

## Research article

## Role of the cytokine system in patients with acute and chronic calculous cholecystitis

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## ABSTRACT

**Introduction and Aim:** Interleukin 1 (IL-1a and IL-1b), IL-6, and tumor necrosis factor-alpha (TNF-alpha), which are mostly released by monocytes and macrophages, are three cytokines that are effective, have multiple uses, and act as proinflammatory proteins during immune response and inflammation. The objective of this study was to evaluate the role of cytokine system in patients with acute calculous cholecystitis (ACC) and chronic calculous cholecystitis (CCC) when the disease got worse after medical and surgical treatment.

**Materials and Methods:** One hundred and thirty-three patients with acute cholecystitis who were hospitalized in our department and were divided into two groups. 79 patients with ACC made up Group 1. 54 patients with CCC made up Group 2. Based on clinical symptoms and an ultrasound scan, the clinical diagnosis was confirmed.

**Results:** A comparison of cytokines revealed that in purulent cholecystitis, the value of IL-6 becomes considerably higher than in ACC, and TNF-alpha becomes significantly higher, whereas IL-1 and IL-4 do not reach significant levels. A comparison of the concentration-time patterns of cytokines in the two groups when they left the hospital showed that IL-1beta and TNF-alpha in group 2 patients were higher than in group 1, but IL-4 was lower.

**Conclusion:** Following drug and surgical treatment, ACC resolves with a significant drop in the levels of pro-inflammatory cytokines and a rise in anti-inflammatory IL-4.

**Keywords:** Cytokines; acute calculous cholecystitis; chronic calculous cholecystitis; interleukins; tumor necrosis factor-alpha.

## INTRODUCTION

Gallbladder inflammatory and destructive diseases affect up to 15% of the population in developed nations (1, 2). Inflammation of the gallbladder is an early sign of gallstone development (3). Gallstones are hard bile fluid deposits in the gallbladder (4). Gallstones are mostly made up of calcium salts, bilirubin, and cholesterol, with trace quantities of protein and other substances (5). A sudden inflammation of the gallbladder is known as cholecystitis. Surgical intervention is usually necessary due to the difficult course of cholecystitis. Due to immune system irregularities prior to surgery, postoperative purulent-inflammatory problems occur often (6). In 80-85% of patients, gallstone disease (GSD) is the underlying factor in acute cholecystitis. Perivesical infiltrate (15%) and gallbladder empyema (12.4%) are the most prevalent purulent manifestations of acute cholecystitis, while subhepatic abscess (3.6%), hydrocholecystitis (7.5%), localized peritonitis (1.3%), and widespread peritonitis (2.3%) are also reported (7-9).

Interleukin 1 (IL-1a and IL-1b), IL-6, and tumor necrosis factor-alpha (TNF- $\alpha$ ), which are mostly released by monocytes and macrophages, are three cytokines that are effective, have multiple uses, and act as proinflammatory proteins during immune response and inflammation (10-12). By promoting the

development of other inflammatory mediators, such as IL-6, which affects the gallbladder's absorption, secretion, and smooth muscle contractility, TNF- $\alpha$  is known to play a significant role in the beginning of inflammation (13-15). The intracellular cytokine IL-1a has both cytokine and transcription factor characteristics (10). By promoting the change from acute to chronic inflammation, IL-6 trans-signaling plays a crucial role in maintaining the disease condition (16).

The objective of this study was to evaluate the role of the cytokine system in patients with acute calculous cholecystitis (ACC) and chronic calculous cholecystitis (CCC) when the disease got worse after medical and surgical treatment.

## MATERIALS AND METHODS

In this study, 133 patients with acute cholecystitis who were hospitalized in the department of surgery of the City Clinical Hospital in Osh, Kyrgyzstan, were divided into two groups. 79 patients with ACC made up Group 1. 54 patients with CCC made up Group 2. A total of 38 individuals were used as the control group, and their biliary tracts were checked for hypo- and hyperkinesis. Based on clinical symptoms and an ultrasound scan, the clinical diagnosis was confirmed.

Most of the participants in the study groups were female: 64% in the control group, 82% in group 1, and

79% in group 2. The average patient is older than 44 years old; in the groups, this percentage was 56%, 71.4%, and 65.7%, respectively. Patients in Group 1 were typically admitted to the hospital within 24 hours after the onset of illness symptoms (79.3%). Laparoscopic surgery accounted for 81% of surgical interventions in both clinical groups.

Blood plasma was evaluated for cytokines such as IL-1, IL-4, IL-6, and TNF- $\alpha$  using an immunoassay photometer analyzer (HumanReader HS, Wiesbaden, Germany).

Statistica v8.0 (StatSoft Inc., Tulsa, USA) was used for the statistical analysis. The acquired information is shown as mean  $\pm$  standard deviation. To evaluate parameter differences, the Student's t test was used. A 95% confidence interval is used to evaluate the range that contains both the upper and lower limits of a difference in the studied groups (P equal to 95%, or P<0.05; at P equal to 99%, or P<0.01; with P equal to 99.9%, or P<0.001). The techniques for determining sensitivity (Se) and specificity (Sp) were identified using a standard method (11). The data acquired was kept confidential, and the International Higher School of Medicine Bioethics Committee gave its approval for this study (Protocol No. 93, dated October 21, 2022).

**RESULTS**

Table 1 shows that the levels of IL-1 $\beta$  (P<0.001), IL-4 (P<0.05), and IL-6 (P<0.001) in the plasma of patients with ACC from Group 1 have gone up significantly. However, the level of TNF- $\alpha$  hasn't changed much (P>0.05). The value of IL-6 rises five times and is much higher. When compared to the values of the control group, IL-6 levels in patients with CCC from this group over this time period significantly rise (P<0.01), while other cytokine indices do not reach significant levels (P>0.05). There is a decrease in IL-1, IL-4, and IL-6 when compared to the parameters of Group 1 (P<0.05–P<0.01); however, there are no significant changes in TNF- $\alpha$  (P>0.05).

A few patients in Group 1 had gangrenous cholecystitis, which was determined surgically. A comparison of cytokines (Figure 1) revealed that in purulent cholecystitis, the value of IL-6 becomes considerably higher (P<0.001) than in ACC, and TNF- $\alpha$  becomes significantly higher (P<0.05), whereas IL-1 and IL-4 do not reach significant levels (P>0.05). The concentrations of IL-1 $\beta$  (P<0.05), IL-6 (P<0.001), and TNF- $\alpha$  (P<0.05) in the blood in this group during discharge from the hospital are significantly lower than their levels prior to treatment (Figure 2), but IL-4 and IL-6 are significantly higher than they were in the control group (P<0.01 and P<0.05, respectively).

In group 2, IL-1 $\beta$  in the blood stayed significantly higher (P<0.05) at the time of discharge from the hospital compared to the time before treatment (Figure 3), but none of the other cytokine parameters changed significantly (P>0.05). Relative to the parameters of the control group, there is an increase in IL-1 $\beta$  and IL-6 (P<0.05).

A comparison of the concentration-time patterns of cytokines in the two groups when they left the hospital (Figure 4) showed that IL-1 $\beta$  and TNF- $\alpha$  in group 2 patients were higher than in group 1 (P>0.05), but IL-4 was lower (P>0.05).

Some of the measurements of the cytokine system in people with acute cholecystitis may show changes in metabolism, cell growth, and immunity, depending on how bad the inflammation in the bile tissue is and how many pro-inflammatory and anti-inflammatory cytokines are active. These modifications also affect immunocompetent cells and phagocytic system malfunction. In the human body, cytokines play a role in chemical signaling that can activate cellular inflammatory mediators such as the products of lipid peroxidation. Pro-inflammatory cytokines are more abundant in the blood as a result of the gallbladder's inflammatory process. The amount of IL-6, which rises in direct proportion to the level of inflammation, is a common parameter of this.

**Table 1:** Parameters of the concentration of cytokines in blood plasma in the study groups

No.	Variables	Statistical parameters	Cytokines in pg/ml			
			IL-1 $\beta$	IL-4	IL-6	TNF- $\alpha$
1.	Control group (n=38)	M $\pm$ m	5.24 $\pm$ 0.47	3.32 $\pm$ 0.31	11.51 $\pm$ 0.92	8.4 $\pm$ 0.65
2.	Group 1 (n=79)	M $\pm$ m P <sub>2-1</sub>	15.8 $\pm$ 52.21 <0.001	7.94 $\pm$ 0.86 <0.05	66.6 $\pm$ 7.54 <0.001	9.1 $\pm$ 1.21 >0.05
3.	Group 2 (n=54)	M $\pm$ m P <sub>3-1</sub> P <sub>3-2</sub>	6.45 $\pm$ 0.72 >0.05 <0.01	4.39 $\pm$ 0.66 >0.05 <0.05	26.7 $\pm$ 4.31 <0.01 <0.001	7.65 $\pm$ 1.09 >0.05 >0.05

All data are expressed as M $\pm$ m = mean  $\pm$  standard deviation. \*p< 0.05. Interleukin = IL-1 $\beta$ , Interleukin-4 = IL-4, Interleukin-6 = IL-6, Tumor necrosis factor- $\alpha$  = TNF- $\alpha$

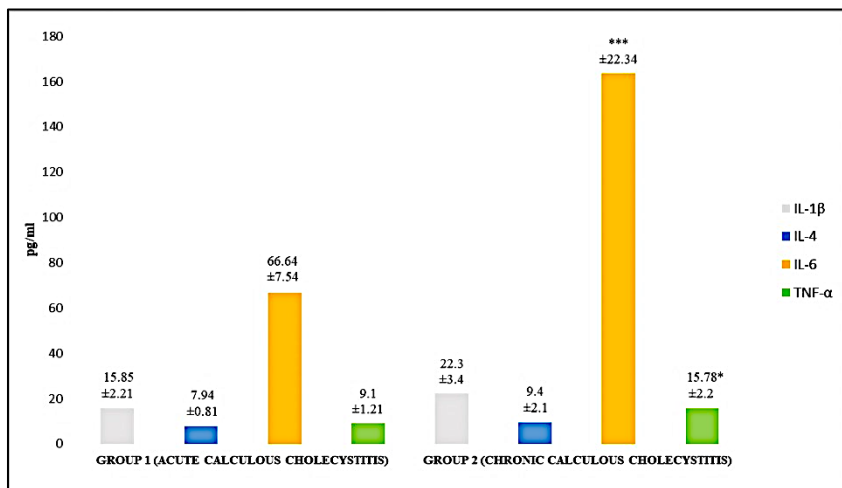


Fig. 1: Comparison of cytokine levels in blood plasma in patients with ACC and gangrenous cholecystitis

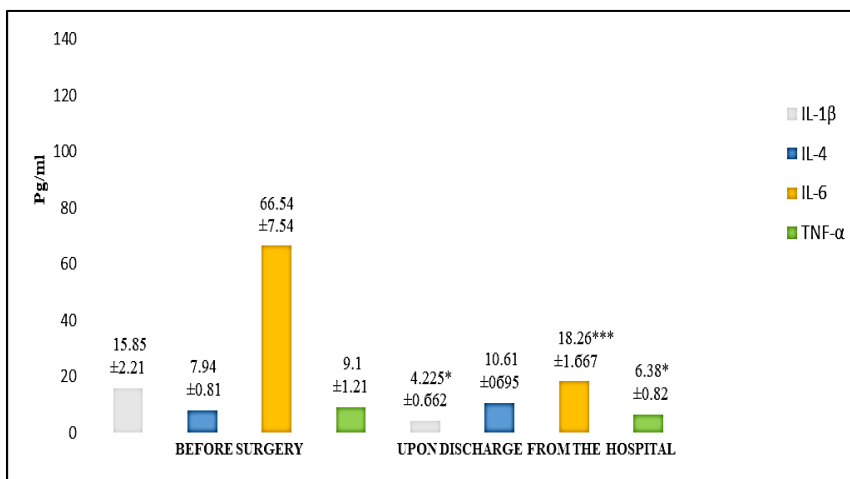


Fig. 2: Comparison of cytokine levels in blood plasma in patients with ACC during the discharge period

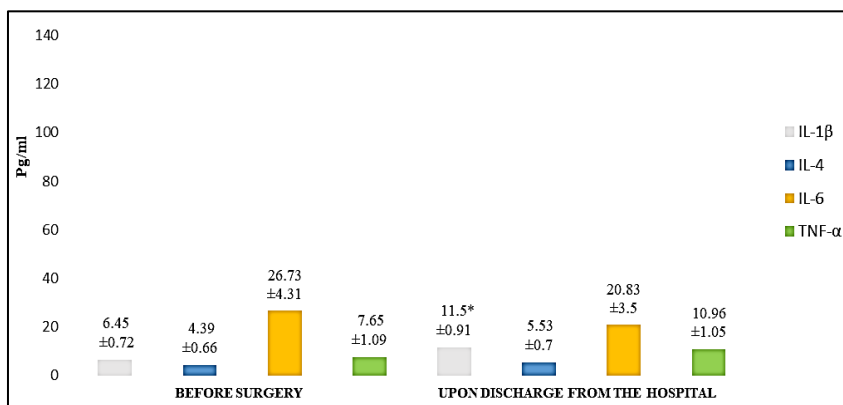


Fig. 3: Comparison of cytokines in blood plasma in patients with CCC during the discharge period

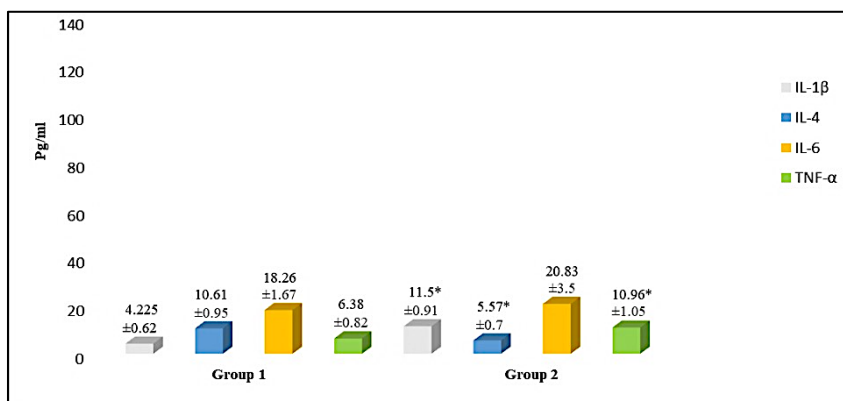


Fig. 4: Comparison of cytokines in blood plasma in patients with ACC and CCC during the discharge period

An increase in IL-4 in Group 1 in the acute period can be regarded as a protective-compensatory reaction to an increase in pro-inflammatory IL-1 $\beta$  and IL-6. This stimulates the production of immunoglobulin G and humoral immunity factors, as well as a decrease in the population of cytotoxic T-cells, prostaglandins, and interferon gamma (IFN- $\gamma$ ). With the resolution of an acute pathological process in the hepato-biliary system, the concentration of pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ) lowers and IL-4 increases compared to group 2 with a planned surgical intervention.

The metabolic processes of glucose and lipids in the liver tissue change in conjunction with an increase in IL-6 concentration in the blood. Intrahepatic cholestasis is a result of this process, which causes calculi to form. An increase in IL-1 $\beta$  concentration in the blood can also be accompanied by the acidification of bile. With inflammation of the gallbladder tissue, a mechanism of formation of bile inhomogeneity in the gallbladder is also possible, which results in bile clots. The activity of IL-4 raises painful biliary symptoms at various levels.

The cytokine system in cholelithiasis and acute cholecystitis characterizes the immune mechanisms of structural changes, which are controlled by immune system cells between antigen-presenting cells and T cells. The interaction of cells of the immune system and the epithelium is determined by cytokines, which, as shown in our studies, significantly change their activity even with respect to functional changes in the gallbladder in patients in the control group.

TNF- $\alpha$  is mostly active because of viral infections, endotoxins, lipopolysaccharides, and other bacterial parts. These parts are activated when cholecystitis gets worse and bile crystals block the bile ducts.

Determination of sensitivity and specificity of cytokine parameters showed that IL-6 reached the highest values, which amounted to 88 and 81%, respectively; IL-1 $\beta$  values were 66 and 71%; IL-4 was 63 and 57%; and TNF- $\alpha$  was 65 and 60%. These parameters indicate that the parameters of the cytokine system can serve as sensitive markers of the course, severity, and effectiveness of treatment for acute cholecystitis.

## DISCUSSION

Considering inflammation, cytokines may occasionally have a systemic effect. Proinflammatory cytokines, which promote neutrophil and macrophage accumulation at the site of infection, promote their phagocytic and bactericidal activities, and trigger the antigen-specific immune response process, are related to the development of the inflammatory process (17). These processes, not only in damaging the gallbladder but also in the entire hepatobiliary system, remain poorly understood to date.

Cytokines play a direct role in the development of inflammation, which helps identify antigens, promotes the expression of the histocompatibility and adhesion complex, activates, multiplies, and differentiates immune system cells to make effector cells, and thus determines the features of the immune response (18).

In all patients with CCC, TNF- $\alpha$  was found to contribute to gallstone development (19). There is no association between TNF- $\alpha$  and IL-4 in patients with CCC when TNF- $\alpha$  protein expression exceeds that of IL-4. This showed individuals with gallstones have an immunological predisposition. According to *Ebadi et al.*, IL-4 inhibits the formation of gallstones, whereas TNF- $\alpha$  increases gallbladder inflammation and speeds up gallstone formation (20). Patients with gallstones had significantly lower quantities of the IL-4 than they had of the IL-1, TNF- $\alpha$ , and IL-6 (19).

There is evidence that factors related to food can affect the risk of cholesterol-related gallstones. Refined sugar, cholesterol, saturated fat, trans fatty acids, and potentially legumes are dietary variables that can increase risk (21). Another risk factor for gallstones is obesity. Fiber, coffee, polyunsaturated and monounsaturated fats, and diet may help avoid the formation of gallstones (21). Having vegetarian food has been linked to a lower risk. A ketogenic diet has been shown to help treat several diseases, such as cancer, high cholesterol, epilepsy, cardiovascular disease, and type 2 diabetes (22, 23). Diabetes, cancer, heart disease, and inflammation are only some of the diseases that can be treated with omega-3 fatty acid supplements (24-27).

Based on the information given about the cytokine system's indicators, it can be said that inflammatory mediators can be very helpful in describing how the human hepatobiliary system breaks down. Inflammatory, congestive, and calculi-forming processes in the gallbladder are accompanied by an increase in the concentration of pro-inflammatory cytokines in the blood. Apparently, the initial sign of cholelithiasis is dyskinesia of the biliary tract, and later, with an increase in the concentration of cytokines, inflammation is revealed in the form of cholecystitis with the formation of gallstones.

## CONCLUSION

In purulent-inflammatory processes, the concentration of IL-6 in the blood increases sharply. Following drug and surgical treatment, ACC resolves with a significant drop in the levels of pro-inflammatory cytokines and a rise in anti-inflammatory IL-4. With the operational resolution of the CCC, such dynamics are not observed. As a result of therapeutic measures in patients, there is a recovery of clinical and functional parameters. But at the same time, the cytokine profile does not reach the values of the control group, which indicates that the normalization

of the functioning of the cytokine system occurs much later after the success of the treatment.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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