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The Expression Levels of HIF-1α and the Long Non-Coding RNA HIF1A-AS1 in the Buffy Coat as Prognostic Markers for Severe COVID-19 Outcomes in Patients with Diabetes and Obesity

Objective — study of pathogenetic mechanisms and the search for effective prognostic markers for COVID-19 are highly relevant.

Materials and methods. Thirty-six patients with comorbidities—obesity (BMI over 30), arterial hypertension, and subcompensated type 2 diabetes—were hospitalized in the infectious disease department of Kyiv City Clinical Hospital No. 4 after 7—12 days of outpatient treatment from the onset of the disease. All patients were confirmed to have COVID-19 by PCR. The control group included 7 practically healthy donors with a BMI < 25 and no signs of diabetes. The severity of the disease was assessed based on pulse oximetry and the duration of oxygen dependence according to WHO classification. According to these criteria, patients were divided into three groups by severity: mild (n = 8), moderate (n = 15), and severe (n = 13) according to the recommendations of the National Health Commission of Ukraine. The severity of COVID-19 was assessed at hospital discharge. Clinical analysis, RNA extraction from peripheral blood leukocytes, reverse transcription, real-time PCR, and the determination of HIF-1α and HIF1A-AS1 expression levels were conducted.

Results and discussion. The expression level of HIF-1α in patients' leukocytes varied widely from 6.8 arbitrary units to 146.9. In the control group, the expression level was (6.8 ± 3.2) a.u., which is 4.3 times lower than in the mild disease group ((29.4 ± 4.1) a.u.). In the moderate disease group, the expression level of HIF-1α almost doubled to (55.728 ± 16.8) a.u. In the severe disease group, the expression level was (146.9 ± 44.9) a.u., which is 5 times higher than in the mild group and 2.6 times higher than in the moderate group ($p < 0.05$). The expression level of the long non-coding RNA HIF1A-AS1 in the control group and patients with mild COVID-19 did not differ statistically (32.096 ± 7.9 and 29.3 ± 7.1). However, in the moderate disease group, the expression increased 5 times compared to the mild group, reaching 146.4 ± 37.4 ($p < 0.05$). In severe COVID-19, the expression level increased 9.8 times compared to the mild group ($p < 0.05$) and 1.9 times compared to the moderate group, reaching 286.7 ± 77.3.

Conclusions. Our data demonstrate that the expression levels of HIF-1α and lnc RNA HIF1A-AS1 in blood leukocytes significantly increase in patients with moderate and severe COVID-19 at the pre-hospital stage, even before clinical deterioration occurs. This suggests that the expression levels of HIF-1α and lnc RNA HIF1A-AS1 can be considered important, complementary prognostic biomarkers for severe COVID-19 in high-risk groups with comorbid conditions, particularly in cases of metabolic disorders. The role of HIF1A-AS1 in the context of respiratory diseases requires further in-depth study and analysis. Targeting HIF-1α through its association with HIF1A-AS1 has the potential to become a therapeutic target in the treatment of severe respiratory diseases accompanied by ARDS.

Keywords

HIF-1α, HIF1A-AS1, hypoxia, diabetes mellitus, obesity, prognostic markers, SARS-CoV-2, COVID-19.

The COVID-19 pandemic has highlighted numerous issues in medical triage and the timely provision of care due to the massive influx of patients into healthcare facilities. Although the risk group for COVID-19 is well-defined [30], and it is well-known that age, sex, and comorbidities, including cancer, cardiovascular diseases, and especially diabetes mellitus, are major risk factors for COVID-19 patients, not all patients in the risk group experience severe disease—most recover without oxygen theapy and intensive care. To address the strain on hospital beds and expand the capacity for outpatient care, it is crucial to study the pathogenetic mechanisms and identify effective prognostic markers for COVID-19.

Established criteria for COVID-19 severity include low albumin levels, high levels of D-dimer, ferritin, procalcitonin, IL-6, C-reactive protein, lactic acid, anisocytosis, and the count of polymorphonuclear leukocytes [5, 14, 22, 26].

In our previous studies, we have thoroughly analyzed the diagnostic significance of markers such as extracellular neutrophil traps (NETs), extracellular plasma DNA, hyaluronic acid levels, and the long non-coding RNA (lncRNA) HAS2-AS in the leukocytes of COVID-19 patients [2, 3]. In this study, we examined the relationship between the expression level of the lncRNA HIF1A-AS1 and the severity of COVID-19 in high-risk patients with diabetes, obesity, and hypertension.

Acute respiratory distress syndrome (ARDS), a severe form of acute lung injury, is one of the life-threatening complications of viral respiratory infections, including SARS-CoV-2 infection [12]. Fluid accumulation in the alveoli during ARDS leads to severe hypoxia [16, 25]. The transcription factor HIF (hypoxia-inducible factor) is the main regulator of the body's response to hypoxia [10—17]. HIF-1α transcriptional activity increases the survival of phagocyte cells and stimulates the expression of critical factors, including vascular endothelial growth factor (VEGF), as well as pro-inflammatory cytokines (TNF, IL-1, and IL-12) at the infection site [18]. It has been shown in numerous studies that viral infection can induce HIF-1α expression, which, upon activation, may favor the pathogen rather than the host [11, 20, 21, 24]. For instance, excessive production of pro-inflammatory cytokines, and ultimately, the cytokine storm, as a key factor in severe pneumonia in patients with H1N1 infection, is mediated by HIF-1α, which can induce the production of pro-inflammatory molecules at the site of inflammation [8].

The role of HIF-1α in the context of COVID-19 has also been demonstrated. Mingfu Tian et al., in their study, present findings showing that the mRNA level of HIF-1α is significantly higher in peripheral blood monocytes (PBMC) of COVID-19 patients compared to healthy individuals [30]. According to the authors, SARS-CoV-2 infection, through the transmembrane protein ORF3a, induces HIF-1α, which in turn enhances viral replication and inflammatory responses [30]. In another study, Jahani also emphasizes that in severe cases of COVID-19, the activation of HIF-1α is responsible for the progression of the cytokine storm [10].

Recently, considerable attention has been given to the investigation of lncRNA, which are involved in numerous physiological and pathological processes through epigenetic regulation and complex signaling pathways. The expression level of HIF-1α is regulated by various factors, including, we believe, potentially through the signaling pathways of the long non-coding RNA HIF1A-AS1.

Long non-coding RNAs are generally defined as endogenous cellular RNAs longer than 200 nucleotides, which are ubiquitously found in eukaryotic genomes but lack protein-coding potential. Long non-coding RNAs play an important role in maintaining cellular homeostasis [34].

The functions of HIFA-AS1 are still insufficiently understood. Only a few studies have documented the biological significance of HIF1-AS1. Increased expression of HIF1A-AS1 has been reported in thoracoabdominal aortic aneurysm [31]. HIF1A-AS1 has also been proposed as a biomarker for colorectal carcinoma [7]. Functionally, HIF1A-AS1 is pro-apoptotic and anti-proliferative in vascular smooth muscle cells, Kupffer cells, and umbilical vein endothelial cells [34, 37].

However, the expression of HIF1A-AS1 in COVID-19 has not been studied.

We publish the results of our own research, which show that the expression levels of HIF-1α and HIF1A-AS1 increase in severe COVID-19 patients from the risk group at the pre-hospital stage, prior to the onset of clinical complications and severe hypoxia. We hypothesize that the expression level of HIF1A-AS1 could be considered a prognostic marker for severe COVID-19 at the pre-hospital stage.

Objective — to study of pathogenetic mechanisms and the search for effective prognostic markers for COVID-19 are highly relevant.

Materials and methods

Description of the Group

Thirty-six patients were hospitalized in the infectious disease department of Kyiv City Clinical Hospital No. 4 after 7—12 days of outpatient treatment from the onset of the disease. All patients had COVID-19 confirmed by real-time polymerase chain reaction (PCR). The patient group included 22 men

and 14 women with comorbid conditions such as obesity (BMI over 30 kg/m^2), arterial hypertension, and subcompensated type 2 diabetes (average blood glucose level — 7.57 mmol/L). The average age of the patients was 61 ± 15.4 years. Glucocorticoids were not administered before hospitalization. The control group consisted of 7 practically healthy donors (average age — 59.0 ± 12.3 years) with a body mass index (BMI) < 25 kg/m^2 and no signs of diabetes.

The severity of the disease was assessed based on oxygen saturation levels (pulse oximetry) and the duration of oxygen dependence according to WHO classification. The severity was categorized into three groups: mild — absence of signs of viral pneumonia and hypoxia; moderate — clinical signs of pneumonia (fever, cough, dyspnea, increased respiratory rate) without severe pneumonia, including $SpO_2 \geq 90$ % on room air; severe — clinical symptoms of pneumonia (fever, cough, dyspnea, increased respiratory rate) and one of the following factors: respiratory rate over 30 breaths/minute, severe respiratory distress, or $SpO_2 < 90$ % on room air. According to these criteria, patients were divided into three groups by severity: mild (n = 8), moderate (n = 15), and severe (n = 13), based on the recommendations of the National Health Commission of Ukraine. The severity of COVID-19 was assessed at the time of hospital discharge or patient death. The average duration of treatment in the mild group was 8.17 days, in the moderate group — 11.0 days, and in the severe group — 20.8 days. In the severe COVID-19 group 5 patients died.

All participants provided written informed consent. The study was reviewed and approved by the relevant national competent authorities: the Research Ethics Committee (No. 6/20 from 11.11.2020) and the Hospital Ethics Committee (No. 104/1-284 from 13.05.2021), which were used to obtain samples from patients and healthy donors.

RNA extraction from peripheral blood leukocytes

Total RNA was extracted from peripheral blood leukocytes using guanidine isothiocyanate-phenol-chloroform extraction with Trizol reagent (Invitrogen). The extracted RNA samples were diluted in 50 μL of distilled water, and RNA concentration was measured spectrophotometrically (NanoDrop ND1000).

Reverse Transcription

The reverse transcription reaction was performed in two stages. First, a mixture of 1 μL Random Hexamer primer, 6 μL total RNA, and 5 μL nuclease-free deionized H_2O was prepared. The samples were incubated at 70 °C for 5 minutes using the Gene Amp® PCR System 2700 amplifier (Applied Biosystems, USA). In the second stage, 2 μL dNTP (10x), 4 μL Buffer RT, 0.5 μL Ribo Lock RNAse inhibitor, and 0.9 μL RevertAid H Minus Reverse Transcriptase were added to the mixture. The samples were then thermostatted at 42 °C for 60 minutes, followed by incubation at 70 °C for 10 minutes.

Real-time polymerase chain reaction

Real-time PCR was performed using the 7500 Fast Real Time PCR thermocycler (Applied Biosystems, USA). In a 96-well plate, 10 μL of a mixture containing 5 μL TaqMan Universal PCR Master Mix, 2 μL cDNA, 0.15 μL probe for lncRNA HIF1A-AS1, 0.2 μL Rox (1 : 9), and 2.65 μL H_2O was added to each well. cDNA denaturation was carried out at 95 °C for 15 seconds. Probe annealing and elongation were performed at 60 °C for 1 minute. The real-time PCR program consisted of 50 cycles. The expression of HIF-1α was determined using the assay: Hs00153153_m1. The expression of HIF1A-AS1 was determined using the assay: Assay ID: Hs04407794_m1. The reference gene was β-actin: Human ACTB (Beta Actin) Endogenous Control (FAM™/MGB probe).

Statistical analysis

Statistical analysis was conducted using the licensed software Origin (https://www.originlab.com/). To determine the significance of differences between groups with normal distribution, a two-tailed t-test was used; otherwise, the Mann—Whitney U test was applied. Correlation analysis was performed using Pearson's correlation coefficient for normally distributed data. Data are presented as mean ± SEM (standard error of the mean of random samples taken from the original population).

Results

Determination of HIF-1α expression in leukocytes of COVID-19 patients

The expression level of HIF-1α in patients' leukocytes varied widely, ranging from 6.8 arbitrary units to 146.9. In the control group, the expression level was (6.8 ± 3.2) relative units (r.u.), which is 4.3 times lower than in the group of patients with mild disease ((29.4 ± 4.1) r.u.) (Fig. 1). In the moderate disease group, the expression level of HIF-1α almost doubled, reaching (55.7 ± 16.8) r.u. However, there was no statistically significant difference between the mild and moderate severity groups (p = 0.24). In patients with severe disease, the expression level of HIF-1α was 146.9 ± 44.9, which is 5 times higher than in the mild group and 2.6 times higher than in the moderate group (p = 0.04 and p = 0.05).

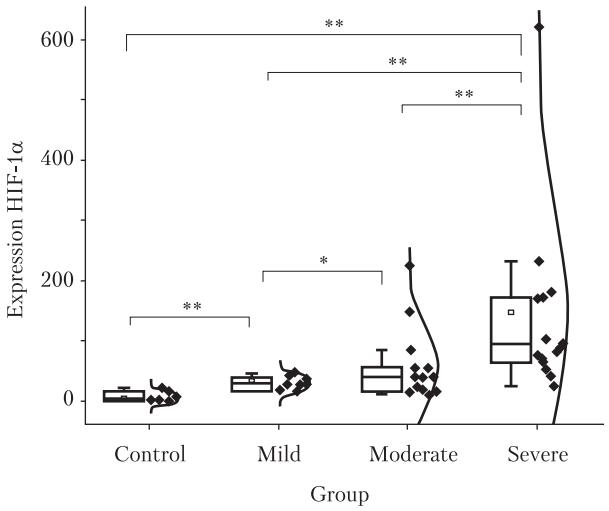

Fig. 1. **Expression level of HIF-1α in pre-hospital COVID-19 patients**

The expression level is presented in relative units to the expression of the reference gene β-actin.
* Significant difference (p > 0.05); ** significant difference (p < 0.05).

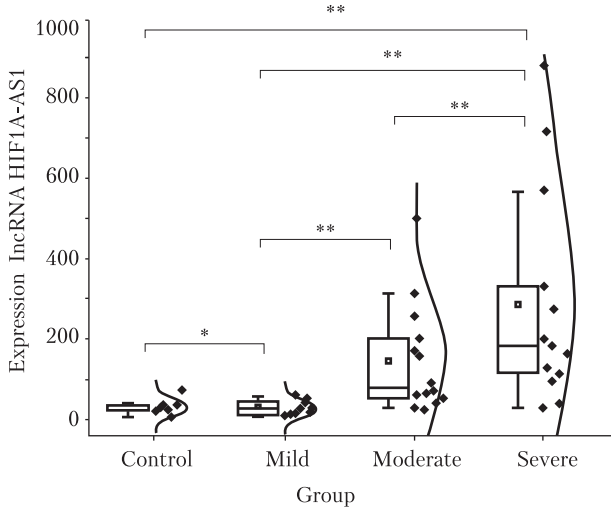

Fig. 2. **Expression level of lncRNA HIF1A-AS1 in pre-hospital COVID-19 patients**

The expression level is presented in relative units to the expression of the reference gene β-actin.
* Significant difference (p > 0.05); ** significant difference (p < 0.05).

Determination of HIF1A-AS expression in leukocytes of COVID-19 patients

It was shown that the expression level of the lncRNA HIF1A-AS1 in the control group of volunteers and patients with mild COVID-19 did not differ statistically (32.1 ± 7.9 and 29.3 ± 7.1) (Fig. 2). In the group of patients with moderate COVID-19, the expression increased 5 times compared to the mild group, reaching 146.4 ± 37.4 ($p < 0.05$). In severe COVID-19, the expression level increased 9.8 times compared to the mild group ($p < 0.05$) and 1.9 times compared to the moderate group ($p > 0.05$), reaching 286.7 ± 77.3.

Discussion

In this study, it was first established that the comorbid course of COVID-19 in the context of diabetes mellitus and obesity is accompanied by increased expression of the lncRNA HIF1A-AS1, depending on the severity of the disease course.

COVID-19 induces inflammation and hypoxia, which induces the transcriptional activity of HIF-1α [35]. Despite HIF-1α being a central regulator of oxygen-dependent gene expression [33], promoting phagocyte cell survival and stimulating the expression of crucial factors, in several studies, it is suggested that the role of HIF-1α may also be pathological and, as a result of activation, could favor the pathogen rather than the host [20].

S. Kondo and colleagues present data indicating that the expression level of HIF-1α protein is elevated due to the degradation of PHD1 and PHD3 by the latent membrane protein 1 oncoprotein of the Epstein—Barr virus [21]. Additionally, influenza viruses stabilize HIF-1α by disrupting proteasome function and reducing the expression of factor inhibiting HIF-1α [8]. The excessive production of pro-inflammatory cytokines, ultimately leading to a cytokine storm — a key factor in severe pneumonia in patients with H1N1 infection — is mediated by HIF-1α, which can induce the production of pro-inflammatory molecules at the site of inflammation [30]. Increased expression of HIF-1α-regulated genes, including those encoding glycolytic enzymes, has been observed in cells infected with HCV. It has been demonstrated that prolonged expression of HCV protein reduces mitochondrial oxidative phosphorylation, and the enhanced utilization of the glycolysis pathway supports cell survival [10].

Phagocytes, under normal oxygen tension in the blood, typically express low levels of HIF-1α. However, when exposed to low partial oxygen pressure at the site of infection, there is a marked increase in HIF-1α expression, which enhances phagocytic activity [15]. The transcriptional activity of HIF-1α increases the survival of phagocytes and stimulates the expression of pro-inflammatory cytokines (TNF, IL-1, and IL-12) at the site of infection [18].

HIF-1α is already being studied in the context of COVID-19. Some studies have shown that HIF-1α may enhance viral replication and inflammatory responses [30] and be responsible for the progression of the cytokine storm [10]. The activity of HIF-1α may be particularly significant in comorbid conditions, especially in coronavirus infection against the background of diabetes mellitus. A.C. Codo et al. demonstrated that monocytes infected with SARS-CoV-2 lead to mitochondria-mediated ROS stabi-

lization of HIF-1α and increased glycolysis [1]. The authors provide evidence that HIF-1α is essential for the induction of glycolysis and the subsequent pro-inflammatory state of SARS-CoV-2-infected monocytes, ultimately contributing to increased glycolysis, which facilitated SARS-CoV-2 replication and cytokine expression. Tissue hypoxia and HIF-1α may play a key role in the pathogenesis of viral infections through the modulation of glycolytic pathways, which can support viral replication. This is particularly relevant in COVID-19 and respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD), where viral infections play a crucial role in exacerbations and disease progression [32, 33].

In the study by Wu Yanghe, it was reported that HIF1A-AS1 enhances apoptosis of Kupffer cells in the liver by upregulating caspase 3 expression, being induced by TNF-α. Inhibition of HIF1A-AS1 significantly reduced TNF-α-induced cell apoptosis [34].

Although the role of HIF1A-AS1 in COVID-19 has not been studied, data on the association of this non-coding RNA with TNF-α are critical. It is well known that TNF-α is one of the primary pro-inflammatory cytokines, which is upregulated in acute lung injury, induces cytokine release syndrome, and facilitates the interaction of SARS-CoV-2 infection with angiotensin-converting enzyme 2 [9]. Elevated levels of TNF-α correlate with the frequency of severe and critical cases of COVID-19, and its increase is associated with in-hospital mortality from this disease [27]. Therefore, it can be hypothesized that the activation of HIF1A-AS1 by TNF-α may exacerbate its toxic effect not only in liver cells but also in other tissues of the body, contributing to the worsening of the disease course.

Data obtained from oncology studies may contradict this hypothesis. For example, J. Zhang et al., in a large-scale study of the role of HIF1A-AS1 in pancreatic cancer, demonstrates the opposite effect of this non-coding RNA, showing that HIF1A-AS1 can induce metabolic dysfunction and disordered protein translation, thereby inhibiting the growth and progression of cancer cells, suggesting a potential therapeutic effect [36].

Our study results show that the expression levels of HIF-1α and, particularly, the non-coding RNA HIF1A-AS1, are significantly increased in the blood leukocytes of patients with moderate to severe COVID-19 compared to the control group and patients with mild COVID-19. Fig. 1 and 2 demonstrate a clear correlation between the expression levels of both markers and the severity of the disease course. We suggest that these findings may allow for the consideration of HIF-1α and lncRNA HIF1A-AS1 expression levels as important, complementary prognostic biomarkers for severe COVID-19.

The study of non-coding RNAs in COVID-19 and other respiratory diseases, such as influenza, holds great promise. An increasing number of studies continue to demonstrate the interconnected involvement of non-coding RNAs in the immune response to SARS-CoV-2 infection by regulating its pathological features.

For instance, exosomal miR-424 has been shown to be an independent predictor of thromboembolic events (pulmonary embolism and acute myocardial infarction) in patients with COVID-19, while miR-103a independently regulated D-dimer levels [6]. Four other non-coding microRNAs (let-7e-5p, miR-651-5p, miR-766-3p, and miR-4433b-5p) exhibit significantly different expression levels between the healthy control group and the group of patients with signs of severe hypoxia, suggesting that these molecules may serve as potential candidates for stratifying patients by severity [4]. Additionally, the expression of several ncRNAs (miR-423-5p, miR-23a-3p, and miR-195-5p) has been proposed as an alternative to classical PCR tests for more accurate identification of COVID-19 and obtaining a detailed disease profile [19].

We hypothesize that the increased levels of lncRNA HIF1A-AS1 could be considered a diagnostic marker for predicting severe COVID-19 in high-risk groups with comorbid conditions, particularly metabolic disorders. However, the role of HIF1A-AS1 in the context of respiratory diseases requires further in-depth study and analysis. Targeting HIF-1α through its association with HIF1A-AS1 is not only an advanced strategy for suppressing glycolysis in cancer treatment [38], but it also has strong potential to become a therapeutic target in the treatment of severe respiratory diseases associated with ARDS.

Conclusions

Using the combination of HIF-1α and HIF1-AS1 as early biomarkers provides a highly accurate tool for predicting the risk of developing clinical hypoxia and monitoring the severe course of COVID-19. Integrating the determination of these indicators into clinical practice will help focus on patients with poor prognosis and optimize treatment.

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Author contributions: collection of clinical data and blood samples, moderation of the study and formation of the group, laboratory performance of the study, analysis and preparation of the manuscript — E.I. Dubrovsky; idea and moderation of the study, laboratory execution of the study, review of the manuscript— T.I. Drevytska; research idea and moderation, review and approval of the final draft of the manuscript — V.Ye. Dosenko; review and approval of the final draft of the manuscript — A.G. Portnychenko.

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Рівні експресії HIF-1α та довгої РНК, що не кодує, HIF1A-AS1 як прогностичні маркери тяжкого перебігу COVID-19 у пацієнтів із діабетом та ожирінням

Пандемія коронавірусної хвороби-2019 (COVID-19) виявила численні проблеми медичного сортування та своєчасного надання допомоги через масовий наплив пацієнтів у медичні заклади. Хоча визначено групу ризику розвитку COVID-19, не всі пацієнти цієї групи мають тяжкий перебіг захворювання, більшість одужують без потреби в кисневій підтримці чи інтенсивній терапії.

Мета роботи — вивчити взаємозв'язок між рівнями експресії HIF-1α і довгої РНК, що не кодує, HIF1A-AS1 та тяжкістю COVID-19 у пацієнтів із групи високого ризику.

Матеріали та методи. В інфекційне відділення Київської міської клінічної лікарні № 4 через 7—12 днів амбулаторного лікування після початку захворювання були госпіталізовані 36 пацієнтів із коморбідною патологією (ожирінням (індекс маси тіла > 30 кг/м^2), артеріальною гіпертензією та субкомпенсованим цукровим діабетом 2 типу. У всіх пацієнтів COVID-19 був підтверджений методом полімеразної ланцюгової реакції. Тяжкість захворювання оцінювали за даними пульсоксиметрії та тривалістю кисневої залежності за класифікацією ВООЗ. Тяжкість захворювання визначалась ретроспективно згідно з критеріями ВОЗ по класифікації захворювання COVID-19. Згідно з ними, пацієнти були розподілені на три групи за ступенем тяжкості: легкий (n = 14), середній (n = 42) і тяжкий (n = 37). Контрольна група — 7 практично здорових донорів з індексом маси тіла < 25 кг/м^2 і відсутністю ознак діабету. Проведено клінічний аналіз, екстракцію РНК із лейкоцитів периферичної крові, зворотну транскрипцію, полімеразну ланцюгову реакцію в реальному часі та визначення рівня експресії HIF-1α і HIF1A-AS1.

Результати та обговорення. Рівень експресії HIF-1α у лейкоцитах становив від 6,8 до 146,9 у.о., у контрольній групі — (6,8 ± 3,2) у. о., що в 4,3 разу нижче, ніж у групі з легким перебігом захворювання ((29,4 ± 4,1) у. о.). У групі із середньотяжким перебігом рівень експресії HIF-1α був майже вдвічі вищим ((55,7 ± 16,8) у. о.), тоді як у групі з тяжким перебігом був у 5 разів вищим, ніж у групі з легким перебігом, і в 2,6 разу вищим, ніж у групі із середньотяжким перебігом ((146,9 ± 44,9) у.о., p < 0,05). Рівень експресії РНК HIF1A-AS1 у контрольній групі та у пацієнтів із легким перебігом COVID-19 не відрізнявся статистично значущо ((32,096 ± 7,9) та (29,3 ± 7,1) у.о.). Однак у групі із середньотяжким перебігом рівень експресії був більшим у 5 разів порівняно з групою з легким перебігом ((146,4 ± 37,4) у. о., p < 0,05). При тяжкому перебігу COVID-19 рівень експресії в 9,8 разу перевищував показник групи з легким перебігом (p < 0,05) і в 1,9 разу — показник групи із середньотяжким перебігом ((286,7 ± 77,3) у. о.).

Висновки. Уперше встановлено, що коморбідний перебіг COVID-19 на тлі цукрового діабету та ожиріння супроводжується зростанням експресії довгої РНК, що не кодує, HIF1A-AS1 залежно від тяжкості перебігу захворювання. Рівні експресії HIF-1α та HIF1A-AS1 у лейкоцитах крові значно зростають у пацієнтів із середньотяжким та тяжким перебігом COVID-19 на догоспітальному етапі, до настання клінічного погіршення. Це свідчить про те, що ці показники можна розглядати як важливі прогностичні біомаркери тяжкого перебігу COVID-19, що доповнюють один одного, у групах ризику з коморбідною патологією, особливо при метаболічних розладах. Роль HIF1A-AS1 у контексті респіраторних захворювань потребує подальшого глибокого вивчення та аналізу. На нашу думку, HIF-1α через зв'язок із HIF1A-AS1 може бути терапевтичною мішенню при лікуванні тяжких респіраторних захворювань, які супроводжуються гострим респіраторним дистрес-синдромом.

Ключові слова: HIF-1α, HIF1A-AS1, гіпоксія, цукровий діабет, ожиріння, прогностичні маркери, SARS-CoV-2, COVID-19.

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