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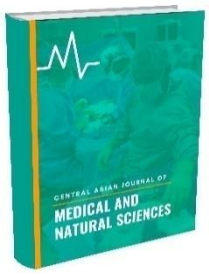

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Assessment of the Cytokine Profile in Patients with Covid-19 Depending on the Severity of the Disease

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Abstract:

Background: NewCoronavirus Infection (COVID-19) is a novel zooanthroponous respiratory infection caused by the Sars-Cov-2 coronavirus. The virus was first identified during an epidemic outbreak in Wuhan City, Hubei Province, China, which has been reported since December 11, 2019. Since then, scientists have been studying the characteristics of the virus to determine the severity of the disease. For a complete and reliable assessment of the severity of the disease in this pathology, the cytokine status was studied.

Methods: To assess the study of the cytokine profile in patients with COVID-19, depending on the severity of the disease, an enzyme-linked immunosorbent assay was used.

Results: It was found that, depending on the severity of the disease, patients with COVID-19 have a pronounced cytokine imbalance. The high level of the studied cytokines is a reflection of the activity and severity of the pathological process. The results obtained indicate the activation of the expression of proinflammatory cytokines, for example, IL-1β, IL-2, IL-6, TNF-α.

Conclusions: The results obtained can be used as a prognostic criterion for the severity of the course and outcome of the disease.

Key words: COVID-19, coronavirus, cytokine profile, moderate stage, severe stage.

Introduction

In recent months, around the world, the number of people who fell ill with a viral infection (COVID-19) caused by the SARS-CoV-2 coronavirus is increasing. On March 11, 2020, WHO declared a COVID-19 pandemic (7). Coronaviruses (Coronaviridae) are a large family of RNA viruses with a single-stranded, positively charged RNA molecule that can infect humans and some animals. In humans, previously known coronaviruses can cause both mild forms of acute respiratory infection and

severe acute respiratory syndrome (SARS). The SARS-CoV-2 genome has been completely sequenced, despite a high degree of RNA nucleotide sequence homology (79% with SARS-CoV and 50% with MERS-CoV), it has some differences from known coronaviruses (8).

Inflammation is the most powerful defense response of the innate immune system, but excessive inflammation can cause tissue and organ damage. A characteristic feature of infections caused by SARSCoV-2, SARS-CoV and MERS-CoV is a pronounced inflammatory reaction in lung tissue, which is reflected in the "cytokine storm", i.e. release of large quantities (5). The kinetics of the response to SARS-CoV-2 is consistent with patterns of conventional antiviral immunity induction and with a crisis that correlates with the probable peak phase of the T-cell response. However, it is unclear whether severe disease is based on immune hyperactivity or failure to resolve the inflammatory response due to ongoing viral replication or immune dysregulation. However, reports of increased levels of thrombus formation and endothelial cell death in patients with COVID-19 indicate damage to the vascular endothelium and involvement of cytokines and immunothrombosis (6).

Despite numerous studies posted on bioRxiv and medRxiv on elevated cytokine levels in severe patients with COVID-19, there is still no unequivocal information and prognosis of standard treatment tactics (4).

In this connection, the purpose of our study was to study and comparatively analyze the levels of immune response mediators in patients with moderate and severe COVID-19.

Materials and Methods

The work was performed in the period from 2021 to 2022 on the basis of the Institute of Immunology and Human Genomics of the Academy of Sciences of the Republic of Uzbekistan in the Department of Cell Therapy.

The study involved men and women aged 26 to 84 living in the Navoi region, whose average age was 58±2.17 years. All patients underwent a complete clinical examination, including laboratory and instrumental studies. The control group consisted of practically healthy women and men of comparable age.

An analysis of the anamnestic data revealed that out of 108 patients: 80 patients were with a moderate form of the disease, 28 patients with a severe form, and one patient was in an extremely severe form of COVID-19. All patients were divided into two groups: group 1 - patients with a moderate form, group 2 - with a severe form of the disease. It should be noted that the exclusion criteria in the study were asymptomatic and mild cases of COVID-19 according to the results of antibody titer G to COVID-19.

Clinical examination of patients at the place of hospitalization included an examination by a therapist and an infectious disease specialist with a detailed history, clinical and instrumental examination (X-ray, ultrasound, MSCT, ECG) and laboratory tests (general blood count, urine), biochemical blood tests, coagulogram.

Cytokine status was studied by enzyme immunoassay (ELISA) using commercial kits of reagents IL-1β, IL-2, IL-6, TNF-α of “Vector-Best”LLC (Russia, Novosibirsk).

Statistical processing of the obtained data was carried out using the computer program Statistica 6.0. The significance of differences in the average values of the compared indicators was assessed by Student's t test (t).

Results and Discussion:

Cytokines are synthesized in the process of implementing the mechanisms of natural or specific immunity, exhibit their activity at very low concentrations, serve as mediators of immune and inflammatory reactions, act as growth factors and cell differentiation factors (2).

Normally, without antigenic stimulation, the cytokine network functions at a minimal level, while the cells of the immune system practically do not secrete cytokines and do not respond to them. In addition, normally functioning mechanisms of the immune system prevent the uncontrolled release of cytokines and other inflammatory mediators, ensuring an adequate response of the body to inflammation (1).

Given the above, our study analyzed and studied the levels of pro-inflammatory cytokines IL-1β, IL-2, IL-6 and TNF-α, which are key in inflammatory processes, in the blood serum of patients and healthy examined patients involved in the development of immune disorders. The results obtained are shown below in Table 1.

Table 1. The level of pro-inflammatory cytokines in the examined patients with moderate and severe form of COVID-19

Cytokines	Control, n=32	Moderateform n=80	Severeform, n=28
IL-1β, pg / ml	7,3.±0,84	24,25±1,33	26,18±2,81
IL-2, pg / ml	5,4±0,14	10,46±0,31	10,28±0,32
IL-6, pg / ml	8,7±1,21	38,95±4,20	45,23±7,65
TNF-α, pg / ml	11,4±1,64	77,08±10,21	65,29±9,51

Note: * Values are significant in relation to the control group (P<0,05-0,001)

IL-1β is a multifunctional cytokine with a wide spectrum of action, which plays a key role in the development and regulation of nonspecific defense and specific immunity. It is one of the first included in the protective response of the body under the action of pathogenic factors. It is synthesized and secreted mainly by macrophages and monocytes. Lymphocytes, fibroblasts can take part in its production (3). Thus, a comparative analysis revealed that the expression of this mediator in the group with a moderate form of the course was 3.3 times, with an average value of 24.25 ± 1.33 pg / ml, compared with the data of the control group 7.3 ± 0.84 pg /ml (P<0.05). Analysis of the obtained data of mediators of the inflammatory response found that the concentration of IL-1β in the group of patients with the group of patients with COVID-19 was 3.5 times higher than in the control group, which averaged 26.18±2.81 pg/ml (P<0.05).

The obtained values can probably be explained by the fact that human vascular endothelial cells, being target cells for this mediator and affected by the virus, secrete proteins similar to platelet growth factor under the inhibited influence of IL-1β. It is likely that these polypeptides stimulate cell migration and proliferation and thereby cause the release of vascular inflammatory mediators, which, with a significant increase in IL-1β, can lead to disseminated intravascular coagulation.

IL-2 promotes the generation, survival, and functional activity of Foxp3+ regulatory T cells (Treg or T-suppressors; Foxp3 is a transcription factor that regulates the transcription of genes responsible for T cell differentiation and cytokine expression), and in its absence, there is a profound deficiency of T - suppressors, leading to the development of autoimmune diseases. Thus, IL-2 has dual and opposite functions: maintenance of regulatory T cells and induction of effector cells, simultaneous control and stimulation of immune responses.

Analysis of data on the content of IL-2 in patients with a moderate course of COVID-19 showed that, depending on gender, there were no significant changes in this cytokine. In general, if in the control group the value of IL-2 averaged 5.4±0.14 pg/ml, then in the examined patients this value averaged 10.46±0.31 pg/ml (P<0.05).

The results of studies of the level of IL-2 in patients with severe course, compared with the group with moderate course, did not show significant changes both in the group as a whole and by gender. Thus, the level of this cytokine was 10.28±0.32, which was 1.8 times higher than the values of the control group - 5.4±0.14pg/ml (P<0.05).

The next stage of our study was to study the concentration of IL-6, which performs both pro-inflammatory and anti-inflammatory roles.

IL-6 is a protein that is produced in various cells and regulates the immune response. IL-6 can be intensively produced in inflammatory, infectious, autoimmune (when the immune system does not work properly and attacks the cells of its own body) diseases, diseases of the cardiovascular system, and some tumor processes. Determining the concentration of IL-6 in the blood can be used as a marker of the activation of the immune system. Normally, IL-6 is present in the blood in small amounts or is not detected at all.

Analysis of the results of the conducted studies showed that the level of IL-6 in patients was significantly increased and averaged 38.95±4.20 pg/ml. Thus, the level of this cytokine was almost 4.5 times higher than the control values - 8.7±1.21 pg/ml, (P<0.05). In our studies, it was found that in all groups the level of IL-6 was significantly increased. Thus, in patients with severe COVID-19, the level of IL-6 was on average 45.23±7.65 pg/ml, which is almost 5.2 times higher than the values of the control group – 8.7±1.21 pg/ml. ml, (P<0.05).

According to the literature, the NSP9 and NSP10 proteins of the SARS-CoV-2 virus may induce the production of IL-6, which is most likely due to a high viral load, and an increase in IL-6 may also be associated with an increase in IL-2, which is observed with secondary hemophagocyticlymphohistiocytosis. And probably, in response to a viral infection, mononuclear phagocytes stimulate the production of interleukins, which leads to the induction of a pathogenic cellular response, and as a result, the development of a cytokine release syndrome, referred to as a "cytokine storm".

Tumor necrosis factor (TNF-alpha, TNF-α). The biological effects of TNF-α depend on its concentration. At low concentrations, it acts at its birthplace as a para- and autocrine regulator of the immune-inflammatory response against injury or infection. It is the main stimulator for neutrophils and endothelial cells, for their adhesion and further migration of leukocytes, proliferation of fibroblasts and endothelium during wound healing. In medium concentrations, TNF-alpha, entering the blood, acts as a hormone, exerting a pyrogenic effect, stimulating the formation of phagocytes, enhances blood clotting, reduces appetite, being an important factor in the development of cachexia in such chronic diseases (3).

Analysis of the results of the study of the level of TNF-α in patients showed that the synthesis of TNF-α in the general group was 6.7 times significantly higher than in the control group (P<0.01) (77.08±10.21 pg/ml versus 11 .4±1.64 pg/ml).

It must be taken into account that an elevated level of TNF-α in the blood correlates with the severity of manifestations of chronic diseases.

Analysis of the results of the conducted studies showed that the level of TNF-α in patients of this group was significantly increased and averaged 65.29±9.51 pg/ml. Thus, the synthesis of this cytokine was almost 5.7 times higher than the control values - 11.4±1.64 pg/ml, (P<0.05).

Conclusion. The data obtained indicate the activation of the innate immune response and the concomitant expression of pro-inflammatory cytokines including IL-1β, IL-2, IL-6, TNF-α, which are involved in host defense against viral infections. It should also be taken into account that the congenital feature of the same link of the cytokine network, on the one hand, can become one of the causes of an unfavorable course and outcome of the infection, and, on the other hand, a risk factor for the development of inflammatory or autoimmune pathology. One possible explanation is damage and death of vascular cells, in particular the endothelium, as a result of viral replication. Virus-induced death of inflammatory cells, including necrosis or pyroptosis, leads to the expression of pro-inflammatory cytokines, recruitment of (uninfected) immune cells and their activation. Despite the apparent ambiguity of the mechanisms of the immune system, the mediators discussed above and the increased activation of innate immunity, including increased expression of T1IFN, IL-1β, IL-6 and TNF-α, definitely contribute to the severity of the disease and mortality from COVID-19, MERS and SARS.

The studied cytokines are produced and act on immunocompetent cells, initiating an inflammatory response. Many authors note that a high level of these cytokines is a reflection of the activity and severity of the pathological process. The imbalance of cytokines is one of the reasons for the aggravation of the inflammatory process in chronic diseases. Numerous studies indicate that pathogens can have multidirectional effects on the production of various cytokines. According to the described mechanism, when infected with coronavirus, proper control over viral replication is disrupted and leads to infiltration of the lungs with activated neutrophils and monocytes, intensive synthesis of pro-inflammatory cytokines with all the ensuing consequences in the form of an acute hyperinflammatory reaction. Probably, activated leukocytes synthesized increased amounts of IL1-β, IL-6, TNF, which sensitized T-lymphocytes to apoptosis, thereby further weakening antiviral protection.

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