

Changes in Spermatogenesis, Lipoperoxidation Processes, and Antioxidant Protection in Men with Pathozoospermia after COVID-19. The Effectiveness of Correction with a Promising Antioxidant Complex

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The indicators of spermatogenesis and the state of LPO and antioxidant protection in men with pathozoospermia after COVID-19 were assessed before and after treatment an antioxidant complex. Blood plasma served as the material for biochemical studies. In the examined patients, the parameters of spermatogenesis, as well as blood concentration of LPO components (diene conjugates and TBA-reactive substances) were analyzed. The total antioxidant activity of the blood was determined as an indicator characterizing the total activity of LPO inhibitors and determining its buffer capacity. In patients recovered from COVID-19, an increase in spermatogenesis disorders and shifts towards the predominance of prooxidant factors were observed. After a course (1 month) of antioxidant complex, patients showed increased sperm motility, decreased leukocyte count in the ejaculate, and restored balance in the prooxidant–antioxidant system towards antioxidant components. The effectiveness of correction of post-COVID disorders largely depends on the degree of damage to the structure and function of cell membranes caused by oxidative stress. The use of the antioxidant complex is a promising option, because it reduces the level of LPO, enhances antioxidant protection of the body, and also normalizes some parameters of spermatogenesis.

Key Words: *COVID-19; astaxanthin; omega-3 fatty acids; oxidative stress; spermatogenesis*

Control of the new coronavirus infection pandemic and its consequences is one of the most pressing social issues in the world. The course of the coronavirus disease COVID-19 is complex and sometimes unpredictable, with possible long-term complications referred to as “post-COVID syndrome”. In men, the

prevalence of SARS-CoV-2 infection with severe consequences in different countries was reported to be much greater than expected [1,2]. Russian and foreign researchers claim that the virus affects not only the lungs, vascular wall, hemostasis system, but also the reproductive system and leads to reduced fertility in men [3]. The pathogenetic mechanisms of the influence of COVID-19 on copulative activity and the state of the endocrine system were studied, the risks of damage to the testicles and spermatozoa have been determined [1-3].

When analyzing the quality of sperm in men with mild to moderate COVID-19, lymphocytic inflammation

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and a decrease in the level of male germ cells to the degree of clinically significant oligospermia, as well as an increase in the frequency of DNA fragmentation were noted [3,4]. Fever against the background of infection disrupts the blood–testicle barrier and promotes the penetration of macromolecular substances into the testicles, due to which some parameters such as the concentration and motility of spermatozoa can decrease within 72–90 days after infection [5].

According to modern concepts, a decrease in the male reproductive function can be due to oxidative stress, a pathological effect of ROS on various stages of spermatogenesis. ROS are continuously generated in the course of cellular metabolism, and their presence within the physiological limits allows for hyperactivation, acrosomal reaction, and capacitation of spermatozoa, which is necessary for successful conception [6]. Normally, ROS accumulation can be inactivated by the antioxidant system; however, excessive ROS production exceeding the protective capabilities of the cell leads to damage to lipids, proteins, cell membranes, and DNA in spermatozoa [5,7], which impairs their mobility and interaction with the oocyte, and can also lead to errors in fusion of the genetic material of the chromosomes from the father and mother and improper development of the embryo.

In the available literature, we found no reports on the effect of COVID-19 on the parameters of spermatogenesis and LPO in men with pre-existing spermatogenesis disorders.

Over the past year, numerous reports have appeared on the triggering role of SARS-CoV-2 in molecular pathogenetic mechanisms of oxidative stress and inflammation, as well as on the possibility of using antioxidants in the complex therapy of patients with COVID-19, in particular, to preserve their reproductive function [5,6,8].

Astaxanthin, a carotenoid of marine origin, is a promising antioxidant manyfold surpassing hydrophobic antioxidants as vitamin E and β -carotene by its antioxidant activity [9–11]. Due to its unique molecular structure, astaxanthin crosses the bilayer membrane and provides protection against oxidative stress. It absorbs and quenches ROS and free radicals (superoxide anion, hydrogen peroxide, and singlet oxygen) in both the inner and outer layers of the cell membrane, unlike most antioxidants working either on the inner (vitamin E and β -carotene) or outer (vitamin C) side of the membrane. The antioxidant effect of astaxanthin is combined with its anti-inflammatory effect due to activation of antioxidant enzymes (in particular, superoxide dismutase) and suppression of prooxidant enzymes (e.g. NO synthase) [9].

Our aim was to study the effect of an antioxidant complex containing ω 3-polyunsaturated fatty acids

(PUFA) and astaxanthin on the parameters of spermatogenesis and processes of LPO and antioxidant protection (AOD) in men with pathozoospermia after SARS-CoV-2 infection.

MATERIALS AND METHODS

The study involved men with pathology of spermatogenesis (pathozoospermia), patients of the Scientific Center for Family Health and Human Reproduction Problems. The feature of the study was that some patients had COVID-19 during the therapy, which made it possible to evaluate the effect of this infection on these patients. It is especially valuable that, in fact, the control group and the reference group (recovered from COVID-19) consisted mainly of the same men ($n=25$; mean age 29.9 ± 5.3 years) before and after the infection. Unfortunately, not all patients treated for infertility underwent complete examination before the disease, so the control group before the disease consisted of 20 patients (mean age 30.2 ± 3.2 years). Clinical examination included collection of complaints and anamnesis, physical examination, laboratory and instrumental diagnostics according to the clinical guidelines “Male Infertility” of the Russian Society of Urology (2019). All patients gave written informed consent to participate in the study in accordance with the WMA Declaration of Helsinki (2013 revision).

Antioxidant complex “Omega-3+astaxanthin, strengthened formula” (Koryakmoreprodukt DV) in gelatin capsules (net weight 0.3 g) contains 77 mg PUFA and 1 mg natural astaxanthin from the microalgae *Haematococcus pluvialis*. The PUFA composition was determined by analyzing their methyl esters by gas-liquid chromatography on a Shimadzu-20 instrument with a flame ionization detector. Carotenoid astaxanthin ($C_{40}H_{52}O_4$, molar weight 596.84 g/mol; reg. EINECS number: 207-451-4, reg. CAS number: 472-61-7) was determined by HPLC in accordance with the manual for methods of analysis of biologically active dietary supplements (R 4.1.1672-03).

The patients received the antioxidant complex in a dose of two capsules 3 times a day over 1 month. This dosage provided 46.5% of the daily body requirement for ω 3-PUFA and 300% adequate daily dose of astaxanthin, but did not exceed the maximum allowable dose (6 mg). The results obtained after taking the drug were compared with the initial values.

The ejaculate was analyzed in accordance with the WHO Guidelines for the laboratory study of ejaculate and sperm-cervical interaction (2021). The first collection of ejaculate and blood from patients who recovered from COVID-19 was performed in 2 months after receiving a negative PCR result of a nasopharyngeal swab for SARS-CoV-2 virus RNA. The blood

was taken in the morning on an empty stomach from the cubital vein into a vacuum tube with anticoagulant EDTA-K3. Patients donated ejaculate according to generally accepted requirements. The ejaculate and blood were collected again immediately after the course (1 month) of the antioxidant complex.

Blood plasma and erythrocyte hemolysate were used as the material for biochemical studies. LPO products were determined by generally accepted methods: diene conjugates (DC), TBA-reactive substances (TBARS) and total antioxidant activity (AOA). The measurements were carried out on an SF-2000 spectrophotometer (Spektr). Analysis of the studied indicators in the group of men before COVID-19 was carried out retrospectively.

When analyzing intergroup differences for independent samples used the methods of mathematical statistics implemented in Statistica 10.0 software (StatSoft, Inc.). The data are presented as $M \pm \sigma$. For comparison in groups before and after treatment, the Wilcoxon's ω test for linked samples was used. The differences were significant at $p < 0.05$.

RESULTS

Analysis of spermograms in men who had COVID-19 revealed a significant decrease in the concentration of spermatozoa (by 54%), their motility (by 33%) and viability (by 23%) in comparison with the pre-disease values. In the ejaculate of men recovered from COVID-19, the number of leukocytes increased by 47% in comparison with the control. The spermogram parameters in men after the treatment improved and approached the reference values, the number of leukocytes significantly decreased (by 50.8%) and the sperm motility index increased (by 22.6%) (Table 1).

In patients recovered from COVID-19, the concentration of TBARS in the blood increased by 49% compared with the control (Table 2). Formed during LPO, TBARS form adducts and insoluble compounds

with various cell components, which determines their mutagenic potential. The concentration of TBARS in the blood reflects LPO activity and serves as a marker of the intensity of oxidative stress in the body. After the course of the antioxidant complex, a significant decrease in TBARS concentration (by 33%) was noted, which attested to a decrease in the toxic effect of LPO products on the body (Table 2).

In men recovered from COVID-19, a decrease in total blood AOA by 15% was found in comparison with the control. After the course of the antioxidant complex, the total blood AOA increased by 20%. A decrease in the content of TBARS and stabilization of the total blood AOA indicate that the balance in the LPO–AOD system was restored.

COVID-19 is a dangerous socially significant disease, the treatment and prevention of which is currently under study and development. Men who have had COVID-19 often complain of decreased libido, weak erection, and ejaculation disorders, which could appear due to the progression of endothelial dysfunction, as well as testicular insufficiency and reduced testosterone production [4,12].

Under preclinical conditions, hyperproduction of ROS was found in SARS-CoV [13]. It is known that respiratory viruses can cause increased production of free radicals due to activation of the ROS generating enzymes (NADPH oxidase, xanthine oxidase, and inducible NO synthase) and the development of mitochondrial dysfunction [8]. The use of antioxidants to reduce the negative effects of oxidative stress is the subject of multiple clinical studies consistently showing a reduction in oxidative stress and DNA fragmentation in spermatozoa [5,7,9,11,15].

The AOD system consists of enzymes such as superoxide dismutase, glutathione peroxidase, and catalase, and non-enzymatic antioxidants – vitamins, trace elements, and substrates with thiol groups that act as LPO inhibitors. Consequently, peroxide damage to spermatozoa depends not only on excessive

TABLE 1. Characteristics of Spermatozoa in the Studied Groups ($M \pm \sigma$)

Parameter	Patients without history of COVID-19 ($n=20$)	Patients recovered from COVID-19 ($n=25$)	Patients receiving antioxidant complex ($n=25$)
Ejaculate volume, ml	3.36±0.26	2.44±1.55	3.13±1.36
Sperm concentration, mln/ml	74.3±37.4	40.40±30.54*	50.50±34.91
Mobility, %	51.59±13.05	34.80±14.48*	45.00±9.24*
Non-motile spermatozoa, %	35.70±9.42	54.80±17.54	48.40±10.68
Normal forms of spermatozoa, %	26.9±21.13	22.00±21.68	25.10±21.45
Viability of spermatozoa, %	67.1±24.23	52.00±22.19*	62.70±22.71
Leukocytes, mln/ml	0.61±0.21	1.16±0.37*	0.59±0.18*

Note. $p \leq 0.05$ in comparison with *patients without history of COVID-19, *patients with history of COVID-19.

TABLE 2. Parameters of Free Radical and Antioxidant Processes in the Studied Groups ($M\pm\sigma$)

Parameter	Patients without history of COVID-19 ($n=20$)	Patients recovered from COVID-19 ($n=25$)	Patients receiving antioxidant complex ($n=25$)
DC, $\mu\text{mol/liter}$	1.09 \pm 0.84	1.08 \pm 0.62	1.14 \pm 0.42
TBARS, $\mu\text{mol/liter}$	1.27 \pm 0.64	2.49 \pm 0.32*	1.67 \pm 0.13*
AOA, arb. units	20.06 \pm 5.79	17.03 \pm 3.18*	21.25 \pm 4.22*

Note. $p\leq 0.05$ in comparison with *patients without history of COVID-19, *patients recovered from COVID-19.

production of ROS, but also on the integrity of the entire antioxidant system.

Improvement in sperm function can be associated with a decrease in ROS as a result of increase in linear velocity and a decrease in DNA damage. For instance, in patients with a history of COVID-19, improvement in the total number of motile spermatozoa and in terms of asthenic condition was noted after a 3-month course of antioxidant complex BESTFertil [14].

Currently, researchers describe the biological functions and clinical benefits of using astaxanthin to protect against the action of ROS [10]. US Food and Drug Administration (FDA) in 1999 approved the use of astaxanthin as a nutraceutical and it was recommended for improving sperm quality. After astaxanthin treatment, changes in the phospholipid composition of the spermatozoon membrane improving its fluidity were registered, which may explain higher capacity of spermatozoa to penetrate the egg. A tendency towards an increase in the testosterone concentration and a significant decrease in the concentration of inhibin B was noted after astaxanthin treatment. This can indicate that impaired secretion of inhibin B in subfertile men is caused by excessive production of ROS.

Recent studies also show that astaxanthin restores steroidogenesis in Leydig cells by reducing the formation of ROS and affects the increase in progesterone and testosterone levels during long-term use [11]. Encouraging results were obtained in an animal model with a high-fat diet. In the group of animals treated with astaxanthin, a significant increase in sperm motility was revealed, and histological examination showed a significant increase in the number of spermatogonia in comparison with these parameters in the group not treated with astaxanthin [15].

The deficit of one or more links of the antioxidant system in tissues leads to the loss of the protection against the action of free radicals, which leads to damage to tissues and organs. The indicator of total AOA characterizes the total activity of peroxidation inhibitors, determining its buffer capacity and reflecting the relative amount of bioantioxidants and their interactions. It depends, among other things, on the

presence of substances that themselves do not have an antioxidant effect or prooxidant action, but are able to enhance or weaken the action of bioantioxidants.

In our study, the positive effect can be due not only to astaxanthin, but also to the potentiating effect of the complex of unsaturated fatty acids that also have ROS quenching properties.

Data obtained by research groups from different countries indicate that the COVID-19 pandemic poses a threat to men of reproductive age. The study of the pathogenetic mechanisms of the influence of infection SARS-CoV on male fertility will improve diagnostic and treatment algorithms, as well as determine further prospects for the use of antioxidant therapy. The assessment of the state of LPO and AOD processes has an important prognostic value in the study of male reproductive health and contributes to the knowledge of the pathogenetic processes of the disease and the rationale for the use of antioxidants and membrane stabilizing drugs to protect body cells from the toxic effects of ROS.

The effectiveness of antioxidant correction and, accordingly, protection of the structure and function of cell membranes from free radical damage proves the feasibility of using the studied antioxidant complex based on astaxanthin in men with impaired spermatogenesis during the rehabilitation period after undergoing COVID-19.

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