

ANNOTATION

**of the dissertation work on the topic: "Multi-omics studies of the relationship between intestinal microbiota and the immune system in severe coronavirus infections", presented for the degree of Doctor of Philosophy (PhD) in the educational program 8D05101-"Biology"
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The relevance of research. The study of the relationship between the gut microbiota and the immune system in severe coronavirus infection is one of the most relevant areas of contemporary biomedical science. It has been established that the gut microbiota plays a key role in regulating the immune response, and its dysbiosis is associated with enhanced inflammatory processes as well as the development of various diseases. Coronavirus infection is characterized by immune system dysregulation and the development of a cytokine storm, caused by excessive production of pro-inflammatory cytokines. Recent studies indicate that the gut microbiota may influence the modulation of the immune response in COVID-19. The gut microbiome not only plays an important role in maintaining immune homeostasis but can also interact with the respiratory system through the "gut–lung axis" and potentially affect the expression of the angiotensin-converting enzyme 2 (ACE2) receptor. Imbalances in the gut microbiota may impact disease severity, clinical outcomes, and vaccine effectiveness.

Currently, research on the gut microbiome in coronavirus infection has been conducted in European and Asian populations; however, in Central Asian countries, including Kazakhstan, this area remains insufficiently studied. Therefore, a comprehensive investigation of the relationship between the gut microbiota and the immune system in severe coronavirus infection will deepen the understanding of disease pathogenesis, identify potential biomarkers, and contribute to the improvement of therapeutic strategies.

The aim of the study is to investigate the relationship between the gut microbiota and the immune system in severe coronavirus infection using multi-omics approaches.

Research Objectives:

1. To investigate the cytokine profile in coronavirus infection compared with a healthy state.
2. To determine the composition of the gut microbiota in coronavirus infection compared with a healthy state.
3. To identify changes in the cytokine profile during the post-COVID period.
4. To examine alterations in the gut microbiota composition during the post-COVID period.
5. To determine the relationship between gut microbiota indicators and immune status parameters in coronavirus infection.

6. To identify biomarkers associated with the severity of coronavirus infection.

Object of the study are peripheral blood and fecal samples collected from 60 patients with severe coronavirus infection, as well as from a healthy control group consisting of 58 individuals.

Study design. Prospective longitudinal study.

Research methods:

- Extraction of DNA from fecal samples and assessment of its concentration;
- 16S rRNA sequencing for microbiota analysis;
- Multiplex analysis of the blood cytokine profile;
- Statistical data processing and bioinformatic analysis of the results.

Scientific Novelty of Study. For the first time in the population of the Republic of Kazakhstan, a correlational assessment of the immune response and the state of the gut microbiota in severe coronavirus infection has been conducted.

For the first time, the levels and dynamics of cytokines have been determined over the course of one year following a severe form of COVID-19.

For the first time, the dynamics and specific features of the gut microbiota during coronavirus infection have been studied.

Practical Significance. The results of the study will make it possible to identify the relationships between the body's immune response and the state of the gut microbiota in severe COVID-19.

Determining the dynamics of cytokine levels over the course of a year in severe COVID-19 will provide an understanding of the characteristics of the immune response to the infection.

The obtained data on cytokine and microbiome biomarkers will supplement the global knowledge base on the mechanisms of coronavirus infection.

The study contributes to the identification of new biomarkers and patterns of interaction between the immune system and the gut microbiome, which will advance fundamental research in immunology and microbiology.

The main provisions submitted for defense:

1. In patients who had recovered from coronavirus infection, the acute phase of the disease was characterized by elevated levels of pro-inflammatory cytokines and decreased levels of anti-inflammatory cytokines. Cytokines MDC, EGF, IP-10, IL-15 and IL-8 were identified as the most significant indicators.

2. According to the gut microbiome analysis, statistically significant differences were found between the groups: the study group showed a predominance of opportunistic bacteria such as *Prevotella 9*, *Lachnospirales*, and *Bacteroides*, while the levels of *Haemophilus*, *Leptotrichia*, *Prevotella*, *Prevotella_7*, *Neisseria*, and *Streptococcus* were reduced.

3. In the post-COVID period, immune markers showed a high concentration of IL-6 and a reduced level of IL-1RA. Significant changes were also observed in the gut microbiome over time: within one year, the number of conditionally pathogenic bacteria decreased, while the number of commensal bacteria increased.

4. During coronavirus infection, a statistically significant commensal bacterial species — *Eubacterium hallii* — was identified. It was found that a decrease in *E. hallii* levels was associated with a reduction in the expression of anti-inflammatory cytokines that help prevent the development of a cytokine storm.

Conclusions:

1. In the study group, compared to the control group, a significant increase in pro-inflammatory cytokines was observed: IL-1A (FDR = 0.03047), FLT-3L (FDR = 0.00302), IL-2 (FDR = 0.03924), IL-6 (FDR = 0.00048), IL-9 (FDR = 0.00778), MCP-3 (FDR = 0.01850), IFN γ (FDR = 0.06763), and IL-17A (FDR = 0.00012). Among anti-inflammatory cytokines, elevated levels were noted for IL-5 (FDR = 0.09064), EGF (FDR = 0.00012), eotaxin (FDR = 0.00012), IL-4 (FDR = 0.00001), IL-12 (p40) (FDR = 0.00082), MDC (FDR = 0.05648), MCP-1, and IL-15 (FDR = 0.00004). When studying the significance of markers of changes, the most important indicators among chemokines were MDC, growth factor EGF and IP-10, and among interleukins - IL-15 and IL-8.

2. In the analysis of gut microbiome structure, alpha diversity showed statistically significant differences between groups by the Observed ($p = 0.005$; $d = 0.59$) and Chao1 indices ($p = 0.05$; $d = 0.53$). Beta diversity by the Bray-Curtis index also revealed significant differences ($R = 0.759$; $p = 0.0001$). Linear discriminant analysis (LEfSe) showed that the study group had a predominance of opportunistic bacteria such as *Prevotella 9*, *Lachnospirales*, and *Bacteroides*, while *Haemophilus*, *Leptotrichia*, *Prevotella*, *Prevotella 7*, *Neisseria*, and *Streptococcus* were reduced. In contrast, the control group had higher levels of *Prevotella 7*, *Haemophilus*, and *Veillonella*.

3. In the post-COVID period, a comparison between 6-month and 12 month time points showed statistically significant changes in immune markers: decreases in levels of eotaxin ($p = 0.01619$), MCP-1 ($p = 0.00794$), MDC ($p = 0.00169$), MIP-1a ($p = 0.00072$), TGF α ($p = 0.00111$), TNF α ($p = 0.06176$), and VEGF-A ($p = 0.02385$), while levels of FGF-2 ($p = 0.01133$), G-CSF ($p = 0.01133$), and IL-15 ($p = 0.03340$) increased after recovery. IL-6 remained elevated compared to the control group for 6 months after the acute phase, whereas IL-1RA expression stayed reduced for six months

4. Alpha diversity (Observed and Chao1 indices) showed statistically significant differences between time points T1 and T5 ($p = 0.005$), T2 and T5 ($p = 0.005$), and T4 and T5 ($p = 0.005$). In terms of microbiome composition, *Prevotella 9* and *Bacteroides* levels decreased from T1 to T5, while

Parabacteroides, *Eubacterium hallii* group, and *Agathobacter* significantly increased during this period.

5. During both the acute and post-COVID periods, *Eubacterium hallii* was identified as a statistically significant commensal species. Its levels significantly increased over time, though they were extremely low during the acute phase. *E. hallii* showed a negative correlation with cytokines MCP-1, MCP-1b, IFN- α 2, eotaxin, IP-10, EGF-2, TNF, G-CSF, as well as interleukins IL-7, IL-10, and IL-12, indicating reduced immune expression with lower *E. hallii* abundance.

6. A predictive model identified excess body weight ($p = 0.05$) and arterial hypertension ($p = 0.05$) as associated with the development of severe COVID-19. Additionally, elevated levels of IL-15, IL-6, and IL-8, along with reduced IL-1RA, were identified as markers predictive of disease severity.

Practical recommendations:

1. Data on immune and microbiome markers have been deposited in an international scientific and technical information database and complement global data on the genetic predisposition to changes in the gut microbiome and immune markers during COVID-19 in Central Asia. NCBI Gene Database: Identifier — SUB13279337, publication date — June 20, 2024.

2. Patients who have recovered from coronavirus infection are recommended to follow the principles of a varied and balanced diet in order to maintain the functional equilibrium of the gut microbiota.

3. Within the framework of identifying biomarkers associated with disease severity in SARS-CoV-2 infection, it is recommended to consider the bacterium *Eubacterium hallii* as a potential marker of the gut microbiota.

Author's personal contribution. Writing a literature review, collecting biospecimens, conducting laboratory tests, interpreting the results. Analysis and generalization of the obtained data, their statistical processing. Collection of scientific materials and literature data, preparation of scientific articles and abstracts for publication. Presentation of the main provisions of the study at international conferences.

Approbation of the thesis. The main results and provisions of the study were presented at the following conferences:

– Republican Conference "Digestive Health - Health of the Whole Body! Fundamentals of Cancer Prevention", June 2, 2023, Astana, Kazakhstan.

– Scientific and Practical Conference "Modern Trends in Laboratory Medicine for Clinical Practice", September 21-22, 2024, Astana, Kazakhstan.

Based on the materials of the dissertation, 6 printed works were published, 1 article in a publication with a percentile of 83 (Q1) according to CiteScore in the Scopus database, 1 article in a publication with a percentile of 66 (Q2), 1 article in a publication with a percentile of 49 (Q3), 1 article in a publication indexed in the Russian Science Citation Index, 2 abstracts were

published in collections of international conferences. 1 author's certificate was received.

Implementation of the results of the work. The results of the work were tested and implemented in the departments of the medical diagnostic center and in the State Enterprise on the Right of Economic Management "City Polyclinic No. 2" of the Akimat of Astana.

Relation of this paper to research projects. This study was conducted with the financial support of projects funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan: BR10965164 "Assessment of the influence of clinical, functional, immunological, and genetic factors on the severity of SARS-CoV-2 coronavirus infection and post-COVID syndrome" (2021–2023) and AP23487014 "Microbiome-mediated interactions in atherosclerosis: SESN and APO family proteins, cytokines, and clinical manifestations".

Volume and structure of the dissertation. The dissertation consists of an introduction, three main chapters, a conclusion, a list of references, and appendices. The total length of the work is 90 printed pages, including 9 tables and 33 figures. The list of references contains 217 sources.