

## DIAGNOSTIC IMPORTANCE OF CLINICAL MARKERS IN COLORECTAL CANCER

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Materials of 79 patients who were examined and treated with the diagnosis of colorectal cancer and adenomatous polyps at the Oncology Clinic of Azerbaijan Medical University were included in the present study; the control group consisted of 14 healthy adults. The age of the patients varied between 36-65, 58 of them were men, and 21 were women. 10 of the healthy persons were female, and four were male. The concentration of calprotectin and cathelicidin in blood serum was carried out by immunoenzymatic method. The principle of the method is based on the use of specific antibodies against the peptide sequence of the studied antimicrobial peptides. In the research work, it is determined that the concentration of cathelicidin in the blood serum of group I patients is higher than the control limits in all patients. Hence, its concentration increases statistically reliably by 2.2 times compared to the control. The reliability coefficient of the difference is calculated as  $p < 0.001$ . Its average statistical value is  $2.10 \pm 0.09$  pg/ml. The group's minimum value is 1.3 pg/ml, and the maximum value is 2.8 pg/ml.

**Keywords:** calprotectin, cathelicidin, colorectal cancer, AMP, cytokines

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### INTRODUCTION

Colorectal cancer (CRC) remains one of the leading causes of cancer-related morbidity and mortality worldwide. According to global epidemiological data, CRC ranks among the top three most frequently diagnosed malignancies and represents a major public health concern. Despite advances in diagnostic and therapeutic strategies, late detection and tumor progression significantly reduce survival rates. Therefore, identification of reliable, sensitive, and non-invasive biomarkers for early diagnosis and prognosis assessment remains a priority in oncological research [1, 2, 3, 4].

The development and progression of CRC is a complex, multistep process involving genetic, epigenetic, inflammatory, and immunological mechanisms. Activation of oncogenes, particularly members of the Ras gene family, occurs through point mutations and gene amplification. The H-Ras oncogene encodes the p21 protein, which plays a crucial role in intracellular signal transduction of growth factors. Under physiological conditions, p21 is transiently activated and subsequently inactivated after transmitting proliferative signals. However, in malignant transformation, persistent activation of p21 leads to uncontrolled cellular proliferation. In contrast, tumor suppressor genes such as p53 and Rb function as negative regulators of cell cycle progression. Mutation or inactivation of p53 disrupts apoptosis and genomic stability, thereby contributing to tumorigenesis [5, 6, 7].

In recent years, increasing attention has been directed toward the role of immune and inflammatory

mechanisms in colorectal carcinogenesis. Cytokines and antimicrobial peptides (AMPs) are key mediators of innate and adaptive immune responses and play a regulatory role in tumor microenvironment modulation. Chronic inflammation is considered one of the major risk factors for colorectal neoplasia, as it promotes cellular proliferation, angiogenesis, and inhibition of apoptosis.

Among inflammatory mediators, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ) are particularly important. IL-6 is involved in tumor growth, survival signaling pathways, and immune dysregulation, while TNF- $\alpha$  contributes to inflammatory cascades and may either promote or inhibit tumor progression depending on microenvironmental conditions. Alterations in serum concentrations of these cytokines have been associated with disease stage and prognosis in CRC patients.

Antimicrobial peptides, including calprotectin and cathelicidin, represent important components of innate immunity. Calprotectin, a calcium-binding protein complex released primarily by neutrophils and monocytes, is widely used as a marker of intestinal inflammation. Elevated levels of calprotectin have been reported in inflammatory bowel diseases and colorectal malignancies. Cathelicidin (LL-37) exhibits antimicrobial, immunomodulatory, and potentially antitumor properties. However, its role in colorectal cancer remains controversial, as both tumor-promoting and tumor-suppressive effects have been described.

Carcinoembryonic antigen (CEA) is a well-established tumor marker used in clinical practice for monitoring CRC progression and recurrence. Nevertheless, its limited sensitivity in early-stage disease highlights the need for complementary biomarkers [8, 9, 10].

Considering the crucial involvement of inflammatory cytokines and antimicrobial peptides in tumor pathogenesis, comprehensive evaluation of IL-6, TNF- $\alpha$ , calprotectin, and cathelicidin may provide valuable diagnostic and prognostic information in colorectal neoplasia. Therefore, the present study aims to investigate the concentration of selected cytokines and antimicrobial peptides in patients with colorectal cancer and adenomatous polyposis, and to assess their clinical significance using statistical and ROC curve analysis.

## MATERIALS AND METHODS

Determining Calprotectin, fecal Calprotectin, and cathelicidin play great practical importance in the differential diagnosis of benign and malignant derivatives of the bowel. The study of IL-6 and TNF- $\alpha$  inflammatory cytokines and AMP is of great scientific and practical importance in investigating pathogenetic immune mechanisms of CRC and adenomatous polyposis and conducting morphological and clinical-biochemical studies. Complex analysis of biochemical and immunological indicators, cytokines, and AMP during colorectal neoplasia has diagnostic information in determining the histological type and stage of the disease.

In order to evaluate the role of immune system disorders in the pathogenesis of Colorectal cancer and adenomatous polyposis (AP), the concentration of Th-1 type cytokines (IL-6 and TNF- $\alpha$ ) in the blood of the study contingent was examined by immunoenzymatic method. The concentration of TNF- $\alpha$  and IL-6 cytokines in blood serum was carried out using the "sandwich" method using the reagent kit of the company "Vector-Best" (Russian Federation). The principle of the method is based on a one-phase enzyme-linked immunosorbent reaction, and the polyclonal antibodies used are specific for the amino acid sequence of IL-6 and TNF- $\alpha$  peptides, allowing us to determine the investigated peptide chains. During incubation, MAb1 - IL-6 MAb2 (polyclonal biotinylated rabbit antibodies against human interleukins) - HRP complexes are formed by the principle of antigen-antibody. After the incubation period, the wells are washed with a special solution, and the chromogenic solution TMB is added to them. The amount of IL-6 is directly proportional to the intensity of the color of the solution in the cell, and the result is determined using a curve built based on the standards.

The concentration of TNF- $\alpha$  was determined in microplate wells coated with specific human monoclonal antibodies. As a result of the reaction, a sandwich complex is formed between monoclonal antibodies sensitive to TNF- $\alpha$  (MAb1) and monoclonal antibodies labeled with peroxidase (MAb2). Residual antibodies not combined with the labeled enzyme are removed with a washing solution. The intensity of the color formed by the formed complex with a specific chromogenic substance is directly proportional to the concentration of TNF- $\alpha$ . The concentration of TNF- $\alpha$  is calculated based on the standard deviation curve.

The concentration of Calprotectin and cathelicidin in blood serum was carried out by immunoenzymatic method using the reagent kit of the company "Eastbiopharm" (Spain). The principle of the method is based on the use of specific antibodies against the peptide sequence of the investigated antimicrobial peptides and their determination by recognizing various epitopes in the polypeptide chain.

The principle of the calprotectin determination method is based on the reaction of monoclonal antibodies against human Calprotectin and biotinylated calprotectin antibodies with Streptavidin-HRP. During the work, 50 µl of standard solution or serum is added to the wells, and 50 µl of Streptavidin-HRP solution is poured over it and incubated at room temperature for 60 minutes. After washing with a special solution, 50 µl of the chromogenic solution is added. After 10 minutes of incubation at 37 degrees, the reaction is stopped with 50 µl of sulfuric acid. To determine the concentration of Calprotectin, a calibration curve based on the intensity of the color of standard solutions is used. Immunoenzymatic tests were performed on an immunoenzymatic analyzer Stat Fax 303 Plus (USA) ( $\lambda= 450$  nm, differential filter 650 nm).

## RESULTS AND DISCUSSION

The concentrations of IL-6 and TNF- $\alpha$  in the blood serum of all groups of patients included in the study, including patients with Adenomatous polyposis, were studied. It was determined that the concentrations of IL-6 and TNF- $\alpha$  in the blood serum of patients with AP did not change statistically reliably compared to control levels. Thus, in this group, the concentration of TNF- $\alpha$  increased by 25.4% compared to the control and was 1.09 $\pm$ 0.14 pg/ml on average. Its minimum level is 0.11 pg/ml, and its maximum is 2.06 pg/ml. The concentration of TNF- $\alpha$  in 4 patients (16.0%) is higher than the control, and in 21 patients (84.0%), it is lower than the control ( $\chi=2.50$ ). Accordingly, the average concentration of TNF- $\alpha$  in the control group is 0.87 $\pm$ 0.20 pg/ml, and within the group, it varies within the limits of 0.28-3.07 pg/ml (Table 1.1.)

**Table 1.1.** Concentrations of some cytokines and CEA in patients with Adenomatous polyposis

| Parameters            | Groups  |   |
|-----------------------|---|---|
|                       | AP (n=25)   | Control   |
| IL-6, pg/ml           | 2,82 $\pm$ 0,27<br>(0,9-5,0)                        | <b>2,40<math>\pm</math>0,36</b><br><b>(0,1-5,2)</b>   |
| TNF- $\alpha$ , pg/ml | 1,09 $\pm$ 0,14<br>(0,11-2,06)                      | <b>0,87<math>\pm</math>0,20</b><br><b>(0,28-3,07)</b> |
| CEA, ng/ml            | <b>1,41<math>\pm</math>0,19</b><br><b>(0,2-3,8)</b> | <b>1,32<math>\pm</math>0,24</b><br><b>(0,2-3,6)</b>   |

- p<0,001; - p<0,01; - p<0,05

In patients with Adenomatous polyposis, IL-6, TNF- $\alpha$ , and CEA oncomarker concentrations, compared to the corresponding indicators of the control group, did not show a statistically significant difference. In the research work, it is known that a certain increase in the concentration of AMP in the blood serum of patients with Adenomatous polyposis is observed. The density of Calprotectin is higher than the control in 14 patients (56.0%) and lower than the control in 11 patients (44.0%) ( $\chi=12.23$ , p<0.001). In this group, the concentration of Calprotectin increased by 30.9% compared to the indicators of the control group and is 119.6 $\pm$ 5.5 ng/ml on average. This difference is statistically reliable, the coefficient of honesty is calculated as p $\leq$ 0.004. Its minimum level is 79.9 ng/ml, and its maximum is 161 ng/ml. Accordingly, the concentration of CP in the control group varies from 67.8 to 110.6 ng/ml and is 91.4 $\pm$ 4.0 ng/ml.

Our research also determined the concentration of IL-6 in the blood serum of patients included in group I (stage I-II) of patients with Primary colorectal lymphomas. It was higher in 5 patients (17.9%) than in controls and 23 patients (82.1 %); it is lower than the control limits ( $\chi=2.84$ ). The concentration of IL-6 in this group increased by 41.5%, i.e., 1.4 times compared to the corresponding indicators of the control group, and is 3.40 $\pm$ 0.34 pg/ml on average.

In 6 (42.9%) Group II (carcinoid tumors) patients, IL-6 concentration is higher than the control, and in 8 (57.1%), it is lower than the control limits ( $\chi=7.64$ , p<0.01). In this group, the concentration of IL-6

varied between the limits of 0.9-6.8 pg/ml, and the average mathematical indicator was 4.01±0.54 pg/ml (Table 1.2.)

**Table 1.2.** The concentration of some cytokines and CEA in the blood serum of patients with Colorectal cancer

| Parameters   | Groups                               |                                       |                          |
|--------------|--------------------------------------|---------------------------------------|--------------------------|
|              | CRC                                  |                                       | Control                  |
|              | I-II grade(n=28)                     | III-IV grade (n=14)                   |                          |
| IL-6, pq/ml  | 3,40±0,34<br>(0,3-6,1)               | 4,01±0,54<br>(0,9-6,8)                | 2,40±0,36<br>(0,1-5,2)   |
| TNF-α, pq/ml | 1,31±0,11<br>(0,37-2,13)             | 1,65±0,23<br>(0,73-3,0)               | 0,87±0,20<br>(0,28-3,07) |
| CEA, nq/ml   | 2,20±0,23, <sup>^</sup><br>(0,4-4,2) | 2,61±0,30, <sup>^^</sup><br>(0,8-4,3) | 1,32±0,24<br>(0,2-3,6)   |

- p<0,001; - p<0,01; - p<0,05 – compared to control, <sup>^^</sup> - p<0,001; <sup>^^</sup> - p<0,01; <sup>^</sup> - p<0,05 – compared to patients with Adenomatous polyposis

According to the results of the research, the density of CP in group I is higher than the control in 23 patients (82.1%) and lower than the control in 5 patients (17.9%) ( $\chi=25.42$ ,  $p<0.001$ ). In this group, the concentration of CP increased by 64.6%, i.e., 1.7 times, compared to the relevant indicators of the control group, and is 150.4±6.6 ng/ml. In this group, the recorded minimum concentration of CP is 92.6 ng/ml, and its maximum concentration is 206 ng/ml. According to the results of the comparative analysis, the density of CP increases by 25.8%, i.e., 1.3 times, compared to that in patients with AP. The coefficient of the integrity of this difference is calculated as  $p1\leq0.002$ .

The concentration of Calprotectin in the blood serum of patients in group II was higher than control limits in 13 patients (92.0%) and lower than control in 1 patient (8.0%) ( $\chi=24.27$ ,  $p<0.001$ ). Based on the obtained results, the concentration of Calprotectin increases statistically reliably by 3.5 times compared to the control group, and the coefficient of the integrity of this difference is  $p<0.001$ . The average mathematical density of this indicator is 3.42±0.48 pg/ml, the minimum density is 0.7 pg/ml, and the maximum thickness is 6.1 pg/ml.

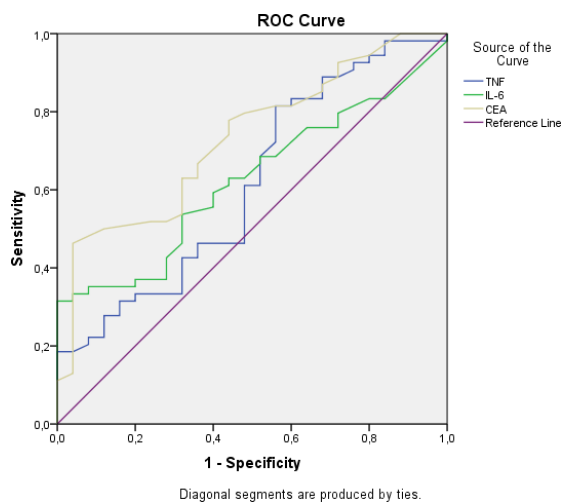
The concentration of CP in the blood serum of IV group (IV stage CRC) patients varies between 119-215 ng/ml and the average mathematical concentration is

169.7±22.3 ng/ml. According to statistical calculations, CP thickness increased by 85.7%, i.e., 1.9 times statistically reliable ( $p<0.001$ ) compared to the control group.

The concentration of cathelicidin in the blood serum of patients in the IV group increased by 70.5%, 1.7 times statistically reliable compared to the control group, and is 108.8±19.6 ng/ml on average. This difference is statistically reliable; the coefficient of honesty is calculated as  $p\leq0.044$ . The concentration of cathelicidin in this group ranges from 57 to 142 ng/ml. A statistically significant increase in the concentration of CEA oncomarker in patients with papillary CRC compared to control by 76.8%, i.e. 1.8 times ( $p\leq0.007$ ), and by 65.4%, i.e. 1.7 times ( $p\leq0.003$ ) compared to patients with AP is an indicator. The concentration of CEA in this group is 2.34±0.18 ng/ml, the minimum hand is 0.4 ng/ml, and the leading indicator is 4.3 ng/ml. In the follicular form, the concentration of CEA increases by 77.2%, i.e., 1.8 times ( $p\leq0.017$ ) compared to the control, and by 65.8%, i.e., 1.7 times ( $p\leq0.020$ ) compared to AP. In the research study, a more significant increase in the concentration of CP, lactoferrin, and defensin is observed among AMP. Thus, the attention of Calprotectin was on average 154.3±5.9 ng/ml, 68.8%, i.e., 1.7 times ( $p<0.001$ ) compared to the control patients, and 29, 0%, that is, it increases with a statistically significant increase of 1.3 times ( $p<0.001$ ).

Based on the ROC curve of TNF-α, it is determined that its area is 0.616, and its standard error is

0.069 ( $p < 0.100$ ). In the 95% confidence interval, the reference indicators of this indicator vary from 0.480 to 0.751. The specificity is calculated based on the ROC curve of IL-6 is 0.624. Its standard error was 0.063 ( $p < 0.077$ ), with an honesty interval ranging from 0.501 to 0.747. CEA has an upper limit of 0.616, a lower limit of 0.845, and a specificity range of 0.731 with a standard error of 0.058 ( $p < 0.00$ ) at the 95% confidence interval of CEA (Figure)



**Figure.** ROC analysis of Calprotectin

Calprotectin is determined based on the ROC curve; its area is 0.776, and the standard error is 0.053 ( $p < 0.001$ ). In the 95% confidence interval, the reference indicators of this indicator vary from 0.672 to 0.879. Cathelicidin's area on the ROC curve is 0.855; its standard error is 0.042 ( $p < 0.001$ ). Cathelicidin reference values range from 0.773 to 0.937 in the 95% confidence interval. The area of the mean is 0.794 and the standard error is 0.050 ( $p < 0.001$ ), with a lower and upper limit of 0.697-892 at the 95% confidence interval.

## CONCLUSION

The present study demonstrates that inflammatory cytokines and antimicrobial peptides play a significant role in the pathogenesis and progression of colorectal neoplasia. Although IL-6 and TNF- $\alpha$  did not show statistically significant differences in patients with adenomatous polyposis compared to the control group, their levels tended to increase with advancing stages of colorectal cancer, reflecting activation of inflammatory and immune mechanisms during tumor progression.

A more pronounced and statistically reliable increase was observed in antimicrobial peptides, particularly calprotectin and cathelicidin. Serum calprotectin levels significantly increased in both early and advanced stages of colorectal cancer compared to controls and patients with adenomatous polyposis, indicating its potential value as a marker of malignant transformation and tumor progression. Similarly, cathelicidin demonstrated a statistically significant elevation, especially in advanced stages, suggesting its involvement in tumor-associated immune modulation.

ROC curve analysis confirmed the high diagnostic performance of calprotectin and cathelicidin, with larger areas under the curve compared to IL-6 and TNF- $\alpha$ . These findings indicate that antimicrobial peptides possess higher specificity and informativeness in distinguishing colorectal cancer from benign conditions and healthy individuals.

Additionally, although CEA remains a clinically established tumor marker, its diagnostic sensitivity may be enhanced when combined with antimicrobial peptides and inflammatory cytokines. Therefore, комплекс evaluation of calprotectin, cathelicidin, IL-6, TNF- $\alpha$ , and CEA may improve early detection, assessment of disease stage, and evaluation of tumor aggressiveness.

The findings of the present study confirm the significant involvement of immune-inflammatory mechanisms in the development and progression of colorectal neoplasia. While IL-6 and TNF- $\alpha$  showed moderate changes, antimicrobial peptides demonstrated more pronounced and statistically significant alterations.

Serum calprotectin and cathelicidin levels increased progressively from adenomatous polyposis to advanced stages of colorectal cancer. ROC analysis revealed higher diagnostic accuracy and specificity for

these antimicrobial peptides compared to inflammatory cytokines.

Thus, calprotectin and cathelicidin may serve as informative, non-invasive biomarkers for early detection, disease staging, and monitoring of colorectal cancer progression. Their combined assessment with traditional tumor markers such as CEA may improve diagnostic sensitivity and clinical decision-making.

In conclusion, antimicrobial peptides, particularly calprotectin and cathelicidin, can be considered promising non-invasive biomarkers for colorectal cancer diagnosis and progression monitoring. Further large-scale studies are recommended to validate their clinical applicability and to explore their potential integration into routine diagnostic protocols.

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