AZERBAIJAN MEDICAL UNIVERSITY

On the rights of the manuscript

ABSTRACT

of dissertation work presented for obtaining scientific degree
"PhD on Medicine"

ROLE OF ANTIMICROBIAL PEPTIDES IN FORECASTING AND INITIAL DIAGNOSTICS OF THE POSTOPERATIVE PURULENT-INFLAMMATORY COMPLICATIONS IN ACUTE CALCULOUS CHOLECYSTITIS

Specialty: 3213.01 – Surgery

Scientific scope: Medicine

Applicant: **Gasimova Shahla Khayyat**

Baku – 2022

The dissertation work has been executed in the Department "General surgery" of Azerbaijan Medical University.

Scientific adviser: PhD on Medicine, professor
                                           Hajiev Jabbar Niyatulla

Official opponents: Doctor of Medical science, professor
                                           Zeinalov Suraddin Musa

                                           Doctor of Medical science, professor
                                           Kazimov İlham Lazim

                                           Doctor of Medical science
                                           Huseinov Sahib Ahmad

ED.2.06. Dissertation Board acting within Azerbaijan Medical University of Higher attestation Commission under the President of the Republic of Azerbaijan

Head of Dissertation Council: Doctor of Medical science, professor
                                   Hadiyev Surkhay Ismayil

Sceintific secretary of
Dissertation Council: Doctor of Medical science, professor
                                   Jamalov Fariz Hidayat

Chairman of scientific workshop: Doctor of Medical science, professor
                                 Karimov Mahammad Mahammadali
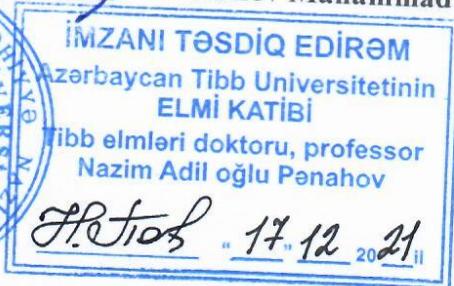
GENERAL DESCRİPTİON OF THE WORK

Relevance and development of the topic: Gallstone disease (GSD) and due to its complication is increased year-by-year, increased tendency to "rejuvenate" complicated cholecystitis, and as well as remaining of the special weight of destructive forms of the disease in elderly patients in high margins and non-reducing of the postoperative death cases turn this pathology to topical medical-social-economic problem[1,2].

Thus, cholelithiasis is determined up to 40% of population in the developed countries of the world. GSD, which number is increased at least twice per ten years is complicated with acute cholecystitis in 82.5-90.0% cases and due to this, cholesystectomy takes second place after appendectomy in the world due to the number of conducted surgical interventions[3].

Although modern technology is applied and modern antibacterial means are used during the surgical treatment of acute calculous cholecystitis (ACC), postoperative purulent-inflammatory complications are seen in 5.4-18.9% cases[4].

One of the reasons of postoperative purulent-inflammatory complications is a strict changes occurred by the acute purulent-inflammatory process in the gallbladder in the immunity, functional activity of immunocomponent cells in patients and as well as in their number. By considering that human organism, in general its immune reaction, as well as cellular and humoral immunity which is integral

---

[1] Hadıyev S.İ. Kəskin xolesistitli xəstələrdə laparosokopik xolesistektomiya / S.İ. Hadıyev, V.A. Paşazadə, H.A. Abbasov // Sağlamlıq, - Bakı: - 2016. № 4, - s. 30 – 34.
[2] Гаджиев Дж.Н. Состояние цитокинового антиоксидантного статуса, функциональных показателей печени и эндогенной интоксикации при разных формах синдрома системной воспалительной реакции у больных с острым калькулезным холециститом / Дж.Н. Гаджиев, Н.Дж. Гаджиев, З.Б. Мамедова // Клиническая хирургия, - Москва: - 2018. № 1, - с. 24 -26.
[3] Леонтьев А.С. Профилактика и лечение постхолецистэктомического синдрома при лапароскопической холецистэктомии с обоснованием роли изменений области большого дуоденального сосочка: / Автореферат диссертации кандидата медицинских наук) / - Томск, 2017. - 42 с.
[4] Lennard T. The influence of surgical operations on components of the human immune system / T. Lennard, B. Shenton, A. Bozzotta // B. J. Surg., - 2005. v. 72, - p. 771-776.

part of such immune reaction, which executes its functions in close mutual relation with each other and the state of phagocytosis play significant key role in the development of purulent-inflammatory process and pathogenesis of surgical infection and acute surgical pathology causes the development of the induced form of secondary immunodeficiency damaging all circles of immunity, their ability to cause secondary immunodeficiency, infectious complaints and even the death by depending on volume of any surgical intervention, heaviness of the condition of patient and pathology, in the recent years, interest to study the changes occurred in immune and cytokine status in ACC patients and relations between immunity parameters and cytokine profiled indicators[5,6].

As we know, protection of organism from infection is realized through congenital (natural) and acquired (adaptive) immunity, which are integral part of single functional complex: at first natural protection factors of resistence and then specific immune reaction join the struggle against reproduction[7,8]. Antimicrobial peptides (AMP) which are considered as significant components of immune system play significant role in modulation of immune reaction and in the first line of protection of macroorganism from infection[9,10].

Thus, key components of the cascade mechanism of the development of purulent-inflammatory process are cytokines and

[5] Гаджиев Дж.Н. Направленная цитокинотерапия в комплексном лечении больных с механической желтухой желчнокаменного генеза / Дж.Н. Гаджиев, Э.Г. Тагиев, Н.Дж. Гаджиев // Вестник хирургии, - Санкт-Петербург: - 2016. № 4, - с. 67 -70.

[6] Tağıyev E.Q. Xoledoxolitiaz mənşəli mexaniki sarılıqlı xəstələrdə cərrahi taktikanın optimallaşdırılması və sitokin disbalansının tənzimi: / Tibb üzrə fəlsəfə doktoru dissertasiyanın avtoreferatı) / - Bakı, 2018, - 48 s.

[7] Захарова И.Н. Роль антимикробных пептидов в защите от инфекций мочевых путей / И.Н. Захарова, И.М. Османов, Л.Я. Климов [и др.] // Медицинский совет, - Москва: - 2019. № 2, - с. 143-150.

[8] Tarr A.W. The role of humoral innate immunity in hepatitis C virus infection / A.W. Tarr, R.A. Urbanowicz , J.K. Ball // Viruses, - 2012. v. 4, no 1, - p. 1-27.

[9] Yang Z. TLRs, macrophages and NK cells: our understandings of their functions in uterus and ovary. Int Immunoph / Z. Yang, B. Kong, D.M. Mosser [et al.] // 2011. v. 11, - p. 1442-50.

[10] Lehrer R. a-Defensins in human innate immunity / R. Lehrer, W. Lu // Immunol. Rev., - 2012. v. 245, - p. 84-112.

antimicrobial peptides[11].

By considering that modern instrumental-laboratory diagnostic methods (USG, CT, laboratory analyses) are not able to determine definite diagnosis to pathomorphological changes in gallbladder, not studying the parameters of antimicrobial peptides in ACC, non-availability of any information about diagnostic and therapeutic diaposone of such indicators, it becomes necessary to realize strict scientific investigations in this sphere.

Purpose of research: Study of the significance of antimicrobial peptides in assessment of the morphological forms of inflammation and forecasting and initial diagnostics of purulent-inflammatory complications in postoperative period in gallbladder in patients with acute calculous cholecystitis disease.

Objectives of research:
1. Study of the level of antimicrobial peptidis in dynamics during surgical treatment of acute calculous cholecystitis.
2. Comparative assessment of antimicrobial peptides, some cytokines and adaptive humoral immunity parameters by depending on morphological form of inflammation in patients with acute calculous cholecystitis.
3. Comparative assessment of the stage of some antimicrobial peptides and cytokines in various bio-environment by depending on morphological form of inflammation.
4. Study of the mutual relations between antimicrobial peptides and cytokine profile and adaptive humoral immunity parameters in acute calculous cholecystitis.
5. Assessment of the diagnostic significance of phagocytic potential of neutrophiles as biomarkers of formation of infectious complications in postoperative period.

Research methods. In conformity with the dissertation work plan, studies cover several stages. In the first stage, data of recent years of national and foreign sources have been analyzed about the current

---

[11] Азизова Г.И. Эндогенные антимикробные пептиды как маркеры инфекционного процесса при талассемии / Г.И. Азизова, А.Р. Дадашова, Г.Р. Вагабова [и др.] // Международный Медицинский журнал, - Харьков: - 2014. № 2, - с. 100-103.

status of surgical treatment of ACC, antimicrobial peptides, and their role in normal and pathological processes and as well as changes observed in immune and cytokine status of organism in ACC. In this purpose, 27 national and 266 foreign sources have been used.

In the second stage studies were realized in dynamics in 18-71 years old patient who held open (15), minilaparotomy (17) and laparoscopic (37) cholecystectomy with diagnosis of ACC. Patients have been divided into three groups by considering TG 13 principles: I group includes 26 patients with cataral form of ACC, II group includes 24 patients with the form of inflegmonosis of ACC and III group includes 19 patients with gangrenous form of ACC. Several antimicrobial peptides, cytokine profile, hepatic functional and as well as endogenic intoxication parameters have been studied in blood in comparative manner in dynamics of the mentioned patients. Several parameters were studied in biopsies taken from liver and in the bladder. Moreover, microbiological study of the bladder has been realized.

In the third stage statistical processing of results separately obtained on each group have been realized.

In the forth stage comparative analysis of studied parameters on groups has been conducted and possible affection of bacteriobilia to studied endogenic antimicrobial peptides, cytokine profile and adaptive humoral immunity parameters has been studied.

In the fifth stage roles of some antimicrobial peptides and cytokines in initial diagnostics of destructive forms of ACC have been proved by aid of reliable medicinal methods.

Key provisions of defense:

1. Number of antimicrobial peptides in acute calculous cholecystitis is increased in local and system levels and this shows the activation of congenital immunity during pathology.
2. Due to high level of studied antimicrobial peptides, especially lactoferrin and hepsidine in plasm is characteristic for destructive process in gallbladder, destructive form of acute cholecystitis may be used as an informative biomarkers in initial diagnostics.
3. Concentration of anti-purulent-inflammatory cytokines   in

TNFα, IL-6 və IL-8 blood serum is completely correlated with depth of inflammation and destructive processes in gallbladder.

4. There are statistical correct strong direct (positive) mutual relations between antimicrobial cytokines and adaptive humoral immunity parameters and syndissertation work of antimicrobial peptides in patients with acute cholecystitis.

5. Expediency of using from antimicrobial peptides and cytokines with high specifity and sensitivity in forecasting and initial diagnostics of postoperative purulent-inflammatory complications has been completely proved with evidence-based medicine methods.

Scientific novelty of research:

Systemic (blood serum) and local (hepatic tissue and bile) levels of antimicrobial peptides and cytokine profile in patients with ACC were studied comparatively and comprehensively, taking into account morphological forms of gallbladder inflammation and diagnostic significance of such parameters has been determined. Moreover, dynamics of change of post-surgical intervention antimicrobial peptides and cytokines has been studied, and by studying the role of antimicrobial peptides and cytokines as informative biomarker in forecasting and initial diagnostics of purulent-inflammatory complications which may be occurred in postoperative period, their significance in clinical practice has been grounded by using from mathematical models by based on principles of theory of probabilities and proved by aid of ROC analysis and evidence-based medicinal methods. Moreover, correlation has been determined between antimicrobial peptides and cytokine profile and adaptive humoral immunity parameters. Affection of bacteriobilia to antimicrobial peptides, cytokine profile and adaptive humoral immunity parameters in blood has been comparatively estimated.

Practical significance of the work:

Inclusion of lactoferrin and hepsidine from antimicrobial peptides in plasm to diagnostics complex of ACC enables to detect destructive forms of inflammation in the gallbladder at an earlier stage and choose the right surgical tactics in a timely manner. Inclusion of serological informative biomarkers – antimicrobial peptides in the

blood, lactoferrin and α-defensin, cytokines such as TNFα and IL-6 to the program of forecasting and initial diagnostics of postoperative purulent-inflammatory complications play an enormous role in achieving of more correct results. Moreover, such biomarkers are essential for monitoring of clinical progress of disease.

Approbation of the work:

Materials of dissertation work have been presented and discussed in following meetings: III Congress of the Association of Extraordinary Medical Assistance of Uzbekistan (Tashkent, October 2017); 4. International medical congress (Baku, November 2-3, 2017); 21st National Surgical Congress 16th Surgical Nursing Congress (Antalya, April 11-15, 2018); International scientific-practical Internet conference "New in Surgery" (Belarus, Minsk, November 1, 2019). Initial discussion of the dissertation work was held in joint meeting of employees of Surgical-profiled departments of AMU and Teaching-Surgical Clinic on July 05, 2019 (protocol N: 13). On May 25, 2021, the next discussion of the work was held in the Approbation Commission, which conducts Scientific Seminars under the Dissertation Council ED 2.06 on the specialty 3213.01 - "Surgery" (Protocol № 4).

Application of work to practice: The obtained results are applied in the Department of General Surgery of AMU and Surgical Departments of AMU Education Surgery Clinic. Moreover, scientific information obtained in result of conducted clinical-biochemical-immunological studies is used in the process of teaching.

The organization where the dissertation work is performed. The topic of dissertation work is included in the plan of scientific research Works of the Department of General Surgery of AMU (state registration number BTEB-217).

Published scientific Works: 6 articles (3 in foreign magazines) and 7 thesis (3 in foreign states) have been published about the topic of dissertation.

The scope and structure of the work

The dissertation consists of an introduction written on 188 pages (233701 characters) introduction (11290 characters), literature review (58451 characters), 3 chapters (11902 + 88918 + 29842 characters),

conclusion, results, practical recommendations (29229 characters) and bibliography. The dissertation contains 33 tables and 15 graphs. The list of used literature includes 293 sources in 27 Azerbaijani, 155 Russian and 111 English languages.

MATERIAL AND METHODS OF RESEARCH

Clinical researches cover 69 patients aged 18-71 years who underwent the intervention with ACC diagnosis during 2016-2018 years in Teaching-Surgical Clinic of Azerbaijan Medical University. "The Tokyo Guidelines" 2013 (TG) has been taken as a basis for assigning acute cholecystitis diagnosis to all patients included in the research contingent. Analysis of patients on age and sex shows that, from 69 patients diagnosed with ACC who underwent surgery, 24 were men ($34.8 \pm 5.7\%$) and 45 ($65.2 \pm 5.7\%$) were women. It is noteworthy that ACC is more common in people aged 51-60 years. At the same time, the "rejuvenation" of gallstones is noticeable. In general, able-bodied individuals dominated among patients. Due to patients applied to clinic in late periods, various complications were seen in 37 persons ($53,6\pm6,0\%$): perivezical infiltrate (8 people), perivezical abscess (7 people), local peritonitis (13 people), general peritonitis (5 people) and acute pancreatitis (4 people). In 36 out of 69 patients ($52.2 \pm 6.0\%$) various somatic diseases were also detected. In addition, somatic diseases were more common than chronic ischemic heart disease (10 people), hypertension (7 people) and diabetes (7 people).

From the patients admitted to the clinic with ACC and complications, 11 ($15\pm0,9\%$) were urgent (within the first 6 hours), 50 ($72\pm0,5\%$) were early (within the first 24-48 hours) and 8 ($11\pm0,6\%$) underwent delayed surgery (after 3 days). At the clinic, all patients underwent cholecystectomy under endotracheal anesthesia under the guidance of active surgical tactics. Among the surgical methods, laparoscopic cholecystectomy (LC), which is considered the "gold standard", is preferred. Thus, in 37 patients who underwent surgery ($53\pm0,6\%$), the gallbladder was removed laparoscopically. Open cholecystectomy (OC) was performed in 15 patients ($21\pm0,7\%$). LC is preferred in patients with group III - gallbladder gangrene, with

various complications of ACC. Minilaparotomycholecystectomy (MLC) was performed by M.D. Prudkov in 17 patients (24±0,6%). There was no need for conversion in any patient. There were no deaths in the postoperative period. However, postoperative local purulent-purulent-inflammatory complications were found in 11 patients (15±0,9%). The frequency of these complications depends on the form of inflammation in the gallbladder.

By considering the purpose of research and set duties, patients were divided into 3 groups: Group I included 26 patients with catarrhal form of ACC, group II with 24 patients with phlegmonous form of ACC, and group III with 19 patients with gangrenous form of ACC. Patients in all three groups received traditional complex basically conservative therapy, generally accepted in the preoperative and postoperative period. In the dynamics of patients in all 3 groups - in the preoperative period, on the 3rd and 7th days of the postoperative period, the concentration of α-defensin (HNPα), endotoxin, neutrophil elastase in the blood plasma was determined by immunoenzyme using a set of NycultBiotech (Netherlands).

The level of protein that increases membrane permeability (BPI) in the blood was studied with the set BPI-IFA-Test (CJSC "Vector-Best", Russia).

Plasma lactoferrin concentration is determined by enzyme-linked immunosorbent assay.

The amount of hepcidin in biopsies taken from the liver during plasma and surgery was determined by direct (direct) immunoenzyme methods by using from ABCAM (USA) monospecific serums and antigenic monoclonal antibodies.

Concentrations of anti-purulent-inflammatory (TNFα, IL-6, IL-8) and anti-purulent-inflammatory (IL-10) cytokines in blood were studied by enzyme-linked immunosorbent assay using Bender Medical Systems (USA) reagents. The TNFα / IL-10 ratio was also calculated in patients.

In 36 patients (12 patients from each group), TNFα and IL-6 levels were also examined in surgical liver biopsies and bladder bile taken during surgery. In addition, the dynamics of IL-6 in the urine of patients was determined. In addition, taking into account the

antimicrobial effect of AMPs, the microbiological landscape of bladder bile taken during the operation was studied.

Studied biochemical, immunological and cytokine profile parameters of 10 healthy people have been accepted as a norm.

Along with performing total blood analysis in all patients admitted to clinic, their coagulogram parameters have been studied, viral hepatitis markers have been determined.

Functional parameters of the liver in the dynamics - total protein in the blood, albumin, total bilirubin and its fractions, as well as liver enzymes - alanine aminotransferase (ALT), aspartate amino-transferase (AST), alkaline phosphatase (AP) and gammaglu-tamyltransferase (GGT) have been studies. Also, the AST / ALT ratio was determined. The amount of OMP in the blood was determined by Nikolaichuk et al. (1991) method.

Mathematical numbers obtained in result of conducted researches were processed by modern statistical methods- parametric and non-parametric methods, correlation analysis was performed between studied parameters. In purpose of assessment of the significance of antimicrobial peptides and cytokines as more informative biomarkers in forecasting and initial diagnostics of occurrence of purulent-inflammatory complications in patients in postoperative period, ANOVA test, correlation analysis, ROC-analysis, evidence-based medicinal methods and Snekedor ration have been used.

Biochemical and immunological analysis were performed in the laboratory of the Department of Biochemistry of AMU, and microbiological examinations were conducted in the Department of Microbiology of AMU.

ANALYSIS OF OBTAINED RESULTS

In comparison with the parameters of healthy persons in pre-operational period in 69 patients involved in research, the amount of antimicrobial peptides in the blood was 2.4 times, BPI - 6.9 times, endotoxin - 8.6 times, neutrophil elastase - 3.2 times, lactoferrin - 2.2 times, hepsidine - 3.5 times more than statistically accurate. Moreover, during the progress of operation, hepcidin levels in liver biopsies taken

from the liver were 2.1 times more than the normal statistically accurate (38.7±2.1 pg / ml). It should be notes that, the amount of antimicrobial peptides varies to different margins depending on the morphological form of inflammation in the gallbladder. Thus, in the catarrhal form of ACC in the pre-operative period, the level of α-defensin in the blood compared to the norm was 66.4% (P<0.001), BPI - 2.9 times (P <0.001), endotoxin - 5.9 times (P<0.001), neutrophil elastase - 2.3 times (P<0.001), lactoferrin - 71.4 times (P<0.001), hepsidine - 3.0 times (P<0.001), and hepcidin in liver tissue 88.6% (P<0.01) increased.

In comparison with catarrhal form of ACC in pre-operative period in the form of phlegmonous inflammation of the gallbladder, α-defensin in blood was 33.5% (P_1<0.001), BPI - 3.0 times (P_1<0.001), endotoxin - 49.1% (P_1<0.001), neutrophil elastase - 33.4% (P_1<0.001), lactoferrin - 22.5% (P_1<0.001), hepsidine statistically inaccurate - 12.1%, and hepcidin statistically inaccurate 11.8% more in liver tissue.

In the gangrenous form of ACC, in relation to the catarrhal and phlegmonous forms of inflammation of the gallbladder, statistically correct α-defensin in the blood was 2.1 times and 58.9%, BPI - 3.5 times and 16.5%, endotoxin - 2.1 times and 38.2%, respectively, neutrophil elastase - 90.3% and 42.7%, lactoferrin - 68.9% and 37.9%, hepsidine - 54.4% and 37.7%, and hepcidin in liver tissue was statistically inaccurate at 25.4% and 12.1% more.

The increase in amount of α-defensine in plasm in all forms of ACC shows direct growth of microbicidal potential of the organism. On the other hand, it is known that, Defensins are potent chemoattractants for monocytes and are actively involved in the effector phase of adaptive immunity. A wide range of changes in α-defensin levels in different forms of ACC reflects the degree of neutrophil activation. In indestructible forms of ACC, a further increase in plasma concentrations of endotoxin is associated with a functional state of the liver. Also, destructive forms of inflammation in the gallbladder and, against this background, purulent-purulent-inflammatory complications lead to increased secretion of BPI. Thus, the metabolism of this endotoxin goes in the liver. A further increase in the amount of neutrophil elastase in destructive forms of ACC

confirms that this endogenous antimicrobial peptide plays an important role in severe purulent-inflammatory reactions.

By combining with iron, lactoferrin synthesized by neutrophils during inflammation causes a deficiency of this metal for microbes and has antibacterial, antiviral, antifungal effects. Since the concentration of lactoferrin in plasma is correlated with the number of neutrophils, as well as the turnover times of these cells, its content in body fluids is considered an indicator of neutrophil activation.

Thus, Lacroferrin, which plays an important role in the body's iron metabolism, as well as has immunomodulatory and antioxidant effects, proves to increase the activity of neutrophils in this pathology as one of the most informative biomarkers of inflammation.

Hepsidine, a hormone that regulates iron metabolism, is synthesized by hepatocytes in the liver and has all the properties of both humoral peptides and cytokines. This antimicrobial peptide is also synthesized by macrophages, fat cells and cardiomyocytes. As noted, although the amount of hepsidin increased in all forms of KDX, both in plasma and in tissue biopsies taken from the liver during surgery, this increase depended on the morphological form of gallbladder inflammation.

In general, in the preoperative period in patients, the concentration of TNFα in purulent-inflammatory serum from purulent-inflammatory cytokines increased statistically correct by 5.4 times, IL-6 - 7.0 times, IL-8 - 4.4 times and anti-purulent-inflammatory cytokine IL-10 - 37.1%. In these patients, the amount of TNFα in the biopsies taken from the liver was 5.2 times ($P<0.001$), the amount of IL-6 was 7.5 times ($P<0.001$) and the level of IL-6 in the urine was 7.6 ($P<0.001$) times higher than in healthy individuals. The average TNFα in bladder bile taken during surgery was 50.2±2.7 pg / ml, and IL-6 at 41.5±2.7 pg / ml. In patients, the TNFα / IL-10 ratio increased 4.6-fold ($P<0.001$) compared with the norm, indicating a deep imbalance in the cytokine profile. Comparative analysis has shown a direct correlation between changes in cytokine profile in patients, both local and systemic, and morphological forms of gallbladder inflammation. Thus, in the catarrhal form of inflammation of the gallbladder, the statistically correct amount of TNFα in the

13

blood was increased 3.8 times, IL-6 - 5.6 times, IL-8 - 3.7 times, and IL-10 - 68.2% compared to healthy people, in biopsies taken from the liver, TNFα - 6.5 times and IL-6 - 4.6 times, IL-6 - 5.1 times in the urine, the concentration of TNFα in the bile was 53.1 ± 4.5 pg / ml, and IL-6 - $36,1\pm3.6$ pg/ml. The TNFα /IL-10 ratio, which characterizes the cytokine balance in these patients, exceeded the norm by 2.2 times ($P < 0.001$).

In comparison with cataral form of inflammation in gallbladder in patients with phlegmonous form of ACC, during pre-operative period, the concentration of TNFα in the serum of pro-purulent-inflammatory cytokines was 34.5% ($P_1 < 0.001$), IL-6 19.5% ($P_1 < 0.01$), IL-8 - 22.9% ($P < 0.01$), on the contrary, the amount of anti-purulent-inflammatory cytokine IL-10 was 13.7% ($P_1 < 0.05$) less. The TNFα / IL-10 ratio was 54.7% ($P_1 < 0.001$) higher in catarrhal inflammation. In these patients, TNFα levels in liver tissue biopsies were 25.5% ($P_1 < 0.05$) lower than in the catarrhal form, but IL-6 levels were 76.6% ($P_1 < 0.01$) in liver tissue and 54.1% in urine ($P_1 < 0.001$) has risen to a high level. Although the concentration of TNFα in the bile taken during surgery was 22.6% ($P_1 < 0.05$) lower than in the catarrhal form, the concentration of IL-6 was statistically inaccurately 16.3% higher. In the gangrenous form of inflammation in gallbladder, cytokine profile parameters were subjected to deeper changers both in local and systematic levels. Thus, in the tissue taken from liver during operation, compared with similar indicators of catarrhal and phlegmonous forms of ACC, the amount of TNFα was 32.7% ($P_1 < 0.05$) and statistically inaccurate 9.7% less, respectively, but the amount of IL-6 was 2.0 times ($P_1 < 0.001$) and was statistically inaccurate by more than 15.9%. Respectively in comparison with cataral and phlegmonous forms of ACC in such patients, in the gallbladder, The amount of TNFα was statistically inaccurate at 5.9% and 36.9% ($P_2 < 0.05$), while IL-6 was statistically inaccurate at 28.5% and 10.5%, respectively. The amount of IL-6 in the urine was 2.0 times ($P_1 < 0.001$) and 32.5% ($P_2 < 0.01$) more. In the gangrenous form of ACC in the preoperative period, respectively, in relation to the catarrhal and phlegmonous forms of inflammation in the serum TNFα concentrations were 2.1 times ($P_1 < 0.001$) and 59.4% ($P_2 < 0.001$), IL-6 - 60.8% ($P_1 < 0.001$) and 34.6%

(P_2<0.001), IL-8-39.7% (P_1<0.001) and although statistically inaccurate is increased by 13.7%, on the contrary, the amount of IL-10 decreased by 49.8% (P_1<0.001) and 41.9% (P_2<0.001). Moreover, TNFα / IL-10 levels are also statistically 4.2 times and 2.7 times higher than catarrhal and phlegmonous forms, respectively.

In general, during preoperative period in patients in comparison with parameters in healthy people, $CD19^+$, one of the indicators of adaptive humoral immunity, was statistically 27.3%, IgA - 35.0%, IgM - 28.6%, IgG - 32.0% and FA is 16.2% lower than normal, the level of SEC increased by 93.9%.

The depth of changes detected both in humoral immunity and hepatic functional parameters and endogenic intoxication parameters were directly depended on morphological form of inflammation in gallbladder.

In general, dynamics of change of endogenic antimicrobial peptides and cytokine profile indicators in patients included in per 3 group in 3rd and 7th days of pre and postoperative periods was shown in table №1.

As seen from the table №1, α-defensine concentration which is microbicide for various microbes, fungus and mucous viruses and extracted by method of degranulation of neutrophiles during local and systematic activation generally in patients in 3rd day of postoperative period, compared to healthy individuals, the statistically significant increase was 31.0%, BPI - 51.0%, endotoxin - 13.7%, neutrophil elastase - 20.4%, lactoferrin - 10.2% and hepsidin - 16.4%. Only after this period, the dynamics decreased, and by the end of the observation, on the 7th day, α-defenzine was statistically inaccurate 13.3% less than the initial value and 2.1 times higher than the norm (P<0.001), BPI - statistically inaccurate 4.7% less than the initial indicator and 6.6 times higher than normal (P<0.001), endotoxin - statistically inaccurate 11.4% less than baseline and 7.7 times higher than normal (P<0.001), neutrophil elastase - statistically inaccurate only 1, 4% less and 3.1 times more than the norm (P <0.001), lactoferrin - 19.8% less than the initial level (P_0<0.001) and 74.2% more than the norm (P<0.001) and hepsidin was statistically inaccurate 7.4% lower than baseline, but 3.3 times higher than normal (P<0.001).

Table 1

**Dynamics of change of endogenic antimicrobial peptides and
cytokine profile indicators in patients**

Indicator	Term of research (day)			Norm
	D/o	3	7	
α-defensine (HNPα), ng/ml	231,1±10,4 105-392 ***	302,7±9,4 163-455 ***;###	200,5±5,1 120-291 ***;##	97,4±6,9 61,3-125
BPI, ng/ml	2,46±0,16 0,74-5,06 ***	3,71±0,24 1,05-7,1 ***; ###	2,34±0,13 0,75-4,39 ***	0,35±0,03 0,16-0,57
Endotoxine, ng/ml	3,26±0,14 1,1-6,4 ***	3,70±0,14 1,6-7,2 ***; #	2,89±0,12 1,2-6,1 ***	0,38±0,15 0,06-1,39
Neutrophiline elastase, ng / ml	167,3±7,1 74,6-303 ***	201,5±6,6 96,3-330 ***; ###	165,0±4,7 88,3-246 ***	53,1±3,5 36-66
Lactoferrin ng/ml	1683,1±51,5 900-2500 ***	1854,2±62,7 850-2800 ***;#	1349,5±43,3 750-2400 ***;###	774,5±6,2 500-1250
Hepsidine, pg/ml	219,4±8,7 68-367 ***	255,4±8,5 96-402 ***;##	203,1±6,7 66-297 ***	62,1±6,2 26-85
TNFα, pg/ml	34,8±1,9 13,7-95,5 ***	43,7±2,5 16,5-110 ***; ##	28,7±1,7 9,3-65,6 ***; #	6,4±0,7 3,6-10,7
IL-6, pg/ml	38,0±1,9 9,8-86,3 ***	43,0±2,5 18,3-116 ***	31,8±1,9 7,2-84,3 ***; ##	5,5±0,9 1,7-10,7
IL-8, pg/ml	180,2±8,2 67-446 ***	201,7±9,6 83-461 ***	138,9±6,2 47-281 ***; ###	41,3±5,7 22-81
IL-10, pg/ml	16,6±0,8 7,6-36,3 ***	32,2±1,1 15,3-58,3 ***; ###	20,9±0,7 10,5-42,1 ***; ###	12,1±1,6 1-18,5
TNFα/IL-10	2,47±0,20 1,02-5,65 ***	1,38±0,07 0,79-2,74 ***; ###	1,39±0,07 0,65-2,45 ***; ###	0,81±0,31 0,38-3,6

Note: Statistical accuracy of the difference between the indicators:
1.According to norm: *-P<0,05; ** - P<0,01; ***-P<0,001.
2. According to initial indicator: #-P_0<0,05; ## - P_o<0,01; ### - P_o<0,001.

Cytokine profile indicators in general by being directed to be increased in postoperative period in the patients, On the 3rd day, the amount of TNFα in the blood was 25.7% ($P_0<0.001$), IL-6 - statistically incorrect 13.2%, IL-8 - statistically incorrect 11.9%, IL-10 - 94,0% ($P_0<0.001$), and the level of IL-6 in the urine is 36.3% ($P_0<0.001$) less than the initial value. During this period, the TNFα / IL-10 ratio falls 44.2% ($P_0<0.001$) below baseline. Only from the mentioned period, concentration of the studied cytokines in blood begins to fall. But, it remains long apart from norm in the 7th day of the postoperative period: in comparison with norm, compared to the norm, the amount of statistically accurate TNFα is 4.5 times, IL-6 - 5.8 times, IL-8 - 3.4 times and IL-10 - 72.2% more. In this period, although TNFα/IL-10 ratio is 43.9% lesser than initial indicator ($P_0<0,001$), it remains 2.6 times more from the level in the healthy people ($P_0<0,001$).
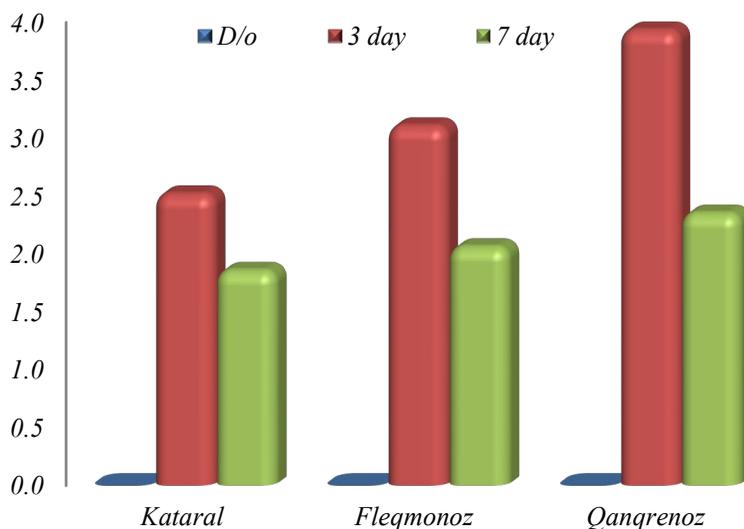
Table 1. Dynamics of change in amount of α-defensine in plasm in various forms of ACC (norm – 1 s.v.).

17
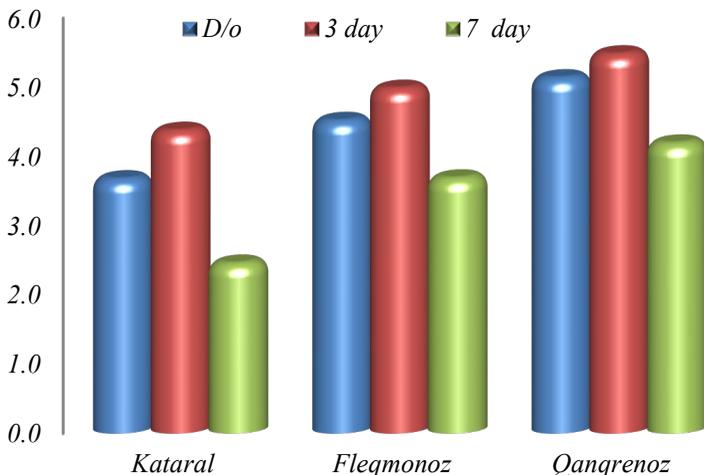
Table 2. Dynamics of change in IL-8 concentration in blood serum in various forms of ACC *(norm – 1 s.v.).*

Dynamic changes observed in endogenic antimicrobial peptides and cytokine profile indicators after surgical intervention on per 3 group follows with the same regularity. But at the end of the research, It is noteworthy that being of endogenic antimicrobial peptides and cytokine profile indicators in gangrenous form of the inflammation in the gallbladder at the end of research far from the norm.

In the following tables 1 and 2, dynamics of change of α-defensine and anti-purulent-inflammatory cytokine IL-8 in blood in per 3 group of patients was issued comparatively.

In general, amount of the CD19$^+$ lymphocytes, every three immunoglobulines in comparison with the indicators of the healthy people in the pre-operative period in patients (A,M,G) and as well as FA are reduced in first 3 days after surgical operation and only after the mentioned period, it is inclined to grow. But, it does not reach norm in 7th day of the research.

In the cataral form of ACC, due to changes revealed in the studied indicators of adaptive humoral immunity in comparison with other forms, in the 7th day of the surgical operation, such indicators

become able to get closer to norm.

In the destructive forms of inflammation in gallbladder, deep changes in adaptive humoral immunity causes to stay very long apart from norm by the ned of study of such indicators in the background of basic conservative therapeutic measures in postoperative period. In the progress of the surgical operation, microbiological researches realized in the bile taken from the gallbladder of 69 patients showed the availability of the bacteriobilia in the bile of 38 of patients $(55,1\pm6,0\%)$. No microbes were revealed in the bile of 31 patients $(44,9\pm6,0\%)$. There was destructive forms of ACC in $39,1\pm5,9\%$ (27 patients) of patients on whom bacteriobilia was revealed. Conducted studies showed that all endogenic antimicrobial peptides studied in blood of patients with bacteriobilia are higher than analogocal indicators of the patients with no microbe in the bile and such difference was continued up to the end of observation. Although TNFα, IL-6 və IL-8 level in blood are in the same level in both group of patients in systematic indicators of cytokine profile in pre-operative period, anti-purulent-inflammatory cytokine IL-10 concentration became 13.7% lower in the patients with bacteriobilia. Amount of TNFα in hepatic tissue and gile of patients with bacteriobilia was not strictly differed from the level of patients with no microbe in bile. But, although amount of IL-6 is 73.9% more in hepatic biopsies in patients with bacteriobilia. in comparison with patients with negative biliculture in bile, it was on the same level in bile. During comparative assessment, it was determined that bacteriobilia negatively affects the adaptive humoral immunity indicators. But, in comparison with patients with no microbe in bile, the amount of OMP in plasm which is endogenic intoxication marker remained in higher levels in whole period of research in patients with bacteriobilia.

The results obtained during studies shows the activation of the congenital humoral immunity in both local and systematic level, on the contrary, the falling of adaptive humoral immunity and reducing of FA in patients during ACC. In the progress of study, several statistical accurate strong direct and contra-correlation relations were determined between endogenic microbial peptides itself, endogenic antimicrobial peptides and cytokine profile and adaptive humoral

immunity indicators. It should be noted that, positive correlation between endogenic antimicrobial peptides and TNFα, IL-6 and IL-8 attracts attention. During purulent-inflammatory processes, due to endogenic antimicrobial peptides and cytokine system function mutually, during ACC, the increase of cytokine profile indicators is accompanied with local level growth of antimicrobial peptides: the increase in IL-6 level in blood stimulates the synthesis of hepsidine, TNFα increases IL-6 synthesis and activates humoral circle of IL-6 circle. Moreover, due to TNFα is an inductor for IL-8, one of active participant of purulent-inflammatory process, strong stimulator of neutrophiles and central mediators of non-specific protection, amount of both cytokines becomes higher in all forms of ACC.

By exclusion of indicators with determined statistical accurate correlation with each other on the basis of conducted ANOVA test and ρ-Spearman analysis, 9 indicators (a-defensine, BPI, endotoxine, elastase, hepsidine, LF, TNFα, IL-6, IL-10) were kept and studies were proceed.

In the further stages, characteristic ROC-curvatures (receiver operating characteristic) were formed in binar classification (there is/ there is no purulent-inflammatory complication in postoperative period) according to sensitivity and specifity indicators (Table 3).
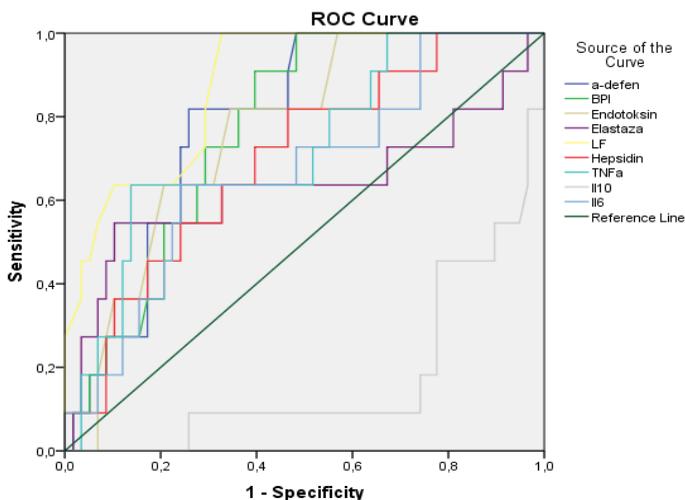
Diagonal segments are produced by ties.

AreaUndertheCurve (Changeness of test results)					
Test Result Variable(s)	Area	Std. Error	Asymptotic Sıg.	Asymptotic 95%Confidence Interval	
				LowerBound	UpperBound
α-defensine	0,786	0,059	0,003	0,670	0,902
BPİ	0,770	0,060	0,005	0,653	0,888
Endotoxine	0,763	0,066	0,006	0,634	0,891
Elastase	0,633	0,115	0,164	0,407	0,859
LF	0,875	0,048	0,000	0,781	0,970
Hepsidine	0,699	0,082	0,037	0,539	0,859
TNFα	0,724	0,083	0,019	0,562	0,886
IL-10	0,172	0,068	0,001	0,038	0,305
IL-6	0,669	0,088	0,077	0,497	0,841

Table 3. Results of ROC-analysis for the studied indicators.

Cut of point was found by using from evidence-based medicinal methods in the further stages on the basis of results of ROC-analysis. By geometrically assessing the farthest point from the reference line as a cutting point, sensitivity and specifity of test was calculated from the mentioned points.

The results obtained during studies shows the activation of the congenital humoral immunity in both local and systematic level, on the contrary, the falling of adaptive humoral immunity and reducing of FA in patients during ACC. In the progress of study, several statistical accurate strong direct and contra-correlation relations were determined between endogenic microbial peptides itself, endogenic antimicrobial peptides and cytokine profile and adaptive humoral immunity indicators.

It should be noted that, positive correlation between endogenic antimicrobial peptides and TNFα, IL-6 and IL-8 attracts attention. During purulent-inflammatory processes, due to endogenic antimicrobial peptides and cytokine system function mutually, during

ACC, the increase of cytokine profile indicators is accompanied with local level growth of antimicrobial peptides: the increase in IL-6 level in blood stimulares the synthesis of hepsidine, TNFα increases IL-6 synthesis and activates humoral circle of IL-6 circle. (J.N.Hajiev et al, 2016, I.P.Danilov et al, 2011). Moreover, due to TNFα is an inductor for IL-8, one of active participant of purulent-inflammatory process, strong stimulator of neutrophiles and central mediators of non-specific protection, amount of both cytokines becomes higher in all forms of ACC.

Our studies did not approve the depressant effect of IL-6 on TNFα claimed by several authors R.M. Khaitov et al, 2011, N.C.Barnes et al, 2011). On the contrary, our studies showed the increase in amount of TNFα in blood serum in the background of high level of IL-6 concentration in all forms of ACC. This status is also approved by strong, direct correlation of IL-6 and TNFα (r=0,25; P<0,040).

Activation of neutrophiles increses the synthesis of defensines by them, and due to defensines are strong chemoattractant for interim circle and immune component cells combining the congenital (non-specific) and adaptive (acquired) immunity, it causes the increase of TNFα, IL-6 and İL-8 in the background of high level of α-defensine in blood in all forms of ACC. Thus, α-defensine by increasing TNFα and IL-8 secretion, it accelerated the development of anti-purulent-inflammatory process. This idea is approved by statistical significant strong positive correlation between α-defensine, TNFα and IL-6.

Due to high level of studied endogenic antimicrobial peptides and cytokines is characteristic for destructive forms of inflammation in gallbladder, such indicators may be used in initial diagnostics of destructive forms of acute cholecystitis as a more informative biomarkers.

In purpose of expression of the forecasting significance of markers studied in further levels by mathematical number, by conducting ANOVA test and calculating the power of affection of per marker in forecasting by Snekedor ratio, 95% upper and lower margins were assessed and the result was visualized as follows :
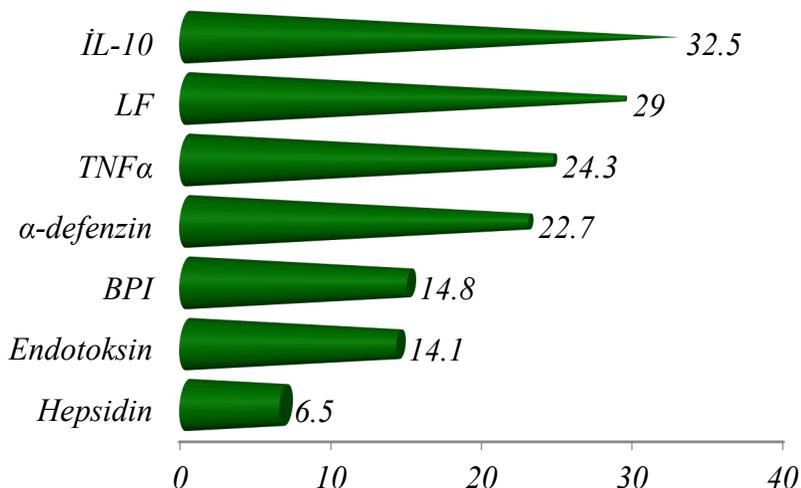
Table 4. Power of affection of markers in forecasting.

The results of conducted studies show that, IL-10(FTG=32,5; 95%EI: 28,5-36,5) and lactoferrine (FTG=29,9; 95% EI: 24,9-33,1) are markers with the greatest power in forecasting and initial diagnostics of formation of postoperative purulent-inflammatory complications in the patients who held cholecystectomy due to acute cholecystitis.

Comparative assessment of biomarkers approved that, inclusion of lactoferrine and α-defensine from antimicrobial peptides and IL-6 and TNFα from cytokines in blood to the program of forecasting and initial diagnostics of the postoperative purulent-inflammatory complications enables to achieve more accurate results.

Thus, studies have shown a decrease in adaptive humoral immunity against local and systemic levels of endogenous antimicrobial peptides and cytokines, which are non-specific factors of congenital humoral immunity during ACC. that it has a negative effect on both congenital and adaptive immunity, that the changes are directly dependent on the form of inflammation in the gallbladder, as well as a number of endogenous antimicrobial peptides and cytokines

in the early diagnosis of destructive forms of cholecystitis, as well as prognosis demonstrated the appropriateness of their use as informative biomarkers.

In the early postoperative period, 14 out of 69 patients (20.2 ± 4.4%) had various complications.

There were no fatalities in the postoperative period. However, local postoperative purulent-inflammatory complications were found in 11 patients (15.9 ± 4.4%): surgical wound infiltrate in 6 patients and purulent ulcer in 5 patients. However, while such a complication in the catarrhal form of ACC was found in only 1 patient, local purulent-inflammatory complications in the phlegmonous form of ACC occurred in 4 patients, and gangrenous ACC in 6 patients.

Thus, the results of the study show once again that the specific gravity of postoperative purulent-inflammatory complications remains high, regardless of the method of cholecystectomy. From this point of view, early diagnosis and adequate treatment are of particular importance in terms of predicting the possibility of such complications in the postoperative period, as well as timely preventive measures.

CONCLUSIONS

1. Activation of congenital humoral circle characterized with increase of amount of α-defensine from endogenic antimicrobial peptides in comparison with the norm of patients with acute calculous cholecystitis plasma-statistically accurate for 2.4 times, BPI- for 6.9 times, endotoxine – for 8.6 times, elastase of neutrophiles – for 3.2 times, lactoferrine- for 2.2 times and hepsidine – for 3.5 times is accompanied with 27.3% decrease of B-lymphocytes, 35,0%, 28,6% and 32,0% of serum immunoglobuline A,M and G correspondingly, 16.2% of phagozytar activity and on the contrary 93.9% of increase of CIC level in humoral circle of adaptive immunity. [1,2,5]

2. Statistically-accurate TNFα concentration increases for 5.4 times, IL-6 – for 7.0 times, IL-8- for 4.4 times and IL-10- for 37.1% in comparison with norm in blood serum in the background of changes formed in the humoral circle of

congenital and adaptive immunity in acute calculous cholecystitis. There are statistically-accurate strong and direct (positive) correlation between endogenic antimicrobial peptides and cytokine profile and adaptive humoral immunity indicators. [4,9,10]

3. Amount of antimicrobial peptides and cytokines in various bio-environments is changed by depending on the form of acute cholecystitis and there are correlations among them. Thus, the increase in the level of endogenic antimicrobial peptides and cytokine profile indicators during acute calculous cholecystitis is directly depended on the form of inflammation in gallbladder. Due to higher concentration of lactoferrine and hepsidine from antimicrobial peptides and TNFα and İL-6 from cytokines concerns the destructive processes in gallbladder, such indicators may play significant role in initial diagnosis of destructive form of acute cholecystitis. [3,6,8,12]

4. tudies performed by using from the evidence-based medicinal methods again proves that, the inclusion of α-defensine and lactoferrine from antimicrobial peptides and TNFα and İL-6 from cytokines as a high informative biomarkers to the program of forecasting and initial diagnosis of postoperative purulent-inflammatory complications enables to achieve more accurate results. [7,9,11,13]

PRACTICAL ADVISES

1. It is advisable to include determination of Concentrations of lactoferrin, hepsidine, tumor necrosis factor (TNFα) and interleukin 6 (IL-6) in the blood to the complex examination measures in purpose of diagnostics of destructive forms of acute cholecystitis in initial period.

2. It is expedient to study the amount of α-defensine, lactoferrine, TNFα and İL-6 in blood as a more informative biomarkers in forecasting and initial diagnostics of possibility of occurrence of purulent-inflammatory complications in this period in the monitoring of postoperative period progress.

List of scientific works published on the dissertation topic

1. Qasımova Ş.X. Kəskin daşlı xolesistitli xəstələrdə bəzi antimikrob peptidlərin səviyyəsinin qiymətləndirilməsi.// 4. International medical congress, Baku, 2-3 november, 2017, p. 77-78.

2. Гаджиев Дж.Н., Гаджиев Н.Дж., Гасымова Ш.Х. Уровень некоторых эндогенных антимикробных пептидов при остром калькулезном холецистите.// Вестник экстренной медицины, Материалы 14-й Республиканской научно-практической конференции «Актуальные проблемы организации экстренной медицинской помощи: Вопросы ургентной хирургии при заболеваниях и травмах гепатопанкреатодуоденальной зоны», Ташкент, 12 декабря, 2017, X(4), с. 51-52.

3. Hacıyev C.N., Hacıyev N.C, Qasımova Ş.X. Akut taşlı kolesistitin formları ile antimikrob peptitler arasındaki bağlantı. // 21. Ulusal Cerrahi Kongresi ve 16. Cerrahi Hemşirelik Kongresi, Antalya, 11-15 Nisan, 2018.

4. Сравнительная оценка содержания некоторых цитокинов и гепсидина в крови и ткани печени у больных острым калькулезным холециститом.// Харківська хірургічна школа, медичний науковопрактичний журнал, Харков, 2018, N 1 (88), с. 31-35

5. Qasımova Ş.X. Kəskin daşlı xolesistitin erkən diqnostikasında laktoferrin və hepsidinin əhəmiyyəti.// Sağlamlıq jurnalı, Bakı 2018 №4 s.63-66.

6. Hacıyev C.N., Qasımova Ş.X. Antimikrob pepdidlər kəskin daşlı xolesistitin destruktiv formalarının erkən diaqnostik meyarı kimi.// A.Ə.Axundbəylinin anadan olmasının 80 illik yubileyinə həsr olunmuş elmi konfransın materialları, Bakı-2018, s. 97-99.

7. Hacıyev C.N., Hacıyev N.C., Qafarov İ.A., Qasımova Ş.X. Kəskin daşlı xolesistlər zamanı əməliyyatdansonrakı irinli-iltihabi ağırlaşmaların baş verməsi ehtimalının proqnozlaşdırılması və erkən diaqnostikası. // Cərrahlıq Jurnalı,

Bakı 2018, №3, s. 33-38.

8. Гаджиев Дж.Н., Гаджиев Н.Дж., Гасымова Ш.Х Определение некоторых ендогенных антимикробных пептидов при остром калькулезном холецистите.// Хірургія Украіни, Кіив 3(67) 2018, с. 25-28.

9. Гаджиев Дж.Н., Гаджиев Н.Дж., Гасымова Ш.Х Антимикробные пептиды и цитокины при остром калькулезном холецистите.// Хирургия журнал им. Н.И.Пирогов, Москва 2018, №10, с. 51-55

10. Qasımova Ş.X. Kəskin daşlı xolesistitli xəstələrdə adaptiv humoral immunitetdə gedən dəyişikliklərin xüsusiyyətləri.// Cərrahlıq Jurnalı, Bakı 2019, №2, s. 75-78.

11. Hacıyev C.N., Hacıyev N.C, Qasımova Ş.X. Bakteriobiliya və antimikrob peptidlər arasında qarşılıqlı əlaqənin xüsusiyyətləri.// Azərbaycan Tibb Jurnalı, Bakı 11-12 may 2019-cu il, s. 102-103..

12. Hajiev J.N., Hajiev N.J., Tagiev E.G., Gasimova Sh.Ch. Correlation between endogenic antimicrobial peptides and cytokine profile indexes in acute calculous cholecystitis// Abstract of the XVIII international euroasian congress of surgery and hepatogastroenterology, Baku, 11-14 September, 2019, p. 169.

13. Гаджиев Дж.Н., Гаджиев Н.Дж., Гасымова Ш.Х., Тагиев Э.Г. Диагностическое значение некоторых эндогенных антимикробных пептидов при деструктивных процессах желчного пузыря //Хирургия Восточная Европа, международный научно-практический журнал, Международная научно-практическая интернет-конференция « Новое в хирургии». Беларусь, г.Минск,1 ноября 2019, с. 28-29.

Conditional abbreviations

OC   – Open cholecystectomy
AMP   – antimicrobial peptides
BPI   – Bactericidal Permeability-Increasing Protein
CIC   – circulating immune complex
CC   – Cholecystectomy
IL-6   – Interleukine 6
IL-8   – Interleukine 8
IL-10 – Interleukine 10
IgA   – Immunoglobuline A
IgG   – Immunoglobuline G
IgM   – Immunoglobuline M
ACC   – acute calculous cholecystitis
APP   – Acute phase proteins
LF   – lactoferrine
LCE   – laparoscopic cholecystectomy
MIC   – Mini-incision cholecystectomy
MMP – Medium molecular peptides
GSD   – Gallstone disease
TNFα – tumor necrotic factor alpha

The defense of the dissertation work will be held in the meeting of ED.2.06 Dissertation Council acting within Azerbaijan Medical University on «*18*» *January* 2022.

Address: A.Gasimzada street, Baku city, AZ1078 (Conference Hall)
You may get acquainted with the dissertation in the library of Azerbaijan Medical University (II floor, Educational building №5 of AMU located at 165, Samad Vurghun street, Baku city)

Electronic versions of dissertation and abstract are posted on official web-site of Azerbaijan Medical University (amu.edu.az)

Abstract was sent to necessary addresses on «*17*» *December* 2021