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ANALYSIS OF THE DYNAMICS OF GENE EXPRESSION IN PATIENTS WITH ACUTE COVID-19 AND IN RECOVERY PERIOD

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Massive infections of people with the previously unidentified SARS-CoV-2 virus have been a shock to the global healthcare system. While many studies have focused on the clinical manifestations of the disease and its treatment methods, understanding the molecular and genetic aspects of infection has proved critical to understanding the pathogenetic mechanisms of host-pathogen interaction. The scientists focused on issues of gene expression and their regulation in response to SARS-CoV-2 infection. In particular, we conducted a study of gene expression within the framework of the project «COVID-19: Scientific and technological justification of the response system to the spread of new respiratory infections, including coronavirus infection», the results of which are presented in the article [1].

Hence, variations in the gene expression profiles of TLR 3 (Toll-Like receptor), TLR 7, TLR 4, ACE 2 (Angiotensing converting enzyme), TMPRSS 13 (Transmembrane serine protease), INF-ɣ (Interferon gamma), and IL 4 (Interleukin) were discerned across all investigated cohorts, including those assessed six months post-recovery.

There is a complex of pathways of immune system to defense with the infection including the involvement of toll-like receptor signaling pathways which contact of viral particles. Moreover, the surge in ACE2 expression demonstrates the multifunctional role as gates for Sars-Cov-2 to enter cells and activation of innate immunity responses. Although, the upregulation of INF-ɣ and IL-4 as proinflammatory cytokines were contributed to the initiation and the progression of the cytokine storm.

Key words: COVID-19; Sars-Cov-2; PCR; RT-PCR; gene expression

INTRODUCTION

Severe COVID-19 is characterized by a number of immune reactions, many of which are associated with alterations in gene expression. Studies such as the Blanco-Melo et al study [4] demonstrates that SARS-CoV-2 induces a unique cytokine profile with reduced expression of type I and type III interferons at a comparably high level of proinflammatory cytokines [4]. This expression profile may explain some of the clinical features of COVID-19. For example, a lack of interferons can lead to a lack of an effective antiviral response in the early stages of infection, which is aggravated by excessive triggering of inflammatory pathways, which leads to acute respiratory distress syndrome (ARDS) [4].

Another key aspect of gene expression is the penetration of the SARS-CoV-2 virus into host cells using the ACE 2 receptor. Thus, the level of expression of the gene of this receptor may affect sensitivity to infection. Studies such as Li MY et al. is confirmed by pointing to age and tissue differences in ACE 2 expression [16]. Individual patterns of gene expression can give an idea of the body's response, and it is their combined interaction that determines the clinical outcome. For example, increased expression of ACE 2 in combination with elevated levels of TMPRS 13 can potentially increase sensitivity to the virus, exacerbating the severity of the disease [5].

Thus, gene expression in acute COVID-19 plays a key role in understanding the molecular mechanisms underlying the response to infection and the clinical manifestations of the disease. Further in-depth study of these mechanisms may contribute to the development of new methods for the diagnosis, treatment and prevention of coronavirus infection.

Aim – to access the dynamics of gene expression in patients with Covid-19.

MATERIALS AND METHODS

Nucleic acid (RNA) extraction from the PBMC (Peripheral Blood Mononuclear Cell) blood of 103 patients was conducted. The RIN for the extracted RNA was determined using the PerkinElmer LabChip GX II Touch 24 (DNA 5K/RNA/CZE) chip electrophoresis system to ascertain the integrity of the nucleic acids. The concentration of the extracted DNA and its purification degree were determined spectrophotometrically using the P 330 nanospectrophotometer (Implen). Prior to further processing, RNA samples were stored at a temperature of -80 °C. Based on the obtained RIN and RNA concentrations, a sample set was formed comprising patients with moderate (29 individuals) and severe (13 individuals) coronavirus infection, 10 individuals from a control group (a sample collection from patients prior to 2019), and patients 6 months post-moderate severity coronavirus infection (18 individuals). The study included 70 participants. Diagnostic testing was carried out to detect SARS-CoV-2 using RT-PCR to confirm the diagnosis (Table 1).

Samples were collected from participants in the control group and patients with severe and moderate coronavirus infection, as well as again 6 months post-recovery. Blood (5 ml) was drawn by venipuncture into tubes containing EDTA (Improvacuter, Gel&EDTA.K2, Improve Medical Instruments, Guangzhou, China). The median day of hospital admission from the onset of illness for patients with moderate severity was 4 [3;7] days, while for patients with severe disease, it was 3.5 [2.2;4.7] days. PBMCs were isolated using the Ficoll reagent.

Total RNA was extracted using the PureLink™ RNA Mini Kit (Invitrogen, ThermoFisher Scientific, USA) following the manufacturer's instructions. cDNA was synthesized from total RNA using 10x random RT primers (Applied Biosystems, USA), 100 mM dNTP (Applied Biosystems, USA), 50 U/µl reverse transcriptase (Applied Biosystems, USA), and 10x reverse transcription buffer (Applied Biosystems, USA) to obtain a final product volume of 20 µl. qPCR was performed using SYBR Green on the CFX96 system (Bio-Rad, France). Each reaction mixture consisted of 5 ng cDNA, 1 µl primer, and Gene Expression Master Mix (Applied Biosystems, USA) with a total reaction volume of 10 µl. The following protocol stages were used: after 30 s at 95 °C for polymerase activation, amplification was allowed to proceed for 44 cycles, each consisting of denaturation at 95 °C for 5 s and annealing/extension at 60 °C for 5 s. To evaluate linear standard curves, from which the efficiency (E) of each PCR cycle was calculated, eight serial dilutions of mixed cDNA for each target gene were analyzed. Fluorescence was recorded at the end of each extension cycle. A melting curve was then generated to confirm the specificity of PCR products. Quantitative assessment was conducted using the CFX Manager software version 3.1 (Bio-Rad, France) with subsequent data analysis using the ΔΔCt method. Ratios were calculated as the geometric mean value of (1 + E)^(−ΔΔCt), where E represents efficiency and ΔΔCt represents target gene expression in the PBMCs of patients with coronavirus infection relative to the PBMCs of control group participants. Expression of TBP and GAPDH genes was evaluated as the endogenous control.

Statistical analysis was carried out using IBM SPSS version 26. We employedthe Kruskal-Wallis test, Pearson's χ^2, or Fisher's exact test to compare differences between groups.

All research procedures were approved by the Bioethics Committee of Karaganda Medical University (protocol No. 6, assigned number 23 dated 07.02.2022). Written informed consent was obtained from all participants.

RESULTS

The results of the obtained threshold cycles were analyzed using the ΔΔCt method to interpret the expression of genes TLR 7, TLR 4, ACE 2, TMPRSS 13, INF-ɣ, and IL 4 among study participants. Expression values were transformed using the log2 FC (Fold change) method for patient groups. Thus, the gene expression values of the control group participants were taken as the baseline (Table 2, Figure 1).

For TLR 7, the log2 FC was 1.12 for patients with moderate COVID-19 severity, -0.01 for patients with severe COVID-19, and -0.42 for the group of patients 6 months post-recovery (p=0.023). For TLR 4, the log2 FC was 1.95 for patients with moderate COVID-19 severity, 2.43 for those with severe COVID-19, and 1.89 for the group 6 months post-recovery (p<0.001). For TMPRSS 13, the log2 FC was 2.15 for patients with moderate COVID-19 severity, 1.93 for those with severe severity, and 1.61 for the group 6 months post-recovery (p<0.001). For IL 4, the log2 FC was 1.15 for patients with moderate COVID-19 severity, -0.55 for those with severe COVID-19, and -0.54 for the group 6 months post-recovery (p<0.001). For INF-ɣ, the log2 FC was 1.97 for patients with moderate COVID-19 severity, 1.19 for those with severe COVID-19, and 0.63 for the group 6 months post-recovery (p<0.001). For ACE 2, the log2 FC was 4.02 for patients with moderate COVID-19 severity, 2.73 for those with severe COVID-19, and 1.34 for the group 6 months post-recovery (p=0.225). For TLR 3, the log2 FC was 2.56 for patients with moderate COVID-19 severity,

Table 1 – Characteristics of Study Participants

Characteristic	Total, n = 70	Prepandemic control, n = 10	Patients with severe COVID-19, n=13	Patients with moderate COVID-19, n= 29	Patients 6 months post-COVID-19 recovery, n= 18	P-value
Age, years, median [IQR]	61 [43;68]	55 [39;66]	56 [34;63]	63 [49;69]	61 [43;70]	0.405
Male sex, n (%)	35 (50%)	3 (30)	4 (30.7%)	16 (55.2%)	12 (66.7%)	0.120
Comorbidity	45(64.3%)	6 (60%)	7 (53.84%)	20 (69%)	13 (72.2%)	0.336

Table 2 – Log 2 FC values for the studied genes

Genes	Patients with Ssevere COVID-19	Patients with moderate COVID-19	Patients 6 months post-COVID-19 recovery	P-value
ACE 2	4,02	2,73	1,34	0,225
TLR 7	1,12	-0,01	-0,42	0,023*
TLR 4	1,95	2,43	1,89	<0,001*
TMPRSS 13	2,15	1,93	1,61	<0,001*
IL 4	1,15	-0,55	-0,54	<0,001*
INF-ɣ	1,97	1,19	0,63	<0,001*
TLR 3	2,56	3,04	1,66	0,357

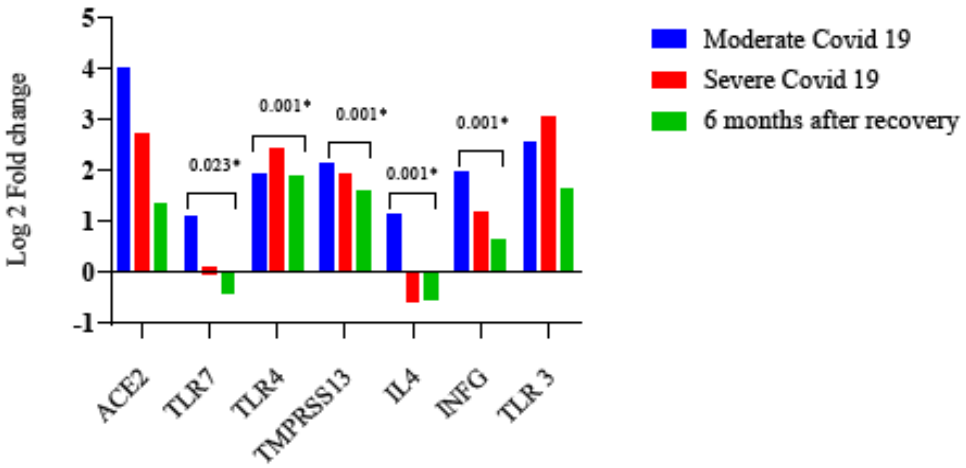

Figure 1 – Dynamics of gene expression across groups

3.04 for those with severe COVID-19, and 1.66 for the group 6 months post-recovery (p=0.357).

Consequently, alterations in the gene expression of TLR 3, TLR 7, TLR 4, ACE 2, TMPRSS 13, INF-ɣ, and IL 4 were observed across all the study groups, including those monitored six months post-recovery. For TLR 7, TLR 4, TMPRSS 13, INF-ɣ, and IL 4, the significance level was less than p <0.05, indicating the statistical significance of the results obtained.

The correlational analysis of gene expression data revealed a high level of correlation between the genes TLR 7, TLR 4, and TMPRSS 13 among patients with both moderate and severe disease intensities. Notably, the INF-ɣ gene displayed a weak negative correlation.

Expression levels of TLR 4 in patients with moderate COVID-19 severity were significantly correlated with the expression of genes such as TLR

7 (r = 0.992, p < 0.0001), TMPRSS 13 (r = 0.992, p < 0.01), and IL 4 (r = 0.988, p < 0.001). The correlation of INF-ɣ with all the genes – TLR 7, TLR 4, TMPRSS 13, and IL 4 – was not statistically significant, with respective values of r = -0.285 at p = 0.643; r = -0.294 at p=0.631; r = -0.281 at p = 0.647; and r = -0.244 at p=0.692. A direct correlational link was also identified between the expression of the TLR 4 gene and the expression of the following genes in patients with severe COVID-19: TLR 7 (r= 0.840, p=0.001); TMPRSS 13 (r = 0.898, p<0.000); IL 4 (r = 0.893, p=0.001).

Our study demonstrates significant variations in the expression of genes involved in the interaction of SARS-CoV-2 with host cells, depending on disease severity. According to our results, patients with a moderate degree of coronavirus infection display a slight increase in the expression of genes TLR 4, TLR 7, and IL 4 (log 2 FC of 1.95; 1.12; 1.15 respectively), which primarily characterizes the involvement of Toll-like receptor signaling pathways upon contact of viral particles with immune system cells, as well as modulating the immune response by stimulating the expression of IL 4, as corroborated by correlation coefficients (r = 0.992, p < 0.0001; r = 0.988, p< 0.001). Toll-like receptors are an integral part of the innate immune system, recognizing molecular structures associated with pathogens. TLR-4 detects viral proteins, whereas TLR-7 and TLR-3 are responsible for sensing viral RNA. In the context of moderate-severity COVID-19, increased expression of these genes reflects the immune system cells' strategy to recognize viral infection and combat it [2, 10, 19].

The expression of genes TLR 3, TMPRSS 13, and ACE 2 markedly increased (log2FC values of 2.56; 2.15; 4.02 respectively), illustrating the active interaction of the SARS-CoV-2 Spike protein and its nucleic acid with the host cell's receptor apparatus. The dynamic expression of the ACE 2 gene during the progression of COVID-19 underscores its dual role – both as the primary route for viral entry and as protection against excessive inflammation [8, 16, 28]. TMPRSS 13 plays a role in receptor interaction by activating the Spike protein [13, 14]. It has been found that TMPRSS 13 activates the spike protein with the same efficiency as TMPRSS2, and TMPRSS 13 mRNA co-expresses with ACE 2 in type II pneumocytes, goblet cells, basal cells, and blood cells, which might serve as targets for SARS-CoV-2 infection. Concurrent increased expression of ACE 2, combined with elevated levels of TMPRSS 13, may potentially enhance susceptibility to the virus, thereby exacerbating disease severity.

The cytokine expression axis of IL 4 and INF-ɣ merits particular attention. Compared to the control group, the expression of these genes is elevated (log 2 FC 1.15; 1.97). It is plausible that a key factor in the development of the cytokine storm and the progression of the disease to severe and critical forms is influenced by the imbalance between IL 4 and INF-ɣ expression [9]. The primary function of INF-ɣ is to modulate adaptive immunity, promoting the activation and differentiation of T-cells. During the acute phase of COVID-19, elevated levels of INF-ɣ indicate an active antiviral response, suggestive of disease progression [25]. Conversely, IL-4, an anti-inflammatory cytokine, facilitates the differentiation of naive T-helper cells into Th2 cells, demonstrating increased expression during the recovery phase. This potentially helps to mitigate the hyperinflammation observed in the acute phase and promotes tissue repair [20]. Thus, the opposing actions of IL 4 and INF-ɣ (enhanced Th1 differentiation under INF-ɣ and Th2 under the influence of IL-4) may influence the severity of COVID-19 disease. Researchers observe a significant shift towards a Th2 response in severe cases, suggesting a pivotal role of IL-4 in the progression of COVID-19 [27]. Despite the elevated expression of IL 4 and INF-ɣ genes, their dynamics are comparable, indicating a balanced inflammatory response to COVID-19.

In the group of patients with severe COVID-19, there was also observed hyperexpression of the genes ACE 2, TLR 4, TMPRSS 13, INF-ɣ, and TLR 3 (log 2 FC 2.73; 2.43; 1.93; 1.19, 3.04 respectively), demonstrating a high involvement of genes encoding the receptor apparatus and INF-ɣ in expression processes. Notably, there is a decreased expression of the genes IL 4 and TLR 7 (log2 FC -0.55 and -0.01 respectively) compared to the expression in the group with patients with moderate COVID-19 severity and the control group. The decreased expression of IL-4 in patients with severe COVID-19 progression might suggest that their immune system is shifted towards a Th1 response, which is characterized by the production of pro-inflammatory cytokines, potentially exacerbating the disease progression and leading to complications such as Acute Respiratory Distress Syndrome (ARDS), the primary cause of death in severe COVID-19 cases [18, 20]. Furthermore, the diminished expression of IL-4 might indicate a weakening of the humoral immune response. This could potentially affect the ability of immunocompetent cells to produce neutralizing antibodies against the virus, prolonging the course of the infection and increasing the risk of complications [22]. Concurrently, a decrease in TLR 7 expression was observed in patients with severe forms of COVID-19. This decline might contribute to a reduced production of interferons, which in turn could exacerbate viral replication and intensify the inflammatory response [12]. The decreased expression of TLR 7 could correlate with

the enhancement of the inflammatory response, characteristic of the «cytokine storm», which is the primary cause of many severe and critical COVID-19 cases [12].

Persistent changes in the gene expression of ACE 2, TLR 4, TMPRSS 13, INF-ɣ, TLR3, IL 4, and TLR 7 (log 2 FC 1.34; 1.89; 1.61; 0.63; 1.66; -0.54; -0.42 respectively) were observed six months post-recovery. The statistically significant expression values of genes (TLR 7, TLR 4, TMPRSS 13, IL 4, INF-ɣ) suggest potential long-term changes in the immune system following infection. This may be pertinent for understanding the long-term consequences of SARS-CoV-2 infection, commonly referred to as Post-Acute Sequelae of SARS-CoV-2 (PASC) or «long COVID» [22]. Patients, six months post-infection, also exhibited an imbalance in the gene expression of IL 4 and INF-ɣ, which might predispose them to maintain a pro-inflammatory status [22]. Comparing expression values across groups, the genes TLR 7, TLR 4, TMPRSS 13, IL 4, and INF-ɣ have p-values less than 0.05, indicating that the observed changes in their expression are statistically significant and not random.

DISCUSSION

Our data demonstrate a complex mechanism of the molecular response of the host organism at various stages of COVID-19. Increasing the regulation of ACE 2 and TMPRSS 13 in the active stages of the disease may be a viral strategy to enhance cell penetration, while the dynamics of expression of genes encoding TLR and cytokines reflect the host's attempts to control and resist infection. An effective response can neutralize and eliminate the virus without causing destructive changes to the patient, while an unbalanced immune response can be catastrophic. A decrease in IL-4 gene expression in patients with severe COVID-19 demonstrates impaired immune responses that may contribute to the severity of the disease. Further research in this area may be promising for understanding the mechanisms of infection pathogenesis: by analyzing gene expression, it is possible to determine which genes are activated or suppressed when interacting with the virus. This could help scientists determine exactly how SARS-CoV-2 interacts with host cells and which molecular pathways are involved in this process.

Firstly, identification of risk groups: changes in gene expression may explain why some people suffer from severe forms of COVID-19, while others experience only mild symptoms or none at all. This will allow us to design more accurate methods for identifying risk groups and develop personalized treatment approaches.

Secondly, the development of new drugs: understanding which genes and protein pathways are activated during infection may lead to the development of new antiviral medications, as well as methods for modulating the immune response.

Finally, predicting the outcome of the disease: analysis of gene expression can also help predict how the disease will develop in a particular patient, which in turn can influence the choice of treatment strategy.

CONCLUSION

Our study confirms that there are significant variations in gene expression that correlate with the severity of Covid-19. In patients with moderate severity of coronavirus infection, increased expression of the TLR 4, TLR 7 and IL 4 genes is observed (log 2 FC 1.95; 1.12; 1.15, respectively). Moreover, the study showed a significant increase in the expression of the TLR 3, TMPRSS 13 and ACE 2 genes, which indicates an active molecular interaction of the SARS-CoV-2 virus with the host cell structures. Taking into account the co-expression of TMPRSS 13 and ACE 2 (log 2 FC 2.15; 4.02) in key cells targeted by the virus, this combination may serve as a factor increasing the risk of developing more severe forms of COVID-19. In the group of patients with severe COVID-19, overexpression of ACE 2, TLR 4, TMPRSS 13, INF-ɣ, TLR 3 genes (log 2 FC 2.73; 2.43; 1.93; 1.19, 3.04, respectively) is also observed, which demonstrates the high involvement of genes encoding the receptor apparatus and INF-ɣ in expression processes.

It was observed the expression of IL 4 and INF-ɣ genes was increased in patients with COVID-19 compared to the control group (log 2 FC 1.15; 1.97, respectively). In the group of patients with severe disease, the expression of IL 4 and TLR 7 genes (log 2 FC -0.55 and -0.01, respectively) was reduced compared with expression in the group with patients with moderate severity COVID-19 and the control group. A decrease in the expression of these genes is also observed 6 months after recovery (log 2 FC -0.54; -0.42). A decrease in IL-4 expression in severe cases may indicate a shift in the immune response to a pro-inflammatory response, exacerbating the course of the disease. In patients 6 months after infection, reduced expression of IL 4 and TLR 7 may become a prerequisite for the development of long-term COVID.

During the correlation analysis, a strong correlation was revealed between the genes TLR 7, TLR 4, TMPRSS 13 in patients with varying degrees of severity of COVID-19. The INF-ɣ gene shows a weak statistically insignificant correlation with all the genes considered. At the same time, for patients with moderate severity of COVID-19, TLR 4 expression levels are closely related to the expression of TLR 7,

TMPRSS 13 and IL 4. The same trend persists for patients with severe disease.

Authors' contributions:

I. Kadyrova – concept development.

I. Kadyrova, V. Barkhanskaya – execution.

I. Kadyrova – processing of results.

I. Kadyrova – scientific interpretation of the results.

I. Kadyrova, V. Barkhanskaya – article writing.

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REFERENCES

1 Референдум на проект COVID-19: Научно-технологическое обоснование системы реагирования на распространение новых респираторных инфекций, включая коронавирусную инфекцию BR11065386 //https://is.ncste.kz/object/view/65386 (дата обращения: 16.02.2024)

2 Aboudounya M. M. COVID-19 and Toll-Like Receptor 4 (TLR4): SARS-CoV-2 May Bind and Activate TLR4 to Increase ACE2 Expression, Facilitating Entry and Causing Hyperinflammation / M. M. Aboudounya, R. J. Heads //Mediators Inflamm. – 2021. – P. 8874339.

3 Alexopoulou L. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3 /L. Alexopoulou, A. C. Holt, R. Medzhitov // Nature. – 2001. – V. 413, No. 6857. – P. 732-738.

4 Blanco-Melo D. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19 / D. Blanco-Melo, B. E. Nilsson-Payant, W. C. Liu // Cell. – 2020. – V. 181, No. 5. – P.1036-1045.

5 Fuentes-Prior P. Priming of SARS-CoV-2 S protein by several membrane-bound serine proteinases could explain enhanced viral infectivity and systemic COVID-19 infection //J. Biol. Chem. – 2021. – V. 296. – P. 100135.

6 Grimm L. Immune Dysregulation in Acute SARS-CoV-2 Infection /L. Grimm, C. Onyeukwu, G. Kenny //Pathog Immun. – 2023. – V. 7, No. 2. – P. 143-170.

7 Hadjadj J. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients /J. Hadjadj, N. Yatim, L. Barnabei //Science. – 2020. – V. 369, No. 6504. – P. 718-724.

8 Hoffmann M. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor /M. Hoffmann, H. Kleine-Weber, S. Schroeder //Cell. – 2020. – V. 181, No. 2. – P. 271-280.

9 Joyce D. A. IL-4, IL-10 and IFN-gamma have distinct, but interacting, effects on differentiation-induced changes in TNF-alpha and TNF receptor release by cultured human monocytes /D. A. Joyce, J. H. Steer //Cytokine. – 1996. – V. 8 (1). – P. 49-57.

10 Kawai T. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors /T. Kawai, S. Akira //Nat. Immunol. – 2010. – V. 11, No. 5. – P.373-384.

11 Kawai T. Toll-like receptor and RIG-I-like receptor signaling /T. Kawai, S. Akira //Ann. N. Y. Acad. Sci. – 2008. – V. 1143. – P. 1-20.

12 Khanmohammadi S. Role of Toll-like receptors in the pathogenesis of COVID-19 / S. Khanmohammadi, N. Rezaei //J. Med. Virol. – 2021. – V. 93, No. 5. – P. 2735-2739.

13 Kishimoto M. TMPRSS11D and TMPRSS13 Activate the SARS-CoV-2 Spike Protein /M. Kishimoto, K. Uemura, T. Sanaki //Viruses. – 2021. – V. 13, No. 3. – P. 384.

14 Laing A. G. A dynamic COVID-19 immune signature includes associations with poor prognosis /A. G. Laing, A. Lorenc, I. Del Molino Del Barrio //Nat. Med. – 2020. – V. 26, No. 10. – P. 1623-1635.

15 Larionova R. SARS-Cov2 acute and post-active infection in the context of autoimmune and chronic inflammatory diseases /R. Larionova, K. Byvaltsev, O. Kravtsova //J. Transl. Autoimmun. – 2022. – V. 5. – P.100-154.

16 Li M. Y. Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues /M. Y. Li, L. Li, Y. Zhang //Infect Dis Poverty. – 2020. – V. 9, No. 1. – P. 45.

17 Paul W. E. What determines Th2 differentiation, in vitro and in vivo? //Immunol Cell Biol. – 2010. – V. 88, No. 3. – P. 236-239.

18 Queiroz M. A. F. Cytokine Profiles Associated With Acute COVID-19 and Long COVID-19 Syndrome /M. A. F. Queiroz, P. F. M. D. Neves, S. S. Lima //Front Cell Infect Microbiol. – 2022. – V. 12. – P. 922422.

19 Root-Bernstein R. Innate Receptor Activation Patterns Involving TLR and NLR Synergisms in COVID-19, ALI/ARDS and Sepsis Cytokine Storms: A Review and Model Making Novel Predictions and Therapeutic Suggestions //Int. J. Mol. Sci. – 2021. – V. 22, No. 4. – P. 2108.

20 Scala S. Fighting the Host Reaction to SARS-COv-2 in Critically Ill Patients: The Possible Contribution of Off-Label Drugs /S. Scala, R. Pacelli // Front Immunol. – 2020. – V. 11. – P. 1201.

21 Schroder K. Interferon-γ: an overview of signals, mechanisms and functions /K. Schroder, P. J. Hertzog, T. Ravasi //J. Leukoc. Biol. – 2004. – V. 75, No. 2. – P. 163-189.

22 Silva M. J. A. Adaptive immunity to SARS-CoV-2 infection: A systematic review /

M. J. A Silva, L. R. Ribeiro, K. V. B Lima //Front Immunol. – 2022. – V. 13. – P. 1001198.

23 Simpson D. S. Interferon-γ primes macrophages for pathogen ligand-induced killing via a caspase-8 and mitochondrial cell death pathway / D. S. Simpson, J. Pang, A. Weir //Immunity. – 2022. – V. 55, No. 3. – P. 423-441.

24 Su Y. Multiple early factors anticipate post-acute COVID-19 sequelae /Y. Su, D. Yuan, D. G. Chen //Cell. – 2022. – V. 185, No. 5. – P. 881-895.

25 Todorović-Raković N. Between immunomodulation and immunotolerance: The role of IFNγ in SARS-CoV-2 disease /N. Todorović-Raković, J. R. Whitfield //Cytokine. – 2021. – V. 146. – P. 155637.

26 Totura A. L. SARS coronavirus pathogenesis: host innate immune responses and viral antagonism of interferon /A. L. Totura, R. S. Baric //Curr. Opin. Virol. – 2012. – V. 2, No. 3. – P. 264-275.

27 Vaz de Paula C.B., de Azevedo M.L.V., Nagashima S., Martins A.P.C., Malaquias M.A.S., Miggiolaro A.F.R.D.S., da Silva Motta Júnior J., Avelino G., do Carmo L.A.P., Carstens L.B., de Noronha L. IL-4/IL-13 remodeling pathway of COVID-19 lung injury // Sci Rep. – 2020. – Vol. 10(1). – P.18689.

28 Zou X. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection /X. Zou, K. Chen, J. Zou //Front Med. – 2020. – V. 14, No. 2. – P. 185-192.

TRANSLITERATION

1 Referendum na proekt COVID-19: Nauchno-tehnologicheskoe obosnovanie sistemy reagirovanija na rasprostranenie novyh respiratornyh infekcij, vkljuchaja koronavirusnuju infekciju BR11065386 // https://is.ncste.kz/object/view/65386 (data obrashhenija: 16.02.2024)

2 Aboudounya M. M. COVID-19 and Toll-Like Receptor 4 (TLR4): SARS-CoV-2 May Bind and Activate TLR4 to Increase ACE2 Expression, Facilitating Entry and Causing Hyperinflammation / M. M. Aboudounya, R. J. Heads //Mediators Inflamm. – 2021. – P. 8874339.

3 Alexopoulou L. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3 /L. Alexopoulou, A. C. Holt, R. Medzhitov // Nature. – 2001. – V. 413, No. 6857. – P. 732-738.

4 Blanco-Melo D. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19 /D. Blanco-Melo, B. E. Nilsson-Payant, W. C. Liu //Cell. – 2020. – V. 181, No. 5. – P.1036-1045.

5 Fuentes-Prior P. Priming of SARS-CoV-2 S protein by several membrane-bound serine proteinases could explain enhanced viral infectivity and systemic COVID-19 infection //J. Biol. Chem. – 2021. – V. 296. – P. 100135.

6 Grimm L. Immune Dysregulation in Acute SARS-CoV-2 Infection /L. Grimm, C. Onyeukwu, G. Kenny //Pathog Immun. – 2023. – V. 7, No. 2. – P. 143-170.

7 Hadjadj J. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients /J. Hadjadj, N. Yatim, L. Barnabei //Science. – 2020. – V. 369, No. 6504. – P. 718-724.

8 Hoffmann M. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor /M. Hoffmann, H. Kleine-Weber, S. Schroeder //Cell. – 2020. – V. 181, No. 2. – P. 271-280.

9 Joyce D. A. IL-4, IL-10 and IFN-gamma have distinct, but interacting, effects on differentiation-induced changes in TNF-alpha and TNF receptor release by cultured human monocytes /D. A. Joyce, J. H. Steer //Cytokine. – 1996. – V. 8 (1). – P. 49-57.

10 Kawai T. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors /T. Kawai, S. Akira //Nat. Immunol. – 2010. – V. 11, No. 5. – P.373-384.

11 Kawai T. Toll-like receptor and RIG-I-like receptor signaling /T. Kawai, S. Akira //Ann. N. Y. Acad. Sci. – 2008. – V. 1143. – P. 1-20.

12 Khanmohammadi S. Role of Toll-like receptors in the pathogenesis of COVID-19 / S. Khanmohammadi, N. Rezaei //J. Med. Virol. – 2021. – V. 93, No. 5. – P. 2735-2739.

13 Kishimoto M. TMPRSS11D and TMPRSS13 Activate the SARS-CoV-2 Spike Protein /M. Kishimoto, K. Uemura, T. Sanaki //Viruses. – 2021. – V. 13, No. 3. – P. 384.

14 Laing A. G. A dynamic COVID-19 immune signature includes associations with poor prognosis /A. G. Laing, A. Lorenc, I. Del Molino Del Barrio //Nat. Med. – 2020. – V. 26, No. 10. – P. 1623-1635.

15 Larionova R. SARS-Cov2 acute and post-active infection in the context of autoimmune and chronic inflammatory diseases /R. Larionova, K. Byvaltsev, O. Kravtsova //J. Transl. Autoimmun. – 2022. – V. 5. – P.100-154.

16 Li M. Y. Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues /M. Y. Li, L. Li, Y. Zhang //Infect Dis Poverty. – 2020. – V. 9, No. 1. – P. 45.

17 Paul W. E. What determines Th2 differentiation, in vitro and in vivo? //Immunol Cell Biol. – 2010. – V. 88, No. 3. – P. 236-239.

18 Queiroz M. A. F. Cytokine Profiles Associated With Acute COVID-19 and Long COVID-19 Syndrome /M. A. F. Queiroz, P. F. M. D. Neves, S. S. Lima //Front Cell Infect Microbiol. – 2022. – V. 12. – P. 922422.

19 Root-Bernstein R. Innate Receptor Activation Patterns Involving TLR and NLR Synergisms in COVID-19, ALI/ARDS and Sepsis Cytokine Storms:

A Review and Model Making Novel Predictions and Therapeutic Suggestions //Int. J. Mol. Sci. – 2021. – V. 22, No. 4. – P. 2108.

20 Scala S. Fighting the Host Reaction to SARS-COv-2 in Critically Ill Patients: The Possible Contribution of Off-Label Drugs /S. Scala, R. Pacelli // Front Immunol. – 2020. – V. 11. – P. 1201.

21 Schroder K. Interferon-γ: an overview of signals, mechanisms and functions /K. Schroder, P. J. Hertzog, T. Ravasi //J. Leukoc. Biol. – 2004. – V. 75, No. 2. – P. 163-189.

22 Silva M. J. A. Adaptive immunity to SARS-CoV-2 infection: A systematic review /M. J. A Silva, L. R. Ribeiro, K. V. B Lima //Front Immunol. – 2022. – V. 13. – P. 1001198.

23 Simpson D. S. Interferon-γ primes macrophages for pathogen ligand-induced killing via a caspase-8 and mitochondrial cell death pathway / D. S. Simpson, J. Pang, A. Weir //Immunity. – 2022. – V. 55, No. 3. – P. 423-441.

24 Su Y. Multiple early factors anticipate post-acute COVID-19 sequelae /Y. Su, D. Yuan, D. G. Chen // Cell. – 2022. – V. 185, No. 5. – P. 881-895.

25 Todorović-Raković N. Between immunomodulation and immunotolerance: The role of IFNγ in SARS-CoV-2 disease /N. Todorović-Raković, J. R. Whitfield //Cytokine. – 2021. – V. 146. – P. 155637.

26 Totura A. L. SARS coronavirus pathogenesis: host innate immune responses and viral antagonism of interferon /A. L. Totura, R. S. Baric //Curr. Opin. Virol. – 2012. – V. 2, No. 3. – P. 264-275.

27 Vaz de Paula C.B., de Azevedo M.L.V., Nagashima S., Martins A.P.C., Malaquias M.A.S., Miggiolaro A.F.R.D.S., da Silva Motta Júnior J., Avelino G., do Carmo L.A.P., Carstens L.B., de Noronha L. IL-4/IL-13 remodeling pathway of COVID-19 lung injury // Sci Rep. – 2020. – Vol. 10(1). – P.18689.

28 Zou X. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection /X. Zou, K. Chen, J. Zou //Front Med. – 2020. – V. 14, No. 2. – P. 185-192.

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АНАЛИЗ ДИНАМИКИ ЭКСПРЕССИИ ГЕНОВ У ПАЦИЕНТОВ С ОСТРЫМ COVID-19 И В ПЕРИОД ВОССТАНОВЛЕНИЯ

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Массовые заражения ранее неидентифицированным вирусом SARS-CoV-2 стали потрясением для мировой системы здравоохранения, а также вызовом для мировой науки. В то время как многие исследования сфокусировались на клинических проявлениях заболевания и методах его лечения, понимание молекулярных и генетических аспектов инфекции оказалось критически важным для понимания патогенетических механизмов взаимодействия хозяина и патогена. Особое внимание исследователями уделялось вопросам экспрессии генов и их регуляции в ответ на инфицирование SARS-CoV-2. В частности, авторами было проведено исследование экспрессии генов в рамках проекта «COVID-19: Научно-технологическое обоснование системы реагирования на распространение новых респираторных инфекций, включая коронавирусную инфекцию», результаты которого представлены в статье [1].

Вариации в экспрессии генов TLR 3 (Toll-Like receptor), TLR 7, TLR 4, ACE 2 (Angiotensing converting enzyme), TMPRSS 13 (Transmembrane serine protease), INF-ɣ (Interferon gamma) и IL 4 (Interleukin) были выявлены во всех исследованных группах, включая те, которые были оценены через шесть месяцев после восстановления.

Механизм защиты иммунной системы включает в себя вовлечение сигнальных путей toll-подобных рецепторов, которые контактируют с вирусными частицами. Более того, увеличение экспрессии ACE2 демонстрирует многофункциональную роль в качестве ворот для проникновения Sars-Cov-2 в клетки и активации реакций врожденного иммунитета. Тем не менее, повышенная регуляция INF-α и IL-4 как

провоспалительных цитокинов способствовала инициации и прогрессированию цитокинового шторма. Таким образом, изменение экспрессии генов TLR 3, TLR 7, TLR 4, ACE 2, TMPRSS 13, INF-ɣ, IL 4 наблюдалось во всех исследуемых группах, включая пациентов, наблюдаемых спустя 6 месяцев. Молекулярное понимание реакции хозяина на вирус может дать представление о патофизиологии заболевания и потенциальных терапевтических мишенях.

Ключевые слова: COVID-19; Sars-Cov-2; ПЦР; ОТ-ПЦР; экспрессия генов

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ЖЕДЕЛ COVID-19 ПАЦИЕНТТЕРІНДЕГІ ЖӘНЕ ҚАЛПЫНА КЕЛТІРУ КЕЗЕҢІНДЕГІ ГЕН ЭКСПРЕССИЯСЫНЫҢ ДИНАМИКАСЫН ТАЛДАУ

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Бұрын анықталмаған SARS-CoV-2 вирусын жаппай жұқтыру Дүниежүзілік денсаулық сақтау жүйесі үшін соққы болды, сонымен қатар әлемдік ғылым үшін сынақ болды. Көптеген зерттеулер аурудың клиникалық көріністеріне және оны емдеу әдістеріне назар аударғанымен, инфекцияның молекулалық және генетикалық аспектілерін түсіну иесі мен қоздырғыштың өзара әрекеттесуінің патогенетикалық механизмдерін түсіну үшін өте маңызды болды.

Зерттеушілер SARS-CoV-2 инфекциясына жауап ретінде гендердің экспрессиясы және оларды реттеу мәселелеріне ерекше назар аударды. Атап айтқанда, біз «COVID-19: коронавирустық инфекцияны қоса алғанда, жаңа респираторлық инфекциялардың таралуына жауап беру жүйесінің ғылыми-технологиялық негіздемесі» жобасы шеңберінде гендердің экспрессиясына зерттеу жүргіздік, оның нәтижелері осы мақалада келтірілген [1].

TLR 3 (Toll-Like receptor), TLR 7, TLR 4, ACE 2 (Angiotensing converting enzyme), TMPRSS 13 (Transmembrane serine protease), INF-ɣ (Interferon gamma) және IL 4 (Interleukin) гендерінің экспрессиясындағы вариациялар барлық зерттелген топтарда, соның ішінде қалпына келтірілгеннен кейін алты айдан кейін бағаланғандарда анықталды.

Иммундық жүйені қорғау механизмі вирустық бөлшектермен жанасатын 'toll' тәрізді рецепторлардың сигнал беру жолдарын тартуды қамтиды. Сонымен қатар, ACE2 экспрессиясының жоғарылауы SARS-Cov-2 жасушаларына ену және туа біткен иммунитет реакцияларын белсендіру үшін қақпа ретінде көп функциялы рөл атқарады. Дегенмен, қабынуға қарсы цитокиндер ретінде INF-α және IL-4 реттелуінің жоғарылауы цитокиндік дауылдың басталуына және өршуіне ықпал етті. Осылайша, TLR 3, TLR 7, TLR 4, ACE 2, TMPRSS 13, INF-ɣ, IL 4 гендерінің экспрессиясының өзгеруі барлық зерттеу топтарында, соның ішінде 6 айдан кейін байқалған пациенттерде байқалды. Иесі вирусқа реакциясы туралы молекулалық түсінік аурудың патофизиологиясы мен ықтимал емдік мақсаттары туралы түсінік бере алады.

Кілт сөздер: COVID-19; Sars-Cov-2; ПЦР; ОТ-ПЦР; ген экспрессиясы