The Crohn's Disease and Proinflammatory Cytokines

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Research Article

Keywords: clinical features of Crohn's disease, Crohn's disease pathogenesis, tumor necrosis factor-α

Posted Date: July 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-753450/v1

Abstract

The aim of the study was to examine the profile of the main proinflammatory cytokines in the serum of patients with Crohn's disease and establish their association with the severity and activity of the disease. A total of 61 patients (29 women (47.5%), 32 men (52.5%) aged from 18 to 40 years (mean age (30.42 ± 2.51) years) with the verified diagnosis of Crohn's disease in the active phase were examined. The control group consisted of 30 virtually healthy people (VHP) of corresponding age. Crohn's disease is characterized by reliable ($p<0.05$) increase of pro-inflammatory cytokines in blood compared to virtually healthy people: TNF-α – by 4.45 times ($p<0.05$), IL-1α – by 5.08 times ($p<0.05$), IL-6 – by 2.16 times ($p<0.05$), IL-8 – by 2.04 times ($p<0.05$), and IFN-γ – by 5.30 times ($p<0.05$), which can be due to the development of the active inflammatory process in the intestine and the systemic reaction of the body. The degree of increase in TNF-α and IFN-γ content, as well as the presence of direct correlations between the Best activity index and the content of these cytokines in the blood of the examined patients, confirm their leading role in the cascade of immune-inflammatory reactions during Crohn's disease.

Introduction

Crohn's disease (CD), like ulcerative colitis (UC), is an inflammatory bowel disease (IBD) with a prevalence ranging from 0.1–58 cases per 100,000 people, depending on the region[1]. More than 2 million people in North America and 3.2 million people in Europe suffer from this disease[2]. The epidemiology of CD has changed over the past decades, demonstrating a trend toward increased prevalence in the developing countries, while in developed countries, its incidence has stabilized[3]. CD is most common among people aged 20 to 30 years[1,2]. The incidence of CD in North America ranges from 0 to 20.2 cases per 100,000 person-years, and that in Europe – from 0.3 to 12.7 cases per 100,000 person-years. The highest incidence of CD among North American countries was recorded in Canada (319 cases per 100,000 population), and among European countries – in Germany (322 cases per 100,000 population).[3] In the United Kingdom, the prevalence of CD increased from 220 to 400 cases per 100,000 population between 2000 and 2017 and amounted to 14.3 person-years per 100,000 population in 2017. Meanwhile, 0.35% of British men and 0.44% of women were diagnosed with CD in 2017[4].

The relevance of CD is not only because its prevalence is increasing annually but also since full-fledged treatment requires significant direct and indirect costs throughout the life of patients, not to mention the psychological and emotional distress of these people and the deterioration of their life quality [5] . For example, in the United States, the average lifetime cost of treating CD is $622,056, of which $273,056 is for outpatient care, $164,298 – for inpatient care, $163,722 – pharmacies, and $20,979 – for the intensive care units [6] .

CD is a heterogeneous inflammatory disease with a multifactorial etiology, including genetic and environmental factors, as well as intestinal microbiota disorders. This disease affects any part of the gastrointestinal tract (GIT) from the oral cavity to the anus and is characterized by chronic, segmental,

and transmural granulomatous inflammation of the GIT with the formation of fistula, abscesses, and stenotic lesions[1,7]. At CD, the small intestine and the large intestine are affected most often[8]. Disorder of the small intestine is observed in 66% of people suffering from this disease. CD with the involvement of the upper gastrointestinal tract disorder occurs in 0.5-4% of cases in the adult population. Typically, lesions of the upper and lower gastrointestinal tracts in CD occur simultaneously.

The etiology of CD is not known exactly, but it is obvious that this disease has a multifactorial nature[8,9]. Currently, the main trigger factors of this disease are believed to be genetic. In particular, more than 30 loci have been identified in the chromosomes that play a direct role in the development of CD. The disease is predominantly associated with the HLA-DR1 haplotype and loci on chromosomes 2 and 6. The role of the HETD2/CARDI5 gene and the OKTN gene has also been studied[10,11].

Among the microorganisms playing a potential role in the development of CD, scientists name Mycobacterium paratuberculosis, i.e., measles virus, but no convincing data confirm them as an etiological factor of this disease[12]. The role of intestinal microflora in the development of CD is also being studied. In this context, the intestinal microbiota is seen as a stimulus, which can lead to a pathological response of the immune system. Such an opinion is related to bacterial endotoxins (lipopolysaccharides, bacterial envelope oligopeptides) that provoke the production of inflammatory mediators, stimulating the migration of cellular elements to the focus of the inflammatory process[9,13].

The importance of stress factors in the development of CD is also not confirmed. However, the practice of clinicians shows that patients with this pathology of the intestine are very sensitive to the action of stress and respond to it with exacerbation of the disease[14]. There is also no consensus among scientists regarding the influence of diet on the occurrence of CD. Nevertheless, this disease is known to be more common in people who eat less natural products and prefer semi-processed foods[9,15].

Autoimmune reactions play a major role in the pathogenesis of CD, since autoantibodies to intestinal mucosal epithelial cells have been detected in such patients[16,17]. Due to the primary increase in the permeability of the intestinal epithelial barrier, in which the cytoskeleton of epithelial cells is damaged, the enterotoxins, i.e., protein substances with antigenic properties, penetrate the bloodstream from the intestine, which leads to sensitization of the body with the production of autoantibodies. A consequence of this is the disruption of the mucosal intestinal system functions like the production of suppressor cytokines and immune areactivity in relation to various mitogens and antigens[17].

In recent years, more and more attention has been paid to the role of various cytokines in the pathogenesis of CD, including tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-2, IL6, IL-8, IL-12, IL-18, and interferon-γ (IFN-γ)[18–20]. By their nature, cytokines are polypeptide molecules with a molecular weight in the range of 5–50 kDa. Their main biological function is the formation and regulation of the body's defense reactions[19, 21]. They are synthesized by almost all cells of the human body, after which they enter the extracellular space, then bind with receptors of corresponding cells, resulting in the initiation of some biological reactions. Cytokines regulate the differentiation and maturation of cells with

immunocompetent properties by stimulating or, on the contrary, suppressing proliferation, migration, secretion, and/or expression of surface receptors and antigens. Besides, cytokines control cytotoxic activity and antibody production[18, 20, 22].

ILs are a subfamily of cytokines being mediator proteins. In CD, proinflammatory cytokines dominate, which stimulate the synthesis of agitrogen monoxide by enterocytes and immune cells, leading to damage of enterocyte cytoskeleton and, as a consequence, an increase in the permeability of the intestinal wall.[18,19] Among proinflammatory cytokines, TNF-α has the greatest significance in the development of CD. Its excess in the body is associated with a number of the following biological processes: activation of T- and B-lymphocytes, neutrophils with the induction of IL-2, INF-γ; activation of macrophages with the induction of IL-2, IL-6 synthesis; activation of free radical synthesis; synthesis of acute-phase proinflammatory proteins in the liver (seromucoid, C-reactive protein, α1-antitrypsin, etc.); development of inflammatory reactions (leukocytosis, sepsis, fever, weight loss); development of endotoxemia; increase in vascular wall permeability with subsequent migration of leukocytes to the inflammation focus; stimulation of adhesion molecules expression on endotheliocytes and leukocytes; inhibition of apoptosis of inflammatory cells. Also, TNF-α together with IL-1 and INF-γ that are involved in granuloma formation. In CD, the concentration of IL-1, IL-2, IL-6, IL-8, and TNF-α sharply increases, while the concentration of anti-inflammatory cytokines (IL4, IL-10, IL-11, etc.) decreases[23].

The significant importance of cytokines in the pathogenesis of CD determines the necessity to perform detailed studies of their content depending on the course, stage, and pathogenesis of the disease. Given the features of the cytokine profile of a particular patient, their examination as predictors of the disease severity and markers of inflammatory process activity would allow developing individual therapy tactics, providing the predictive treatment of CD.

The aim of the study was to investigate the profile of the main proinflammatory cytokines in the serum of patients with Crohn's disease and establish their association with the severity and activity of the disease.

Materials And Methods

A study enrolled 61 patients (29 women (47.5%), 32 men (52.5%) aged from 18 to 40 years (mean age (30.42 ± 2.51) years) diagnosed with Crohn's disease in active phase. The control group consisted of 30 virtually healthy people of the corresponding age. The study was conducted from 2015 to 2019.

Patients who met the following requirements were included in the study: the diagnosis of CD in the active phase (confirmed by the results of colonoscopy and histology); age from 18 to 40 years; a voluntary consent form signed by the patient to participate in the study.

Patients with at least one of the following criteria were excluded from the study: CD in remission stage; age less than 18 years and older than 40 years; other inflammatory diseases of GIT; acute somatic pathology; other chronic somatic pathology in acute or sub/decompensation stage; infectious diseases; cancer pathology of any localization; mental diseases; pregnancy, lactation.

Verification of CD was performed in accordance with 2018 Recommendations of the American College of Gastroenterology[6], and the European Crohn's and Colitis Organization[24], based on the results of colonoscopy and histological examination of biopsy specimens of the affected colon area.

All patients included in the study underwent a detailed interview with an examination of complaints, medical and life history, physical examination, general clinical, biochemical, and instrumental examinations.

Disease activity was determined by calculation of the Best's activity index, which is based on an assessment of clinical manifestations of the disease: frequency of mushy stool, abdominal pain, general condition, other symptoms (including extraintestinal and intestinal complications), abdominal muscle tension, administration of antidiarrheal drugs, hematocrit, body weight. According to Best's activity index grading, clinical remission is indicated by a value of less than 150 points, low activity (mild disease severity) by 150–300 points, moderate activity (moderate disease severity) by 301–450 points, and high activity (severe disease severity) by more than 450 points.

The following laboratory tests were performed: general clinical blood and urinalysis, coprological examination, fecal occult blood test, determination of blood glucose, total protein, albumin and globulin, urea, creatinine, C-reactive protein, total, direct and indirect bilirubin, hepatic transaminases activity, and coagulogram. Blood sampling for the biochemical study was performed from the ulnar vein in the morning on an empty stomach.

The blood content of cytokines (TNF-α, INF-γ, IL-1α, IL-6, IL-8) was determined by enzyme immunoassay (ELISA) using ELISA Kit test systems (Diaclone SAS, France) on Stat Fax 3030 Plus analyzer (USA). Blood cells were incubated in the presence of lipopolysaccharide (as a mitogen), phytohemagglutinin, and concavalin-A, as well as in a culture medium (RPMI-1640) for 24–48 hours at 37°C in a 5% CO2 atmosphere. The samples were then centrifuged for 10 min (400 g), and the supernatant was used for further testing.

All patients underwent obligatory colonoscopy for endoscopic verification of the diagnosis. Each colonoscopy was accompanied by biopsy for histological verification of CD diagnosis. Besides, every patient underwent an ultrasound examination of the abdominal and pelvic organs, electrocardiography, and chest X-ray examination.

Statistical processing of the results was performed using methods of variance statistics, such as Student's t-criterion, Mann-Whitney U-criterion, and Fisher's F- criterion. The correlation analysis was performed using the Spearman method. Microsoft XP Excel (2013) and Statistical Package for the Social Sciences (SPSS) 17.0 were used for statistical data processing.

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The subjects have given their written informed consent and that the study protocol was

approved by the National Committee for Ethics on Human Research of Al-Ahliyya Amman University. Written informed consent was obtained from participants to participate in the study.

Results

The mean age of CD diagnosis in examined patients was (27.75 ± 3.62) years.

The study of the features of CD clinical course showed that the most typical symptoms for the included patients were asthenia-vegetative being observed in 61 people(100.0%), abdominal pain in 55 people (90.2%), flatulence in 46 people (75.4%), diarrhea/frequent stool in 40 people (65.6%), an admixture of mucus and/or blood in stool in 32 people (52.5%), weight loss in 39 people (63.9%), increased body temperature in 37 people (60.7%). Besides, 30 patients (49.2%) with CD had extraintestinal manifestations, among which joint lesions were in 13 (21.3%) patients, skin lesions in 10 patients (16.4%), gallstone disease in 8 patients (13.1%), urolithiasis in 7 patients (11.5%), and eye lesions in 7 patients (11.5%). It should be noted that among 30 patients (49.2%), extraintestinal symptoms with involvement of several organs and organ systems were recorded in 16 patients (53.3%).

When analyzing the Best's activity index, 18 patients (29.5%) corresponded to low disease activity, 37 patients (60.7%) had moderate activity, and high activity of the disease was noted in 6 (9.8%) patients. The mean value of the Best's activity index in patients was (346.90 ± 25.37) points, which corresponds to a medium degree of disease severity. Besides, Best's activity index was higher in persons with a combined involvement of the large and small intestine lesions compared to those with terminal ileitis or isolated involvement of the large intestine.

Compared to virtually healthy people, the study of the proinflammatory cytokines content in the blood of CD patients showed that the level of TNF-α increased 4.45-fold ($p < 0.05$), that of IL-1α – 5.08-fold ($p < 0.05$), IL-6–2.16-fold ($p < 0.05$), IL-8–2.04-fold ($p < 0.05$), and INFγ5.30-fold ($p < 0.05$) (Table 1).

Table 1
Serum proinflammatory cytokine content in Crohn's disease patients, (M ± m)

Indicator	**Virtualy healthy people (n = 30)**	**Crohn's disease (n = 61)**
TNF-α**, pg/ml	30.88 ± 1.65	137.46 ± 7.31*
IL-1α, pg/ml	10.14 ± 0.97	51.55 ± 3.10*
IL-6, pg/ml	9.27 ± 0.40	20.03 ± 1.09*
IL-8, pg/ml	12.62 ± 1.01	25.74 ± 1.34*
INF-γ, pg/ml	39.35 ± 2.54	208.63 ± 7.15*
Note. *The difference is statistically significant in comparison with virtually healthy people ($p < 0.05$);** TNF – tumor necrosis factor; IL – interleukin; INF – interferon.		

It has been established that the blood content of the proinflammatory cytokines increased with higher disease activity (Best's activity index). In particular, TNF-α content in the blood of patients with low cytokines activity was 3.34 times ($p < 0.05$) higher than that in virtually healthy people, with moderate activity – by 4.41 times ($p < 0.05$), with high activity – by 5.70 times ($p < 0.05$), while the content of this cytokine in persons with moderate disease activity was 1.32 times ($p < 0.05$) higher than in persons with low activity ($p < 0.05$), 1.30 times ($p < 0.05$) higher in persons with moderate disease activity, 1.71 times ($p < 0.05$) higher in persons with low activity of the disease (Fig. 1).

A similar trend was observed in the IFN-γ content in the blood. Thus, in persons with low disease activity, this indicator was 4.24 times ($p < 0.05$) higher than that in virtually healthy people, in those with moderate activity – 5.08 times ($p < 0.05$), and with high activity – 5.99 times ($p < 0.05$). At that, IFN-γ content in people with moderate activity was 1.28 times ($p < 0.05$) higher compared to patients with low disease activity, and those with high activity had 1.20 times ($p < 0.05$) higher content compared to moderate activity and 1.54 times ($p < 0.05$) higher compared to low activity.

IL-1α content was 3.65 times ($p < 0.05$) higher in those with low disease activity, 5.00 times ($p < 0.05$) by moderate activity, and 6.14 times ($p < 0.05$) by high activity, respectively. In those with moderate CD activity, this cytokine content was 1.37 times ($p < 0.05$) higher than by low activity ($p < 0.05$) higher than in low activity, and in patients with high activity – 1.27 times ($p < 0.05$) higher than in moderate activity and 1.73 times ($p < 0.05$) higher than in low activity (Fig. 2).

Compared to virtually healthy people, the IL-6 content in the blood of patients with low disease activity was 1.96 times higher ($p < 0.05$), with moderate activity – 2.15 times higher ($p < 0.05$), and with high activity – 2.55 times ($p < 0.05$). The content of IL-8 was 1.68 times ($p < 0.05$) higher, 1.96 times ($p < 0.05$), and 2.39 times ($p < 0.05$) higher, respectively. Statistically significant difference in IL-6 and IL-8 content was noted only between patients with low and high disease activity ($p < 0.05$). In particular, individuals with high CD activity had 1.31 times ($p < 0.05$) higher IL-6 blood content compared to patients with low disease activity, and a 1.43fold ($p < 0.05$) higher IL-8 content compared to patients with low disease activity, respectively.

The following correlations have been established: between the Besta activity index and the content of TNF-α ($r = 0.84$, $p < 0.05$), INF-γ ($r = 0.61$, $p < 0.05$); between TNF-α and INFγ content ($r = 0.67$, $p < 0.05$), IL-1α ($r = 0.49$, $p < 0.05$), IL-6 ($r = 0.40$, $p < 0.05$), and IL8 ($r = 0.51$, $p < 0.05$); INF-γ and IL-1α ($r = 0.53$, $p < 0.05$), IL-6 ($r = 0.37$, $p < 0.05$), IL-8 ($r = 0.44$, $p < 0.05$); IL-1α and IL-6 ($r = 0.55$, $p < 0.05$), IL-8 ($r = 0.36$, $p < 0.05$); IL-6 and IL-8 ($r = 0.60$, $p < 0.05$).

Discussion

According to the results of the study, the age of CD diagnosis in examined patients was quite late and amounted to (27.75 ± 3.62) years, which coincides with the data of other studies[25,26]. The patients

included in the study demonstrated typical clinic features of CD with a predominance of astheno-vegetative and abdominal pain syndromes, stool disorders, weight loss, etc. However, rather a large part of patients (49.2%) showed extraintestinal lesions, of which the damage of joints as arthropathies (in 13 patients or 21.3%) and skin lesions mainly as erythema nodosum (in 10 patients or 16.4%) were the most common. The development of skin lesions in CD patients is associated with the TRAF3IP2 gene and HLA-B*27, HLA-B*58, and HLADRB1*0103 antigens[27]. In general, the occurrence of extraintestinal lesions in CD is associated with the ability of the intestinal mucosa to induce an immune response in extraintestinal sites, which is due to the presence of common epitopes in intestinal microorganisms, particularly in the synovial membrane. That is, an adaptive immune response is stimulated as a result of the bacteria movement through the intestinal barrier, which had a high permeability[1,28]. Hence, the results of this study regarding the main clinical manifestations of CD and extraintestinal lesions coincide with the available literature findings[7,28,29].

According to the results of this study, the CD was characterized by a significant increase ($p < 0.05$) of such pro-inflammatory cytokines as TNF-α, INF-γ, IL-1α, IL-6 and IL-8 in the blood content, whose values varied within a fairly wide range (see Table 1). Such high content is attributed to the development of an active inflammatory process in the intestine and a systemic reaction of the body, which is confirmed by the presence of direct correlations between the Best's activity index and the content of TNF-α ($r = 0.84$, $p < 0.05$) and IFN-γ ($r = 0.61$, $p < 0.05$), as well as direct correlations between all the proinflammatory cytokins studied. In the author’s opinion, this is an evidence of their synergistic effects, leading to excessive proinflammatory response, disorders of regulatory mechanisms and, as a consequence, chronicity of the disease.

Compared to virtually healthy people, the increase in TNF-α content is 4.45 times ($p < 0.05$), that of IFN-γ – 5.30 times ($p < 0.05$), and IL-1α – 5.08 times ($p < 0.05$) was the most significant, which is comparable to the results of studies by other scientists. This increase in TNF-α and IFN-γ content in the examined patients confirms their leading role in the cascade of immune-inflammatory reactions during CD. In particular, being synthesized by macrophages, monocytes, T- and B-lymphocytes, detritus cells, neutrophils, keratinocytes, and endotheliocytes, TNF-α has a wide range of biological effects. Thus, it activates proliferation of fibroblasts and lymphocytes; increases the expression of adhesion molecules (both cellular and vascular) necessary for migration of lymphocytes to the inflammatory zone; stimulates the synthesis of leukotrienes, prostaglandins, matrix metalloproteinases, nitrogen monoxide; decreases body weight.[17,19] At CD, TNF-α directs circulating inflammatory cells to its focus, resulting in edema formation. This cytokine also initiates coagulation processes and participates in granuloma formation.[19] An increase in TNF-α content in blood leads to an increase in production of other pro-inflammatory cytokines, which is confirmed by the presence of direct correlations between TNF-α content in blood and IFF-γ ($r = 0.67$, $p < 0.05$), IL-1α ($r = 0.49$, $p < 0.05$), IL-6 ($r = 0.40$, $p < 0.05$), and IL-8 ($r = 0.51$, $p < 0.05$).

As shown in this study, another cytokine playing an important pathogenetic role in the development of CD is INF-γ, also called immune interferon. This cytokine is produced by T-lymphocytes (CD4 + and CD8+)

and NK cells. IFN-γ is involved in the stimulation of Tx0 to Tx1, maintenance of Tx1/Tx2 balance, regulation of cellular and humoral immune response (it enhances cellular immunity development while inhibiting its humoral link), mediates the relationship between lymphocytes and macrophages, perform antiviral and anti-tumor activity[17,19,22].

The patients with CD included in the study were characterized by a significant increase in IL-1α content (by 5.08 times ($p < 0.05$) compared to virtually healthy people), but no correlation between this cytokine content in blood and disease severity (Best activity index) has been established. The increase of IL-1α in serum during CD can be explained by the fact that this cytokine is one of the main inflammation mediators, but less specific concerning the disease under study. The IL-1α is necessary for the activation of T-cells during their interaction with antigen being the main mediator of short-distance activity (it remains inside the cell or can have a membrane form, as well as may appear only in insignificant amounts in the extracellular space)[19].

The increase of IL-6 and IL-8 content in the blood was less significant (Table 1). Besides, no correlation between the content of the indicated cytokines and the disease severity (Best's activity index) was found, which may indicate their secondary role in the CD pathogenesis.

Conclusions

Thus, the clinical course of Crohn's disease was most characterized by astheno-vegetative (in 100.0% of patients) and abdominal pain syndrome (in 90.2% of patients), stool disorders (in 65.6% of patients), weight loss (in 63.9% of patients). Among extraintestinal manifestations, the most frequent were joint lesions (in 21.3% of patients) and skin lesions (in 16.4% of patients). Crohn's disease was characterized by a significant ($p < 0.05$) increase (in comparison with practically healthy individuals) in the content of proinflammatory cytokines in the blood: TNF-α – 4.45 times ($p < 0.05$), IL-1α – 5.08 times ($p < 0.05$), IL-6– 2,16 times ($p < 0.05$), IL8 2.04 times ($p < 0.05$), INF-γ – 5.30 times ($p < 0.05$), which is caused by development of active inflammatory process in intestine and systemic reaction of the body. Blood content of proinflammatory cytokines in the examined patients increased with the increase of the disease activity (Best's activity index). The degree of increase in TNF- α and INF-γ content as well as the presence of direct correlation links between the Best activity index and the content of the above cytokines in the blood of the examined patients confirm their leading role in the cascade of immune-inflammatory reactions in Crohn's disease.

Prospect for further research. The prospect for further research is to study the quantitative and qualitative composition of the intestinal microbiome in patients with Crohn's disease, as well as the possibility of using probiotic drugs in the complex treatment scheme for this disease.

Abbreviations

CD – Crohn's disease

GIT – gastrointestinal diseases

Declarations

Acknowledgments

Not applicable.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Author Contributions

AQ, KK, and DB contributed equally to the experimentation. AQ and KK wrote and edited the article. DB and KK equally designed and conducted the experiment. AQ and DB studied scientific literature about the topic. All authors read and approved the final manuscript.

Data availability

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The research was conducted ethically in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study protocol was approved by the National Committee for Ethics on Human Research of Al-Ahliyya Amman University. Written informed consent was obtained from participants to participate in the study.

Consent for publication

Consent for publication was obtained from all individual participants included in the study.

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Figures

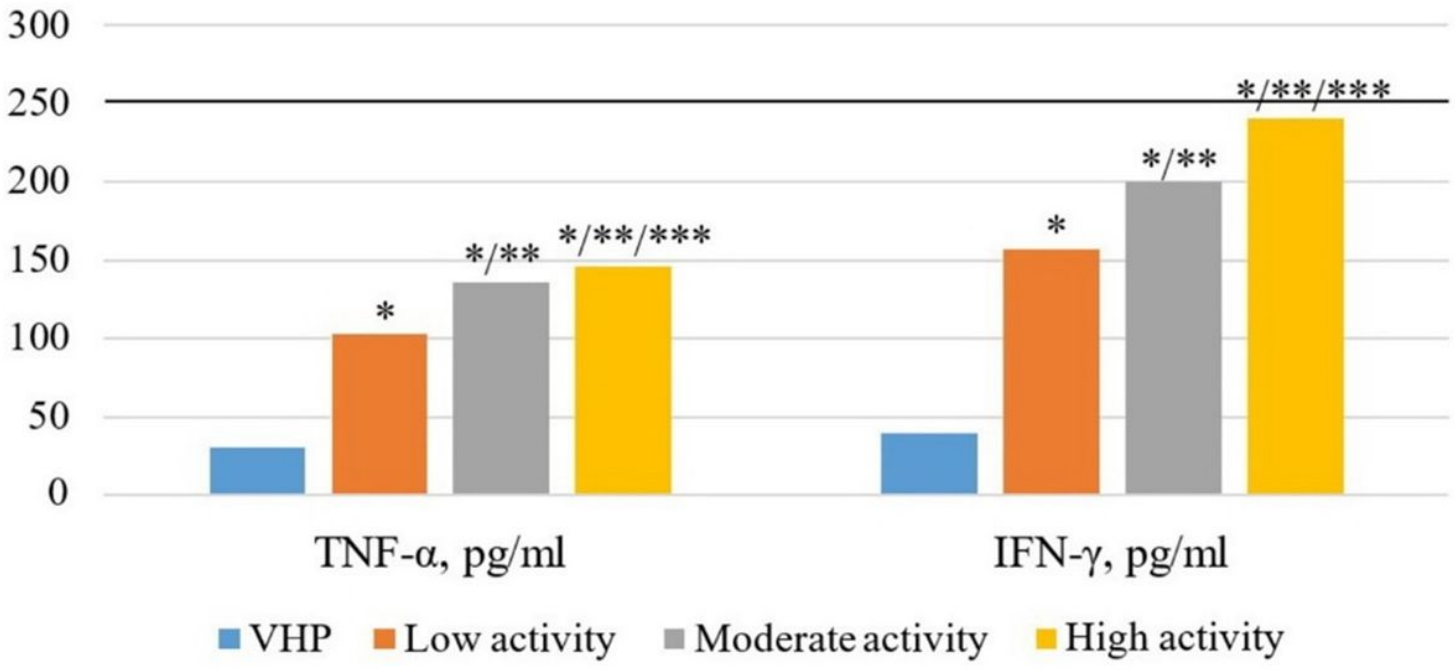

Figure 1

TNF-α (pg/ml) and IFN-γ (pg/ml) content in the serum of patients with Crohn's disease depending on the disease activity. Note. * the difference is statistically significant compared to virtually healthy people ($p < 0.05$); ** the difference is statistically significant compared to the indicator with low disease activity ($p < 0.05$); *** the difference is statistically significant compared to the indicator with moderate disease activity ($p < 0.05$).

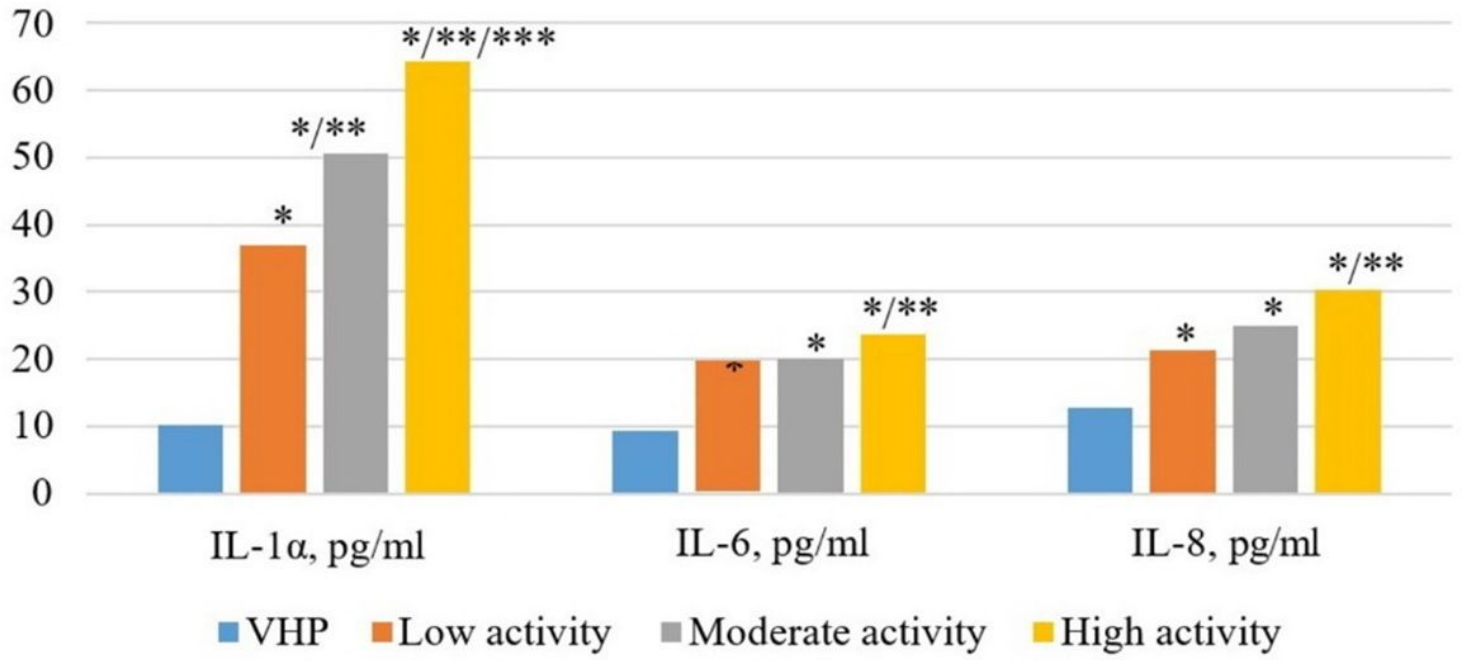

Figure 2

Serum levels of IL-1α (pg/ml), IL-6 (pg/ml), and IL-8 (pg/ml) in patients with Crohn's disease depending on the disease activity. Note. * - the difference is statistically significant compared to virtually healthy people ($p < 0.05$); ** - the difference is statistically significant compared to the indicator with low disease activity ($p < 0.05$); *** - the difference is statistically significant compared to the indicator with moderate disease activity ($p < 0.05$).