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IMMUNE REACTIVITY WHEN USING SAFFLOWER AND COTTONSEED OIL IN CONDITIONS OF EXPERIMENTAL ACUTE TOXIC HEPATITIS IN RATS

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XULOSA

Immun mexanizmlar toksik gepatit patogenezida asosiy rol o‘ynaydi, samarali gepatoprotektorlarni izlash esa eksperimental tibbiyotning muhim vazifasi bo‘lib qolmoqda.

Maqsad. *Kalamushlarda eksperimental o‘tkir toksik gepatit sharoitida immunologic markerlar dinamikasini baholash va saflor hamda paxta moyining immunomodulyator ta’sirlarini taqqoslash.*

Materiallar va usullar. *Tajriba paratsetamol yordamida o‘tkir toksik gepatit chaqirilgan 20 ta oq erkak kalamushda o‘tkazildi; so‘ngra ularning bir qismida saflor, ikkinchisida esa paxta moyi bilan tuzatish ishlari olib borildi.*

Natijalar. *Nazorat guruhida IL-1β darajasi intact hayvonlarga nisbatan 1,73 marta oshdi (13,3±1,59 ga qarshi 7,7±0,08 pg/ml, p<0,05), saflor moyi esa bu ko‘rsatkichni 18% ga kamaytirdi (11,3±0,98 pg/ml). TNF-α konsentratsiyasi nazorat guruhida normal qiymatdan 3,47 marta yuqori bo‘ldi (38,6±5,42 ga qarshi 11,1±1,29 pg/ml, p<0,05), biroq saflor moyi bilan davolashda 36% ga kamaydi (23,5±2,72 pg/ml). VEGF-A darajasi nazorat guruhida 125,6±39,2 pg/ml gacha oshdi (normadan 8,6 marta yuqori), saflor moyi esa un iikki martadan ortiq pasaytirdi – 53,0±12,3 pg/ml gacha (p<0,05).*

Xulosa. *Saflor moyi paxta moyiga nisbatan kuchliroq immunomodulyator va yallig‘lanishga qarshi ta’sir ko‘rsatdi, sitokin muvozanatini normallashtirdi va jigar regeneratsiyasini qo‘llab- quvvatladi.*

Kalit so‘zlar: *o‘tkir toksik gepatit, saflor moyi, paxta moyi, immunmarkerlar, sitokinlar.*

РЕЗЮМЕ

Иммунные механизмы играют ключевую роль в патогенезе токсического гепатита, а поиск эффективных гепатопротекторов является важной задачей экспериментальной медицины.

Цель исследования. *Изучить динамику иммунологических показателей и сопоставить иммуномодулирующее действие сафлорового и хлопкового масел при остром токсическом гепатите у крыс.*

Материалы и методы. *Эксперимент проведён на 20 белых крысах-самцах, у которых модель острого токсического гепатита вызывали парацетамолом, с последующей коррекцией сафлоровым или хлопковым маслом.*

Результаты. *У животных контрольной группы уровень IL-1β увеличился в 1,73 раза по сравнению с нормой (13,3±1,59 против 7,7±0,08 пг/мл, p<0,05), тогда как при применении сафлорового масла показатель был на 18% ниже контроля (11,3±0,98 пг/мл). Концентрация TNF-α в контроле превышала норму в 3,47 раза (38,6±5,42 против 11,1±1,29 пг/мл, p<0,05), но на фоне сафлорового масла снижалась на 36% по сравнению с контролем (23,5±2,72 пг/мл). Уровень VEGF-A в контроле вырос до 125,6±39,2 пг/мл (в 8,6 раз выше нормы), тогда как при коррекции сафлоровым маслом он снижался более чем в 2 раза – до 53,0±12,3 пг/мл (p<0,05).*

Вывод. *Сафлоровое масло оказывает более выраженный иммуномодулирующий и противовоспалительный эффект по сравнению с хлопковым маслом, способствуя нормализации цитокинового профиля и регенерации печени.*

Ключевые слова: *острый токсический гепатит, сафлоровое масло, хлопковое масло, иммунные маркеры, цитокины.*

The immune system plays a key role in the pathogenesis of toxic hepatitis, determining the degree of inflammatory response and the rate of liver recovery. Damage to hepatocytes leads to activation of both innate and adaptive immunity, which is accompanied by changes in the levels of pro- and anti-inflammatory cytokines. Studying these processes in experimental models allows for a deeper understanding of the mechanisms of the im-

mune response to liver damage. Particular attention is paid to studying the effects of various hepatoprotective agents on immunological parameters. Safflower oil, due to its antioxidant and immunomodulatory properties, is a promising agent for correcting the immune response in toxic hepatitis [3,5,8,12].

Evaluation of the dynamics of immunological markers during treatment allows us to identify the optimal timing of therapeutic effects and predict the effectiveness of liver restoration. The levels of key cytokines, the activity of lymphocytes and the phagocytic system reflect the balance between inflammatory and regenerative processes. Comparative study of safflower oil and other oils, such as cottonseed oil, makes it possible to determine the advantage of one or another vegetable oil. These data are especially important for developing therapy strategies aimed at minimizing damage and restoring liver function. In addition, assessment of the immune response helps to identify potential side effects and correlate them with clinical safety [1, 6, 9, 11].

Integration of immunological data with biochemical and antioxidant indicators allows for a comprehensive assessment of the effectiveness of therapy. Timely regulation of pro- and anti-inflammatory processes is an important condition for the successful treatment of toxic hepatitis [2, 4, 7, 10].

Conducting studies on rats allows for control of experimental conditions and accurate quantitative data on the dynamics of the immune response. These studies serve as the basis for subsequent translation of the obtained results to clinical models. Ultimately, studying the effect of safflower oil on immune mechanisms allows for the development of substantiated approaches to the treatment and prevention of toxic liver damage.

THE AIM OF THE STUDY

To evaluate the dynamics of immunological parameters and determine the differences in the immunomodulatory effects of safflower and cottonseed oils in experimental acute toxic hepatitis in rats caused by paracetamol.

MATERIALS AND METHODS OF RESEARCH

The study involved 20 healthy male outbred white laboratory rats aged 2-3 months with a body weight of 203.1±1.32 g. Before the experiment, the animals underwent a 10-14-day quarantine under standard vivarium conditions. The animals were kept at an air temperature of +20.2 ± 5 °C, relative humidity of 55 ± 10% and a 12:12 light regimen, with access to standard feed (water) and specialized laboratory nutrition.

After acclimatization, all animals were randomly assigned to groups using a random number generator (simple randomization method). Treatment assignment (control, toxic model, safflower oil) was based on a pre-compiled list. The study was conducted under partial blinding conditions: the investigator assessing the biochemical and antioxidant parameters was not aware of the group assignment of the animals.

The animals were divided into four groups:

I – intact (healthy animals), (n=5);

II – control group (OTG without treatment), (n=5);

III – group (OTG + safflower oil 10 ml/kg), (n=5);

IV – group (OTG + cottonseed oil 10 ml/kg), (n=5).

Acute toxic hepatitis was induced by intragastric administration of an aqueous solution of paracetamol at a dose of 1000 mg/kg using a tube once a day for two days. Beginning on the third day, the animals of the protective groups were administered the test oil (obtained by the cold-pressed method, manufactured by Botanic Herbs Company LLC) at a dose of 10 ml/kg using a syringe dispenser every day, while the control and model groups received an equivalent volume of physiological solution.

To determine the concentration of IL-1β, IL-4, IL-6, TNFα, VEGF-A, βKlotho in the blood serum of the study groups, a three-stage “sandwich” method was used - this is a type of three-phase ELISA “Vector-Best” (Novosibirsk, Russian Federation), (βKlotho Abbexa, USA).

The study was approved by the Ethics Committee of the Institute of Human Immunology and Genomics of the Academy of Sciences of the Republic of Uzbekistan (protocol No. 2025-0001) and was carried out in accordance with the principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes of 2010.

Statistical processing of the obtained data was performed on a personal computer using the Student’s t-test and the standard software package “Microsoft Excel”. The arithmetic mean (M), standard deviation (m), standard error (m), Student’s t-test (t) with the calculation of the probability of error (p) were calculated.

Differences in mean values were considered reliable at a significance level of $p<0.05$. In this case, existing guidelines for statistical processing of clinical and laboratory research data were followed.

RESEARCH RESULTS

At the early stage of toxic hepatitis, a pronounced immune response is formed, which is determined by the balance of pro- and anti-inflammatory cytokines, the activity of lymphocytes and the phagocytic system. The seventh day of the experiment is critical for assessing the initial response of the body to liver damage and therapeutic effects. A comparative analysis of the effect of safflower and cottonseed oil on key immunological parameters was carried out. Changes in cytokine levels (IL-1β, IL-4, IL-6, TNFα, VEGF-A, βKlotho) were considered. These data allow us to assess the initial effectiveness of therapy and determine the potential of safflower oil as an immunomodulator in toxic hepatitis (Table).

IL-1β is a key proinflammatory cytokine involved in the activation of macrophages and enhancement of the inflammatory cascade in toxic hepatitis. In the control group, its level (13.3±1.59 pg/ml) was 1.73 times higher than that of intact animals (7.7±0.08 pg/ml) ($p<0.05$), reflecting the development of a pronounced inflammatory reaction. In group III, which received safflower oil, the IL-1β concentration was 11.3±0.98 pg/ml, which was

1.47 times higher than the norm and 1.18 times lower than the control value, indicating a moderate decrease in inflammatory activity. In group IV (cottonseed oil), the indicator was 13.1±0.95 pg/ml, 1.70 times higher than the norm and was practically no different from the control ($p>0.05$), indicating the absence of a significant anti-inflammatory effect (Fig. 1). The data obtained suggest that the decrease in IL-1β in the safflower oil group is associated with suppression of NF-κB activation and a decrease in cytokine production, while cottonseed oil did not affect this regulatory pathway.

The effect of the studied samples of safflower and cottonseed oil on immunological parameters in rats with acute toxic hepatitis on day 7, (M±m; n=20), pg/ml

Indicators	IL-1β	IL-4	IL-6	TNFα	VEGF-A	βKlotho
Intact (Healthy) (n=5)	7.70±0.08	1.19±0.27	2.57±0.02	11.1±1.29	14.6±5.28	2.84±0.88
Control (water) (n=5)	13.3±1.59	4.06±0.43	4.57±0.32	38.6±5.42	125.6±39.2	56.5±26.4
Group III (n=5)	11.3±0.98	2.61±0.49	3.44±0.12	23.5±2.72	53.0±12.3	12.7±1.68
IV group (n=5)	13.1±0.95	3.74±0.49	3.62±0.23	28.5±2.53	104.9±25.3	14.8±3.30

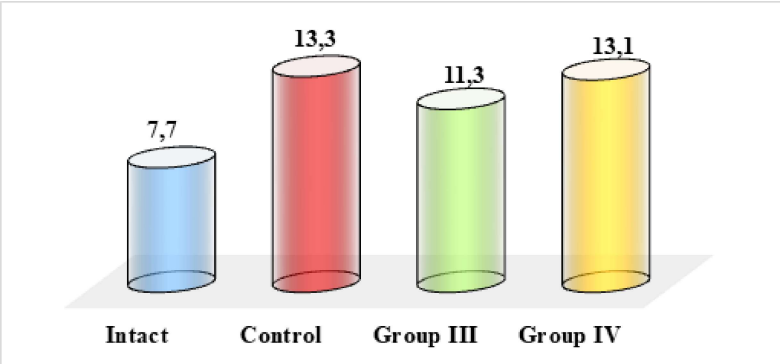

Fig. 1. Interleukin 1 β level in rat blood serum (pg/ml)

IL-4 is an anti-inflammatory cytokine that regulates the balance of the Th1/Th2 response and helps suppress the production of proinflammatory mediators. In the control group, its level (4.06±0.43 pg/ml) exceeded the level in intact animals (1.19±0.27 pg/ml) by 3.41 times ($p<0.05$), indicating compensatory activation of the Th2 component of immunity in toxic hepatitis. In group III treated with safflower oil, the IL-4 concentration was 2.61±0.49 pg/ml, which is 2.19±0.49 times higher than the norm, but 1.55 times lower than the control value ($p<0.05$), reflecting a moderate decrease in the activity of the Th2 response. In group IV (cottonseed oil), the indicator was 3.74 pg/ml, 3.14 times higher than the norm and only 1.09 times lower than the control, indicating the absence of a pronounced effect (Fig. 2). The decrease in IL-4 in the safflower oil group is probably associated with modulation of the cytokine profile due to polyunsaturated fatty acids that stabilize the membranes of immunocompetent cells.

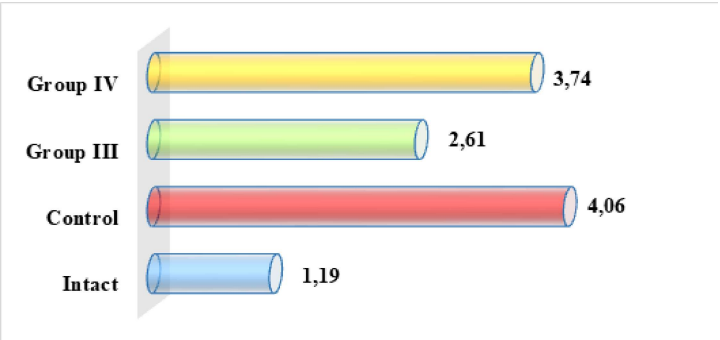

Fig. 2. Interleukin 4 level in blood serum of rats (pg/ml)

IL-6 is a pleiotropic proinflammatory cytokine that plays a key role in the development of a systemic inflammatory response and stimulation of acute phase protein synthesis in liver damage. In the control group, its level (4.57±0.32 pg/ml) was 1.78 times higher than in intact animals (2.57±0.02 pg/ml) ($p<0.05$), reflecting the acti-

vation of inflammatory mechanisms in toxic hepatitis. In group III, which received safflower oil, the IL-6 concentration was 3.44±0.12 pg/ml, 1.34 times higher than the norm and 1.33 times lower than the control value, indicating a pronounced anti-inflammatory effect.

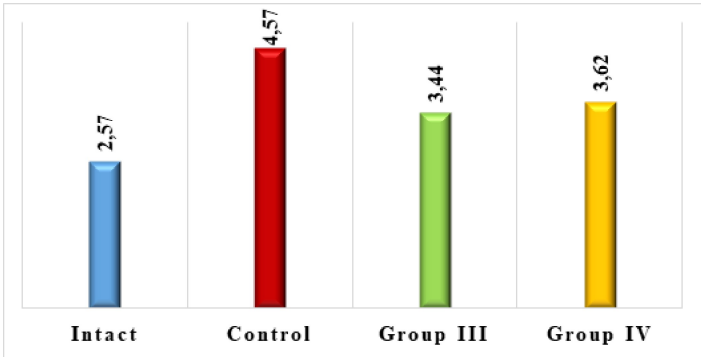

Fig. 3. Interleukin 6 level in blood serum of rats (pg/ml)

In group IV (cottonseed oil), the cytokine level was 3.62±0.23 pg/ml – 1.41 times higher than the norm and 1.26 times lower than the control ($p<0.05$), indicating a less pronounced decrease in inflammatory activity (Fig. 3). The results indicate that safflower oil more effectively corrects IL-6 hyperproduction, probably due to the higher content of linoleic acid and antioxidant components.

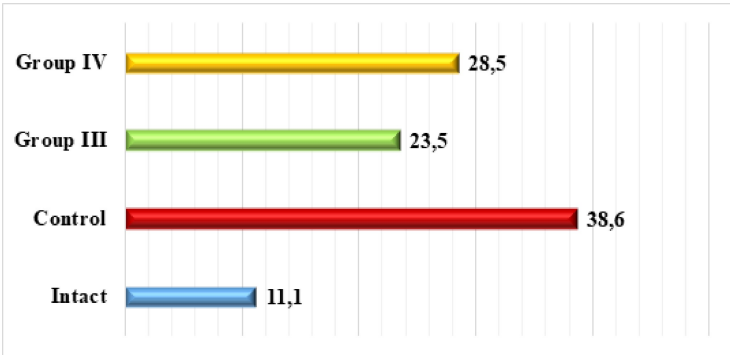

Fig. 4. TNFα level in blood serum in rats (pg/ml)

TNF-α is a key mediator of the systemic inflammatory response involved in the induction of hepatocyte necrosis and activation of the cascade of proinflammatory cytokines in toxic liver damage. In the control group, its concentration (38.6±5.42 pg/ml) exceeded the level in intact animals (11.1±1.29 pg/ml) by 3.47 times ($p<0.05$), which reflects pronounced activation of inflammatory and cytotoxic processes. In group III, which received safflower oil, the indicator decreased to 23.5±2.72 pg/ml, which is 2.12 times higher than the norm, but 1.64 times lower than the control value ($p<0.05$), indicating a significant anti-inflammatory effect. In Group IV (cottonseed oil), the TNF-α level was 28.5±2.53 pg/ml, 2.57 times higher than normal and 1.35 times lower than the control, demonstrating a moderate reduction in inflammation (Fig. 4). These data show that safflower oil is more effective in suppressing TNF-α hyperproduction, probably due to its higher content of polyunsaturated fatty acids and antioxidant activity.

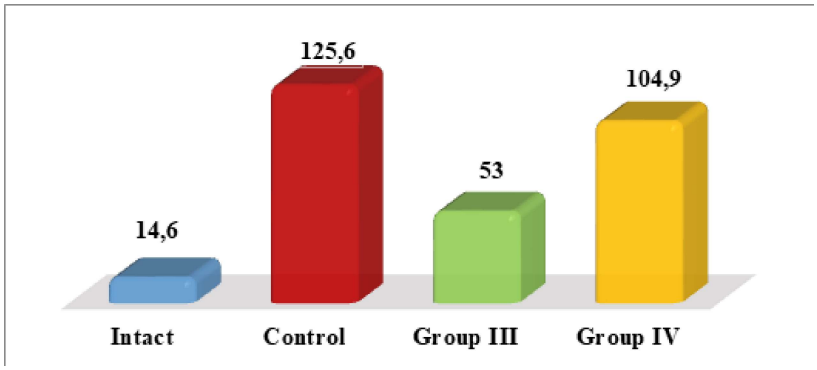

Fig. 5. The level of vascular growth factor in the blood serum of rats (pg/ml)

VEGF-A is a vascular endothelial growth factor that regulates angiogenesis and vascular permeability, which is key in liver regeneration, but in toxic hepatitis its overexpression can increase edema and inflammation. In the control group, the VEGF-A level (125.6±39.2 pg/ml) was 8.6 times higher than in intact animals (14.6±5.28 pg/ml) ($p<0.05$), which reflects excessive activation of angiogenic processes against the background of damage. In group III (safflower oil), the indicator was 53±12.3 pg/ml - 3.63 times higher than the norm, but 2.37 times lower than the control ($p<0.05$), indicating normalization of the vascular response. In group IV (cottonseed oil), the VEGF-A level reached 104.9±25.3 pg/ml, 7.19 times higher than normal and only 1.2 times lower than the control ($p<0.05$), which indicates a less pronounced therapeutic effect (Fig. 5).

Thus, safflower oil more effectively limits pathological hyperproduction of VEGF-A, promoting controlled regeneration without excessive inflammatory angiogenesis.

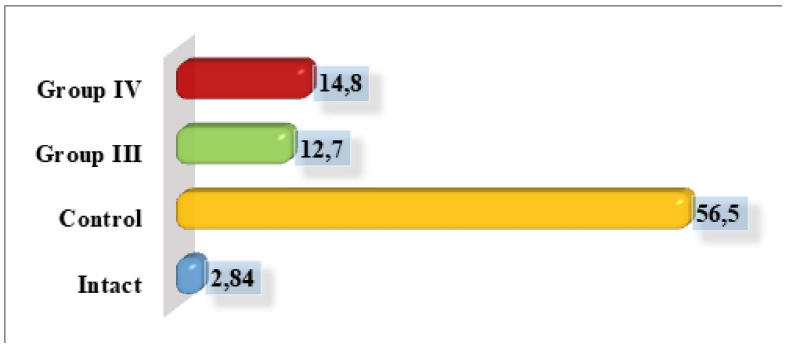

Fig. 6. Concentration of Klotho protein in blood serum in rats (pg/ml)

βKlotho is a membrane cofactor of FGF receptors involved in the regulation of energy metabolism, antioxidant protection and liver regeneration processes. In the control group, the βKlotho level (56.5±26.4 pg/ml) exceeded the values of intact animals (2.84±0.88 pg/ml) by 19.89 times ($p<0.05$), which reflects compensatory hyperexpression of the protein against the background of toxic damage to hepatocytes. In group III (safflower oil), the concentration was 12.7±1.68 pg/ml - 4.47 times higher than the norm and 4.45 times lower than the control ($p<0.05$), indicating a decrease in excessive activation of regenerative pathways. In group IV (cottonseed oil), the value reached 14.8±3.30 pg/ml, which is 5.21 times higher than the norm and 3.82 times lower than the control ($p<0.05$), indicating a partial but less pronounced correction compared to group III (Fig. 6). These data confirm that safflower oil has a more balanced effect on βKlotho expression, preventing excessive response and promoting physiological regeneration.

Thus, the conducted analysis showed that in toxic hepatitis there is a significant increase in the levels of proinflammatory cytokines (IL-1β, IL-4, IL-6, TNFα) and angiogenesis mediators (VEGF-A), as well as βKlotho compared to intact animals, which reflects a pronounced systemic inflammatory and regenerative response. The most dramatic changes were observed in the control group without correction, where the indicators exceeded the initial values \u200b\u200bseveral times. The use of the studied oils contributed to a decrease in the excessive production of cytokines and VEGF-A, while safflower oil showed a more pronounced normalizing effect than cottonseed oil. The correction was also accompanied by a decrease in βKlotho hyperexpression, which may indicate a more balanced inclusion of liver recovery mechanisms. Thus, the obtained results confirm the pathogenetic significance of the studied markers and the effectiveness of safflower oil for modulating inflammatory and regenerative processes in toxic hepatitis.

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УДК: 616.24-007

ЦИТОКИНОВЫЙ ПРОФИЛЬ ПРИ ХОБЛ: ЗНАЧЕНИЕ ИЛ-8 И ИЛ-18 В СИСТЕМНОМ ВОСПАЛЕНИИ И СТРАТИФИКАЦИИ ТЯЖЕСТИ

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XULOSA

Maqsad. *O’pka surunkali obstruktiv kasalligi (O‘SOK) bilan kasallangan bemorlarning zardobidagi IL-8 va IL-18 darajalarini baholash hamda ularning klinik va funktsional parametrlar bilan bog‘liqligini aniqlash. Materiallar va usullar: Tadqiqotga O‘SOK tashhisi tasdiqlangan 53 nafar bemor va 8 nafar sog‘lom ko‘ngilli jalb qilindi. IL-8 va IL-18 darajalari IFA usuli yordamida aniqlandi.*

Natijalar. *IL-8 va IL-18 darajalari zardobda nazorat guruhiga nisbatan O‘SOK guruhida ishonchli ravishda yuqori bo‘ldi (mos ravishda p=0,003 va p=0,019). IL-8 va S-reaktiv oqsil darajalari o‘rtasida kuchli ijobiy korrelyatsiya aniqlandi (rho=0,983; p=0,008). IL-18 uchun esa xavf omillari soni bilan salbiy bog‘lanish (rho= –0,371; p=0,049) va MOS 25% ko‘rsatkichi bilan ijobiy bog‘liqlikka moyillik kuzatildi.*

Xulosa. *IL-18 tizimli yallig‘lanish uchun sezgir marker va O‘SOK prognozining potentsial indikatori sifatida qaralishi mumkin. IL-8 yallig‘lanish faolligini aks ettiradi, biroq funktsional parametrlar bilan bog‘lanmagan.*

Kalit so‘zlar: *O‘SOK, interleykin-8, interleykin-18, sitokinlar, tizimli yallig‘lanish, inflammasoma, biomarkerlar.*

SUMMARY

Objective. *To assess serum levels of IL-8 and IL-18 in patients with chronic obstructive pulmonary disease (COPD) and determine their relationship with clinical and functional parameters.*

Materials and methods: *The study included 53 patients with confirmed COPDand 8 healthy volunteers. Serum IL-8 and IL-18 levels were measured by ELISA.*

Results. *Serum levels of IL-8 and IL-18 were significantly higher in the COPD group compared to the control group (p=0.003 and p=0.019, respectively). A strong positive correlation was observed between IL-8 and C-reactive protein (rho = 0.983; p=0.008). For IL-18, there was a negative correlation with the number of risk factors (rho = –0.371; p=0.049), and apositivetendency toward an association with MEF25%.*

Conclusions. *IL-18 may be considered a sensitive marker of systemic inflammation and a potential predictor of COPD prognosis. IL-8 reflects inflammatory activity but shows no association with functional respiratory parameters.*

Keywords: *COPD, interleukin-8, interleukin-18, cytokines, systemic inflammation, inflammasome, biomarkers.*

ВВЕДЕНИЕ

Хроническая обструктивная болезнь лёгких (ХОБЛ) остаётся одной из ведущих причин смертности и инвалидизации во всем мире, с постоянно растущей заболеваемостью и увеличением бремени для здравоохранения [1,2]. Патогенез ХОБЛ характеризуется комплексным воспалительным процессом, включающим как элементы врождённого, так и адаптивного иммунного ответа. Ключевыми патофизиологическими проявлениями являются инфильтра-