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CYTOKINE PROFILE OF PATIENTS WITH ENT DISEASES ON THE BACKGROUND OF COVID-19

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BACKGROUND

Patients with COVID-19 are characterized by the development of a "cytokine storm" - a state of uncontrolled release of a large number of inflammatory mediators. **The aim of the study** was to assess the levels of regulatory cytokines in the blood serum of patients with ENT diseases in hospitalized patients with moderate and severe severity of COVID-19. **Material and methods**. 84 patients with a confirmed diagnosis of coronavirus infection were under observation. Of these, 16 (19.04%) patients had a mild course, 44 (52.4%) patients with a moderate degree, and 24 (28.6%) patients with a severe degree. The diagnosis of COVID-19 was established based on the detection of SARS-CoV-2 RNA by PCR in swabs from the nasopharynx and oropharynx. The levels of serum cytokines (IL-1β, IL-4, IL-6, IFNα and IFNγ) were studied by ELISA using the kits of Vector Best JSC (Novosibirsk, Russia). **The results** of the study showed that the levels of both pro-inflammatory cytokines (IL-1β and IL-6) and anti-inflammatory (IL-4) cytokine in patients with ENT diseases on the background of COVID-19 are sharply increased. Moreover, the elevated level is more pronounced in severe COVID-19. Interferons in COVID-19 have multidirectional synthesis: IFNα is reduced, and IFNγ is increased. Therefore, the cytokine storm in COVID-19 is a severe clinical condition caused by systemic hyperinflammation.

KEYWORDS: Patients with ENT diseases, COVID-19, cytokines.

INTRODUCTION

The gates of SARS-cov2 infection are epithelial cells of the upper and lower respiratory tract, as well as enterocytes of the small intestine, containing the angiotensin-converting enzyme II receptor (ACE2).[2,4] Some patients may develop minimal symptoms of acute nasopharyngitis or enteritis. In the vast majority of cases, this period remains without manifestation. Many infected people carry this condition in an erased form, constituting the main pool of latent virus excretors.[5,12] In people with weakened local immunity, the virus enters the bloodstream and spreads throughout the body (viremia). The glycoprotein of coronaviruses is also specifically tropic for endotheliocytes, which also contain the angiotensin-converting enzyme II receptor. This is related to the phenomenon of pantropism of the new coronavirus - all parenchymal organs (lungs, liver, kidneys, etc.), as well as mucous membranes, including the respiratory tract, are affected. In the latter case, patients are able to shed the virus when coughing, sneezing, talking and breathing.[12,13] Primary viremia and systemic specific endovasculitis are accompanied by symptoms of fever, general infectious intoxication, as well as diffuse damage to the lungs and other parenchymal organs and the rapid development of their functional failure. Data on the duration and intensity of immunity against SARSCoV-2 are currently accumulating.[5,7,15]

It should be assumed that in premorbidly healthy people, specific post-infection immunity is long and intense, which makes it possible to use the blood plasma of recovered people for specific immunotherapy. With latent (inapparatny) and erased forms of infection, as well as in weakened patients, immunity is not stable and re-infection is possible.[1,2,3,17]

The aim of this study was to assess the levels of regulatory cytokines in the blood serum of patients with ENT diseases in hospitalized patients with moderate and severe severity of COVID-19.

MATERIALS AND RESEARCH

Methods. 84 patients with a confirmed diagnosis of coronavirus infection were under observation. Of these, 16 (19.04%) patients had a mild course, 44 (52.4%) patients with a moderate degree, and 24 (28.6%) patients with a severe degree. The diagnosis of COVID-19 was

established based on the detection of SARS-CoV-2 RNA by PCR in swabs from the nasopharynx and oropharynx. Among the patients admitted to the hospital, there were predominantly men, which amounted to 68 people (80.9%) and 16 women (19.04%). The age of the patients ranged from 19 to 47 years. Moreover, the largest percentage of patients was noted in the group of patients from 40 to 50 years old.

Laboratory studies were carried out by determining the complete blood count, biochemical studies and determining the level of serum cytokines (IL-1β, IL-4, IL-6, IFNα and IFNγ) by enzyme immunoassay using standard kits of Vector Best JSC (Novosibirsk, RF). The control group consisted of 30 practically healthy people.

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.

RESULTS AND ITS DISCUSSION

During the examination, the most frequent complaints of patients were fever, headache, general weakness, sore throat, hypo- or anosmia, nasal discharge (runny nose), loss of taste, sore throat (Table 1).

Table 1: Signs and symptoms of COVID-19 patients.

	Mild form, n=16	Medium-severe, n=44	Severe, n=24
Fever (°C)			
<37,3	12 (75%)	6 (13,6%)	-
37,3–38,0	4(25%)	23 (52,3%)	8 (33,3%)
38,1–39,0	-	10 (22,7%)	13 (54,2%)
> 39,0	-	0	2 (8,3%)
Cough	8 (50%)	35 (79,5%)	19 (79,2%)
Headache	2 (12,5%)	15 (34,1%)	11 (36,7%)
Runny nose	6 (37,5%)	27 (61,3%)	8 (33,3%)
Gastrointestinal form of the gastrointestinal tract	3 (21,4%)	8 (18,2%)	4 (16,7%)
Sore throat	12 (75%)	40 (90,9%)	22 (91,7%)
Muscle pain	1 (7,1%)	2 (4,5%)	3 (12,5%)
Anosmia, dysgeusia	-	30 (68,2%)	21 (87,5%)
Skin rashes	-	2 (4,5%)	1 (4,2%)
Chills	-	3 (6,8%)	2 (8,3%)

In patients with COVID-19, the main manifestations of ENT diseases in the moderate form proceeded as acute rhinosinusitis, acute pansinusitis, unilateral hemisinusitis, acute ethmoiditis, sinusitis, or acute rhinopharyngitis.

Patients with severe COVID-19 had more severe fever, headache, and general weakness. In this category of patients, the clinical and endoscopic picture was more pronounced with manifestations of high fever, difficult nasal breathing, nasal discharge, headache, sore throat, impaired sense of smell and taste, often cough, less often dyspeptic syndrome, pain in the eye socket was noted in 2 patients. Manifestations of acute rhinosinusitis were expressed in the form of hemisinusitis or pansinusitis, or bilateral sinusitis with lesions mainly of the maxillary and ethmoid sinuses. 2 patients with a severe form of COVID-19 were hospitalized with a diagnosis of cavernous sinus thrombosis.

Of the comorbidities, 16% of hospitalized patients had diabetes mellitus (32%), arterial hypertension and coronary artery disease in 11 patients (13.09%), and obesity (2.4%), chronic rhinosinusitis (14.3%), chronic tonsillitis (2%), conjunctivitis (2%).

In the study of the general analysis of blood from the level of leukocytes, leukocytosis was more often noted. So, in moderately severe patients, on average, it was 10.49 ± 0.54 thousand / μl and in severe patients - 12.31 ± 0.75 thousand / μl. When analyzing data on the level of lymphocytes, it was found that patients with COVID-19 had lymphopenia, and more pronounced in patients with severe severity (18.41 ± 0.79% vs. 28.5 ± 0.92% normal, P <0.01).

In patients with ENT diseases on the background of COVID-19, the fibrinogen level was on average 1.5 times higher than in the control group (P<0.05), in the group with severe course - 1.7 times higher (P<0, 01) than in the control group - 266.41 ± 8.37 g/l. The level of D-dimer in patients with moderate course was also increased by 1.5 times than in the control group. In severe course, the level of D-dimer was 2 times higher than normal.

COVID-19 is accompanied by excessive inflammation and elevated serum levels of cytokines and chemokines, suggesting the development of cytokine release syndrome or cytokine storm.[8,9,10,16] Complications of ENT diseases in COVID-19 begin due to a cytokine storm, cells of the immune system produce too many signaling cytokine molecules, severe inflammation develops, which harms both the virus and the person himself.[18,19]

As can be seen from the data presented in Table 1, in patients with a moderate course of coronavirus infection, the level of IL-1β was more than 2 times higher, and in patients with a severe course, it was 2.8 times higher than the control values (P<0.001). As is known, IL-1β is an important and one of the most universal regulators of immunity and inflammatory reactions with a wide range of biological effects, which include the induction of the synthesis of other cytokines.[12,22] It is likely 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 possible 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.[16,20]

Table 1: The level of cytokines in blood serum in patients with COVID-19, (M±m).

Cytokines,pg/ml	Control group, n=30	Medium- severe, n=44	Severe course, n=24
IL-1β	29,7 ± 1,7	68,4 ± 2,1*	84,4 ± 2,5* ^
IL-4	4,8 ± 0,19	10,2 ± 1,2*	18,7 ± 1,4* ^
IL-6	4,5 ± 0,2	19,9 ± 1,8*	38,2 ± 2, 1* ^
IFNα	21,7 ± 1,6	15,4 ± 0,7*	11,2 ± 0,6* ^
IFNγ	23,8 ± 1,7	48,6 ± 1,9*	54,8 ± 2,1* ^

Note: * Values are significant in relation to the control group
** Values are significant in relation to the group with an average course of the disease (P<0.05 - 0.001)

A unique feature of the cytokine storm COVID-19 is a sharp increase in the level of anti-inflammatory IL-4 in patients with ENT diseases against the background of coronavirus infection.[8,13] As can be seen from the presented data, the level of the anti-inflammatory cytokine IL-4 was more than 2 times higher than the control values (P<0.01). In patients with severe COVID-19, the level of IL-4 was 4 times higher than the control data (P<0.001) and 1.8 times higher than in patients with moderate severity (P<0.01). IL-4 is a key regulator of the immune response; it is synthesized by activated CD4+ T-lymphocytes and activated B-lymphocytes.[15,21] Elevated levels of IL-4 may play an immunoactivating role in the pathogenesis of COVID-19.

IL-6 is one of the key pro-inflammatory cytokines during the development of infection, especially in mucosal areas.[22] The level of IL-6 in patients with moderate severity was 4.4 times higher than in the control group (P<0.001). And in patients with severe course, the level of IL-6 was 8.5 times higher than the values of the control group (P<0.001). Moreover, values above the average were found in 38.4% of cases. A number of authors have found a strong correlation between serum levels of IL-6 and respiratory failure.[12]

Coronavirus creates a high viral load, which is facilitated by its multilevel inhibitory effect on the interferon system. Coronavirus uses numerous mechanisms to suppress interferonogenesis and evade the immune response. Suppression of the interferon system in coronavirus infection is associated with the severity of clinical manifestations of the disease.[17,20]

Analysis of the results of the study showed that in patients with moderate severity of COVID-19, the level of IFNα was 1.4 times lower than in the control group (P<0.05), and the range of individual values was from 10 to 18 pg/ml. While in patients with severe severity, the level of IFNα dropped to 11.2 ± 0.6 pg/ml, which was almost 2 times lower than the control values (P<0.001), (Fig. 1).

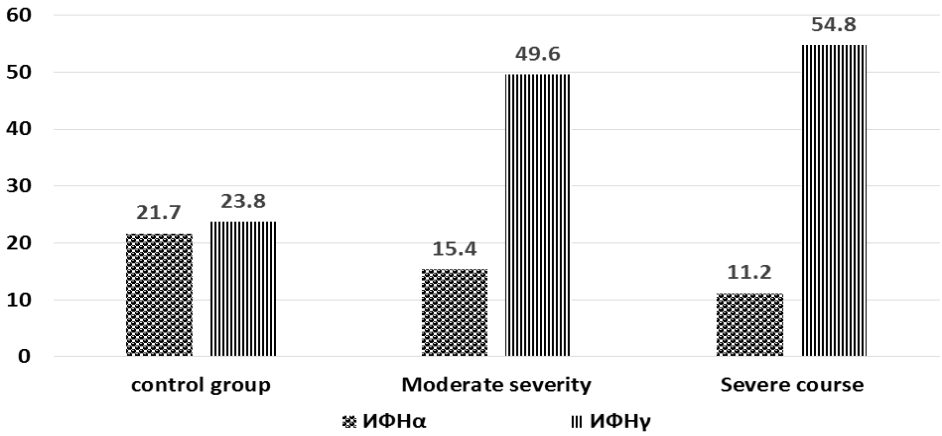

Fig. 1: The level of interferon α and γ in ENT patients on the background of COVID-19.

SARS-CoV2 effectively suppresses the expression of type 1 interferons.[18] The resulting tissue damage and expression of pro-inflammatory cytokines and chemokines from infected monocytes/macrophages contributes to excessive immune cell infiltration and cytokine responses.[14] Apparently, insufficient synthesis of IFNα plays a decisive role in the imbalance of innate immunity responses in COVID-19.[18] This was confirmed in a mouse model of respiratory infection caused by SARS-CoV, where an unbalanced synthesis of IFNα and the release of leukocytes into lung tissue were observed. The essence of the imbalance was the low synthesis of IFNα at the initial stage of infection, which was accompanied by a lack of proper control over the development of coronavirus infection. In response to intense viral replication, the synthesis of pro-inflammatory cytokines was triggered, and then hyperproduction of IFNγ, and increased synthesis of IFNγ led to an increase in the inflammatory response with a massive release of leukocytes into the lung tissue.[12]

IFN-γ is secreted by several immune cells, including macrophages, NK cells, T cells, and plays a role in directly stimulating major inflammatory effector cells. Thus, IFN-γ is considered the main effector cytokine in various disorders. IFNγ has a significantly lower antiviral activity compared to IFNα, interacts with its own specific receptors and has important immunoregulatory functions. In our studies, the level of IFNγ sharply increased in ENT patients with moderate severity of COVID-19, averaging 48.6 ± 1.9 pg/ml, which is more than 2 times higher than in the control group ($P<0.001$).

Moreover, in patients with severe course, the level of IFNγ was even higher - 54.8 ± 2.1 pg / ml ($P<0.05$ compared with the group of patients with moderate severity and $P<0.001$ compared with the data of the control group). Therefore, a very high level of IFNγ synthesis, accompanied by the synthesis of a number of other cytokines, can not only have a protective effect, but also cause the development of severe clinical manifestations of a viral infection.

Thus, cytokine storm is a severe clinical condition caused by systemic hyperinflammation, which leads to serious changes, in particular the ENT organs, including the lungs, and even death.

CONCLUSIONS

1. The immunodeficiency state in COVID-19 in patients with ENT diseases, which develops due to lymphopenia, is the main pathogenetic mechanism of the “cytokine storm” syndrome, when there is an increased synthesis of pro- (IL-1β and IL-6) and anti-inflammatory (IL-4) cytokines. Moreover, the maximum value of these cytokines was recorded in patients with severe COVID-19.
2. Synthesis of type I and II interferons in patients with ENT diseases on the background of COVID-19 changed depending on the severity of the disease. The level of IFNα in moderately severe patients was reduced by 1.4 times, and in severe patients - by 1.9 times. At the same time, the level of IFNγ in patients with moderate severity was 2 times higher than the control values, and in severe patients - more than 2.3 times higher.

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