of complications. This versatile picture of clinical conditions, which can appear in the early postoperative period, is directly associated with the great diversity of immune cells in the pancreas. There are major structural differences

&Author Contributions: Todorov RS, Dardanov D, Katibova SN, Dimitrov K, Bulanov D, Ionkov A, Bozev V, These authors have contributed equally to this article.

in the immune cells of the exocrine and endocrine pancreas, [1, 2] stated that the endocrine pancreas and the exocrine pancreas have pools of immune cells differing in their origin and function. These cells secrete cytokines-interleukins (IL) such as IL-12 and IL-6, which are pro-inflammatory, and IL-10, which is anti-inflammatory [3-5].

IL-6 is a pro-inflammatory cytokine that is secreted by T lymphocytes and macrophages during infection, trauma, and tissue damage [7]. Sathyanarayan et al. [8] reported the major role of IL-6 in development of postoperative pancreatitis. IL-6 is a predictor of the inflammatory process in the pancreas and is associated with the severity of inflammation. An increase in IL-6 blood levels can predict developing organ damage and organ insufficiency [7]. A change in the concentration of IL-6 is followed by the development of postoperative pancreatitis [8, 9]. The IL-6 concentration increases during inflammation and declines during repair and regeneration.

IL-10 exhibits anti-inflammatory effects in the human body because it inhibits cytokine secretion from macrophages, T lymphocytes, and B lymphocytes. IL-10 is secreted mainly by monocytes, and to a lesser degree, lymphocytes, T helper cells, and mastocytes. Its main functions are immuno modulation and regulation of the inflammatory process. IL-10 is a fundamental regulator of inflammation in the gastrointestinal tract [10-13]. A significant reduction in the severity of pancreatitis was observed in experimental models in which IL-10 was administered before or during development of pancreatic inflammation [14]. Demonstrated a reduction in the severity of pancreatitis and histological silencing of pro-inflammatory and necrotic processes after IL-10 administration [15]. Reported an increase in the serum concentration of IL-10 during the early postoperative period in patients with a mild form of pancreatitis. They also reported low serum concentrations of IL-10 in patients with a severe form of pancreatitis [16].

Serum blood levels of ILs-IL-6 and IL-10 have a major role in the evolution of pancreatitis in the early postoperative period. Pancreatitis can progress as an acute inflammation and present various clinical manifestations if pro-inflammatory ILs are abundant, or a transient postoperative hyperamylasemia may occur in cases when serum levels of anti-inflammatory IL-10 are high.

Postoperative pancreatitis complications can be separated into two main groups based on literature reviews and clinical and experimental trials. Some authors suggest that postoperative pancreatitis is a severe complication that should be treated conservatively and operatively if needed. Other authors defend an opposite opinion that only postoperative hyperamylasemia exists, which is a transitory condition with no risk of complications [17] stated that approximately 10% of patients developed postoperative hyperamylasemia with no clinical complications after abdominal surgery. They suggest that this condition does not require active treatment [18] suggest that postoperative hyperamylasemia should be considered and treated as postoperative pancreatitis due to its ability to evolve.

The aim of the present study was to test and determine serum blood concentrations of IL-6 and IL-10 in the postoperative period in patients who received surgery on the abdomen. The study also aimed to determine the accuracy of immunological markers to differentiate postoperative hyperamylasemia from postoperative pancreatitis.

4. Materials and Methods

At the University Hospital for Active Treatment Alexandrovska, Clinic of General and Liver-Pancreatic Surgery, a cohort of patients with increased amylase concentrations in the postoperative period between January 2017 and December 2018 were studied and followed with immunological and biochemical tests for interleukin IL-6 and IL-10. Regardless of the type of abdominal surgery to which they were subjected, all patients who presented with an elevated amylase concentration in the postoperative period were selected. The study protocol and ethics approval were postulated by a clinical council of the Department of General and Operative Surgery, Medical University Sofia, which serves as a scientific-ethical committee for the Clinic of General and Liver-Pancreatic Surgery at the University Hospital for Active Treatment Alexandrovska. The clinical council stated that immunological and biochemical tests for IL-6 and IL-10 are standard procedures in the diagnostic and treatment process at the Clinic of General and Liver-Pancreatic Surgery and do not need a specific ethics approval.

Two standard tubes of peripheral venous blood were taken from each patient. The tubes were of the SST type (serum with gel and clot activator) for the separation of serum from blood components. Both tubes were centrifuged at 10,000 rpm using an ultracentrifuge for 15 minutes. Serum supernatant was dispensed into 2-ml Eppendorf tubes and frozen immediately at −82°C. Two tubes of serum were frozen from each patient to measure IL-6 and IL-10. Enzyme-linked immunosorbent assay kits were used to perform the IL assay, to evaluate the serum concentrations of IL-6 and IL-10 in a total of 78 patients. Patients were tested for IL-6 and IL-10 following all established protocols, norms, and standards for enzyme-linked immunosorbent assay, as per the manufacturer's instructions (eBioscience).

5. Results

(Figure 1) shows the concentration of IL-6 in pg/ml. In most patients, the IL concentration increased above normal. A normal concentration of IL-6 (when measured in serum using an SST tube with a clot activator) ranged from 00.0 to 12.7 pg/ml.

In seven patients, the concentration of IL-6 remained within the normal range. In the clinical follow up, these seven patients did not develop acute postoperative pancreatitis, and no complications emerged. The remaining patients developed complications affecting the gastrointestinal, cardiovascular, pulmonary, or excretory systems, all of which are clinical features of postoperative pancreatitis.

The IL-10 concentration was markedly increased in 10 patients, suggesting suppression of the inflammatory response (Figure 2). In the other patients, the concentration of IL-10 remained low, with values ranging from 0.1 pg/ml to 5.0 pg/ml. No standard set point for the normal concentration range of IL-10 existed. In healthy individuals, IL-10 could not be detected in plasma. In pathological conditions, the plasma IL-10 concentration depended on the type of disorder.

In the present study, all patients with an increased concentration of IL-10 (higher than that of IL-6 in the same patient) were clinically observed as presenting with postoperative hyperamylasemia. The IL-6 concentration increased above normal in several patients, but IL-10 concentrations exceeded IL-6 concentrations. In these patients, acute postoperative pancreatitis was not clinically observed, but transient hyperamylasemia was. In other patients, when the absolute concentrations of IL-6 were greater than those of IL-10, the clinical development of postoperative pancreatitis and subsequent complications with varying severities were observed. (Figure 3) compares the values of IL-6 and IL-10 in all patients. Red represents patients in whom the IL-10 concentration was greater than the IL-6 concentration.

The working hypotheses were the following: i) IL-6 and IL-10 are directly relevant in the development of postoperative pancreatitis; ii) the concentrations of IL-6 and IL-10 are prognostic factors in the early postoperative period and mark the boundary at which patients will develop postoperative pancreatitis and its various clinical manifestations; iii) the postoperative period is accompanied by transient postoperative hyperamylasemia and absence of complications. To test these hypotheses, the value of the interleukins was statistically analyzed using SPSS v23 software.

Checking whether there is a statistically significant difference existed between the concentrations of IL-6 and IL-10 in patients who developed postoperative pancreatitis and those with transient hyperamylasemia was necessary. In essence, this is a statistical hypothesis test that can be confirmed or rejected with a definite probability of certainty.

H0: No difference exists in the mean concentrations of IL-6 and IL-10 in patients with postoperative pancreatitis versus those with transient hyperamylasemia.

H1: A difference is present in the mean concentrations of IL-6 and IL-10 in patients with postoperative pancreatitis versus those with transient hyperamylasemia.

As already stated, the risk of error with which the hypothesis was tested was 5%. In order to determine the hypothesis test method, it is necessary to verify that the two conditions for applying a parametric test method are fulfilled. Because ILs were quantified, only the second condition (i.e., normality of distribution) was verified. Two hypotheses for normality of distribution were stated:

H0: The distribution of IL-6 and IL-10 is normal.

H1: The distribution of IL-6 and IL-10 is different from normal.

The level of significance in both tests was less than the error of 0.05 (Table 1); therefore, the alternative hypothesis, which states that the distribution of the two ILs is not normal, was accepted. This conclusion can be argued with a 95% probability of certainty. Since one of the two conditions for applying a parametric method was not fulfilled, a non-parametric method was used for verification. The applied statistical method checks the difference between two averages of two independent groups (1st, patients who developed postoperative pancreatitis; 2nd, patients with transient hyperamylasemia), the method used was the Mann–Whitney U test.

Test results for the difference between patients who developed postoperative pancreatitis and those with transient hyperamylasemia are shown in (Table 2&3).

The results of the hypothesis test show that the level of significance was <0.05, which means that both the ILs are considered valid alternative hypotheses.

With a 95% confidence interval (CI), a statistically significant difference exists in IL-6 concentration between patients who developed postoperative pancreatitis and those with transient hyperamylasemia.

With a 99% CI, a statistically significant difference exists in IL-10 concentration between patients who developed postoperative pancreatitis and those with transient hyperamylasemia.

The IL-10 significance level was lower than that of IL-6, suggesting that IL-10 is a more reliable indicator for determining the likelihood of postoperative pancreatitis and its course of development.

Subsequently, hypotheses were designed to test for a correlation between the two immunological markers and their value in predicting the pattern of postoperative pancreatitis and transient hyperamylasemia.

Measurement of the relationship between the two ILs can be accomplished by correlation analysis, which is a measure of the presence of an investigative bond, marked by R, and ranges from -1 to +1. The closer the correlation coefficient to +1, the stronger the relationship between the two indicators. The closer the correlation coefficient to 0, the weaker the relationship between the two indicators. The following limits may be conditionally accepted:

$0 < |R| < 0.3$ – Weak correlation

$0{,}3 < |R| < 0.5$ – Moderate correlation

$0{,}5 < |R| < 0.7$ – Significant correlation

$0{,}7 < |R| < 0.9$ – Strong correlation

$0{,}9 < |R| < 1.0$ – Very strong correlation

As is depicted graphically (Figure 4.), no correlation was found between the concentrations of IL-6 and IL-10. The correlation coefficients are extremely low—R- 0.040 (Table 4), but more importantly, they are not statistically significant (sig. = 0.726 > 0.05 and rejects the H0 (zero hypothesis)).

As was statistically proven, the clinical course of the postoperative period was smooth with the manifestation of transient hyperamylasemia only in patients with low IL-6 and high IL-10 concentrations. Contrary to the above, in patients with a high concentration of IL-6 and a low concentration of IL-10, the postoperative period was clinically mediated by postoperative pancreatitis and its various clinical manifestations.

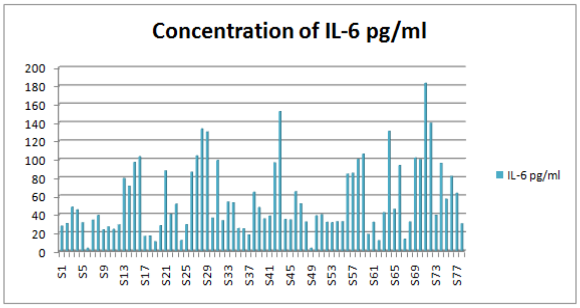

Figure 1: Concentrations of IL-6.

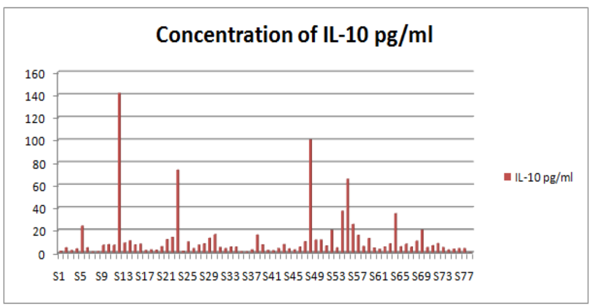

Figure 2: Concentrations of IL-10.

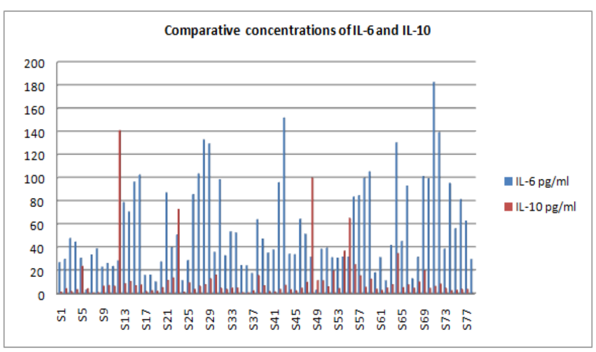

Figure 3: Comparitive Concentrations of IL-6 and IL-10.

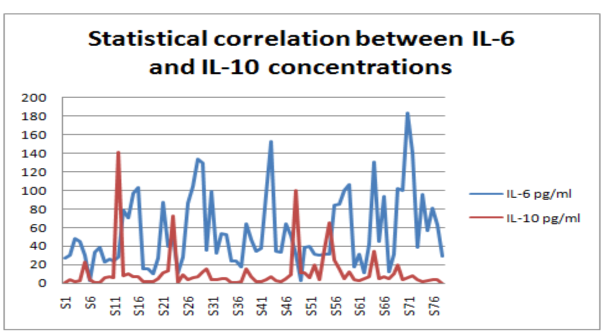

Figure 4: Statistical correlation between IL-6 and IL-10 Concentrations

Table 1: Statistical test for normality:Kolmogorov–Smirnova and Shapiro–Wilk tests.

Tests of Normality						
	Kolmogorov–Smirnov[a]			Shapiro–Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
IL-6	0.182	78	0	0.892	78	0
IL-10	0.304	78	0	0.467	78	0
a. Lilliefors Significance Correction						

Table 2: Statistics with the Mann–Whitney U test.

Group Statistics					
	Clinical Status	N	Mean	Std. Deviation	Std. Error Mean
IL-6	postoperative pancreatitis	71	58.3009	38.85991	4.61182
	postoperative hyperamylasemia	7	25.8449	17.19951	6.5008
IL-10	postoperative pancreatitis	71	7.1695	6.27507	0.74471
	postoperative hyperamylasemia	7	61.7014	48.90847	18.48566

Table 3: Statistical difference between patients who developed postoperative pancreatitis and patients with transient hyperamylasemia.

TestStatistics[a]		
	IL-6	IL-10
Mann–Whitney U test	123	56.5
Wilcoxon, W	151	2612.5
Z	−2.194	−3.358
Asymp. sig. (two-tailed)	**0.028**	**0.001**
a. GroupingVariable: State		

Table 4: Statistical correlation between IL-6 and IL-10.

Correlation			
		IL-6	IL-10
IL-6	Pearson'scorrelation	1	−.040
	Sig. (two-tailed)		0.726
	N	78	78
IL-10	Pearson'scorrelation	−.040	1
	Sig. (two-tailed)	**0.73**	
	N	78	78

6. Discussion

The present study shows a direct association between the immunological markers, IL-6 and IL-10 serum levels, and the pathway of development of postoperative pancreatitis. Statistically proven IL-6 and IL-10 serum levels predict postoperative pancreatitis progression as an acute inflammation and development of various clinical manifestations, or progression into a transient postoperative hyperamylasemia with absence of complications. Results show that, with a very high CI, IL-6 is a predictor of the inflammatory process in the pancreas and is associated with the severity of inflammation. An increase in IL-6 blood levels can predict developing organ damage [7-9]. IL-10 exhibits anti-inflammatory effects and along with a very high CI is a predictor of significant reduction in the severity of developing pancreatitis or evolution of pancreatitis into a transient postoperative hyperamylasemia [14-16]. The results from that specific clinical trial confirm the results obtained by other authors and also elucidate the direct connection between clinical evolution of postoperative pancreatitis and IL-6 and IL-10. Postoperative pancreatitis is a complex process with many cytokines involved in its regulation. IL-6 and IL-10 have a major role in pancreatitis development, but as every cell transduction mechanism, it is a sophisticated process with several cytokines involved in it. The results obtained will elucidate a better understanding of postoperative pancreatitis evolution and also help in optimizing treatment process by early prediction of development of various clinical manifestations and complications.

7. Conclusion

The present study shows a direct link between the immunological markers, IL-6 and IL-10, and the evolution of postoperative pancreatitis and transient hyperamylasemia. With a 95% confidence interval, a statistically significant difference was found between patients who developed postoperative pancreatitis and those with transient hyperamylasemia in the IL-6 study. With a 99% certainty, a statistically significant difference was found between patients who developed postoperative pancreatitis and those with transient hyperamylasemia in the IL-10 study. These results show that IL-6 and IL-10 can be used to clinically elucidate differences in the development of postoperative pancreatitis and transient hyperamylasemia in the early postoperative period; thus, these markers can aid correct diagnosis and effective treatment.

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