6. Haidl G., Haidl F., Allam J. P., Schuppe H. C. Therapeutic options in male genital tract inflammation. *Andrologia*. 2019;51(3):e13207. <https://doi.org/10.1111/and.13207>
7. Kulchenko N. G. Qualitative Criteria for Morphological Evaluation of Spermatogenesis in Azoospermia. *Difficult patient*. 2018;16(7):48-50.
8. Zandieh Z., Vatannejad A., Doosti M., Zabihiadeh S., Haddadi M. [et al.] Comparing reactive oxygen species and DNA fragmentation in semen samples of unexplained infertile and healthy fertile men. *Irish Journal of Medical Science*. 2018;187(3):657-662. <https://doi.org/10.1007/s11845-017-1708-7>
9. Gautam R., Singh K. V., Nirala J., Murmu N. N., Meena R. [et al.] Oxidative stress-mediated alterations on sperm parameters in male Wistar rats exposed to 3G mobile phone radiation. *Andrologia*. 2019;51(3):e13201. <https://doi.org/10.1111/and.13201>
10. Kulchenko N. G. Oksidativnyj stress v razvitii neobstruktivnoj azoospermii. *Difficult patient*. 2017;15(4-5):44-46.
11. Meena R., Kumari K., Kumar J., Rajamani P., Verma H. [et al.] Therapeutic approaches of melatonin in microwave radiations-induced oxidative stress-mediated toxicity on male fertility pattern of Wistar rats. *Electromagnetic Biology and Medicine*. 2014;33(2):81-91. <https://doi.org/10.3109/15368378.2013.781035>
12. Kulchenko N. G. Antioxidant therapy for patospermia. *Vestnik medicinskogo instituta «REAVIZ»: reabilitaciya, vrach i zdorov'e*. 2018;31(1):41-48.
13. Schuppe H. C., Pilatz A., Hossain H., Diemer T., Wagenlehner F. [et al.] Urogenital infection as a risk factor for male infertility. *Deutsches Ärzteblatt International*. 2017;114:339-346. <https://doi.org/10.3238/arztebl.2017.0339>
14. Adams J. A., Galloway T. S., Mondal D., Esteves S. C., Mathews F. Effect of mobile telephones on sperm quality: A systematic review and meta-analysis. *Environment International*. 2014;70:106-112. <https://doi.org/10.1016/j.envint.2014.04.015>
15. Kulchenko N. G. Inflammation and infertility. What is common? *RUDN Journal of Medicine*. 2017;21(4):402-407.
16. Ihsan A. U., Khan F. U., Khongorzul P., Ahmad K. A., Naveed M. [et al.] Role of oxidative stress in pathology of chronic prostatitis/chronic pelvic pain syndrome and male infertility and antioxidants function in ameliorating oxidative stress. *Biomedicine & Pharmacotherapy*. 2018;106:714-723. <https://doi.org/10.1016/j.biopha.2018.06.139>
17. Gromov A. I., Bujlov V. M. Radiation diagnostics and therapy in urology: national manual. M.: GOEHTAR-Media, 2011:544.
18. Kandola K., Bowman A., Birch-Machin M. A. Oxidative stress- a key emerging impact factor in health, ageing, life-style and aesthetics. *Int. J. Cosmet. Sci.* 2015;37(2):1-8.

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THE FREQUENCY OF POLYMORPHISM –262 C>T CAT GENE OF INFERTILE MEN IN THE MOSCOW REGION

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ЧАСТОТА ПОЛИМОРФИЗМА –262 С>Т ГЕНА САТ У БЕСПЛОДНЫХ МУЖЧИН МОСКОВСКОГО РЕГИОНА

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Follow up, 138 men of reproductive age of the Moscow region were performed. The standard clinical examination with double spermogram analysis and determination of reactive oxygen species in the ejaculate was produced. Using the polymerase chain reaction of DNA synthesis, we examined DNA samples of blood leukocytes for the presence of –262 C>T polymorphisms of the CAT gene. The semen analysis of 70 (50.7 %) patients showed pathospermia (main group). In 68 (49.3 %) men, we discovered normozoospermia (control group). In 35 (50.0 %) patients with pathospermia identified local mutations of the gene CAT. Also, 2/3 of the participants were registered with the heterozygous genotype (262CT), and 7(10 %) men were marked by the genotype homozygous for the defective allele (262TT). In 1/3 of men with normozoospermia were found-

ed local mutations of the CAT gene in both heterozygous and homozygous types. We defined a direct correlation between the level of reactive oxygen species and the frequency of CAT gene polymorphisms –262 C>T in infertile men ($r=0.7$). In conclusion, the CAT C–262T (rs1001179) gene polymorphism occurs in 50 % of cases in infertile men of Moscow region. Allele –262t of the CAT catalase gene can be considered as a genetic risk factor for oxidative stress and pathospermia.

Keywords: male infertility, oxidative stress, CAT gene, polymorphism, pathospermia

Обследовано 138 мужчин репродуктивного возраста Московского региона. Всем мужчинам проводили стандартное клиническое обследование с двукратным анализом спермограммы и определением активных форм кислорода в эякуляте. Методом полимеразной цепной реакции синтеза ДНК изучали образцы ДНК лейкоцитов крови на наличие полиморфизмов –262 C>T гена CAT. По данным спермограммы у 70 (50,7 %) пациентов выявили патоспермию (основная группа). У 68 (49,3 %) мужчин определили нормозооспермию (контрольная группа). У 35 (50 %) пациентов с патоспермией выявили локальные мутации гена CAT. При этом у 2/3 мужчин был зарегистрирован гетерозиготный генотип (262СТ), а у 7 (10 %) – гомозиготный генотип по дефектному аллелю (262ТТ). У 1/3 мужчин с нормозооспермией обнаружены локальные мутации гена CAT как по гетерозиготному, так и по гомозиготному типу. Выявлена прямая корреляция уровня активных форм кислорода и частоты полиморфизмов –262 C>T гена CAT у infertильных мужчин ($r=0,7$). Таким образом, полиморфизм гена каталазы CAT C–262T (rs1001179) встречается в 50 % случаев у infertильных мужчин Московского региона. Аллель –262T гена каталазы CAT можно рассматривать как генетический фактор риска развития оксидативного стресса и патоспермии.

Ключевые слова: мужское бесплодие, оксидативный стресс, ген CAT, полиморфизм, патоспермия

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CAT – catalase
ROS – reactive oxygen species

SNP – single nucleotide polymorphisms

Every fourth married couple in the world seeks medical care with the problems of the natural conception of a child [1, 2]. At the same time, in 50 % of cases, violations in the spermogram are detected, which means the malefactor of infertility [3, 4, 5]. Spermogram parameters do not always allow to recognize the cause of male infertility [6]. It becomes even more difficult to correct the pathospermia when no visible reasons for its occurrence can be found, and doctors are forced to state idiopathic infertility [3, 7]. Currently, oxidative stress is considered to be one of the leading causes of male infertility [3, 5]

Oxidative stress is an imbalance between the level of reactive oxygen species (ROS) and the ability of the human biochemical system to block reactive oxygen species [2, 3]. In case of a violation of antioxidant protection in a man, the cell membrane of the sperm cell becomes damaged, DNA fragmentation and apoptosis of germ cells occur [1, 2]. It causes a violation of quantitative and qualitative indicators of the sperm, up to the total death of male gametes [3, 5].

In the human body, there is a «natural» antioxidant defense, which is represented by a group of enzymes, including catalase. Catalase (CAT) can break down toxic forms of hydrogen peroxide (H_2O_2), converting it into H_2O and O_2 [8]. Cat enzyme is localized in chromosome 11p13. The locus of polymorphism –262 C>T (rs1001179) is situated in the promoter region of the gene CAT and affects the gene expression levels of CAT and the activity of the enzyme [8, 9].

To date, the question of the genetic causes of the enzymatic antioxidant system in infertile men has been little studied. The link of C–262T polymorphism with infertility in men in the Moscow region has not been previously studied.

The purpose of the research was to determine the frequency of polymorphism –262 C>T CAT gene of infertile men in the Moscow region.

Material and Methods. The study was prospective. Patients were included in the study on a blind principle. A total of 138 men of reproductive age from the Moscow region were included. Inclusion criteria: age of patients older than 18 years, absence of severe somatic and endocrine pathology, the reproductive capacity of their wives was clinically confirmed. Exclusion criteria were: genetic abnormalities (detected by karyotyping), leukospermia, sexually transmitted infections, obstructive infertility, varicocele.

All the men underwent a standard clinical examination, a two-fold analysis of semen (according to the standard who, 2010). The reactive oxygen species were determined in the native sperm by luminol-dependent chemiluminescence (LUM-5773, Russia).

We performed a genetic analysis of the genomic DNA of peripheral blood leukocytes using the DNA-EXTRAN-1-blood kit produced by «Syntol» (Russia).

Using a real-time polymerase chain reaction for DNA synthesis (Real-Time-PCR), we studied the DNA samples for the presence of –262 C>T polymorphisms of the CAT gene. For PCR, we used ready-made kits made by «Syntol» (Russia). PCR was performed on a CFX96 amplifier (Bio-Rad, USA) with CFX Manager TM software.

Statistical processing was performed with the help of spreadsheets «MS Excel 2003» (Microsoft Office, USA). The differences were considered significant at $p<0.05$.

Results and Discussion. The average age of all patients was 32.4±5.6 years. According to the spermograms, we revealed pathospermia (quantitative and qualitative disorders of the ejaculate) in 70 (50.7 %)

patients. These patients were included in the main research group. In the ejaculate of 68 (49.3 %) men, we revealed normozoospermia (the control group). The number of patients with different sperm characteristics in the main group is shown in Figure.

Fig. The number (frequencies) of the patients of main group with spermogram characteristics

In men with normozoospermia, the average number of reactive oxygen species was 13.4 ± 4.1 RLU/s $\times 10^6$, in patients with pathospermia – 27.9 ± 8.4 RLU/s $\times 10^6$ ($p < 0.05$). The frequency distribution of polymorphisms –262 C>T of the CAT gene in men of the Moscow region is presented in the Table.

Table
The frequency of polymorphisms –262 C>T of CAT gene in the groups men of Moscow region

CAT single nucleotide polymorphisms (SNP)	CC		CT		TT	
	n	%	n	%	n	%
Main group (pathospermia, n=70)	35	50	28	40	7	10
Control group (normozoospermia, n=70)	47	69.1	19	27.9	2	3.0
P	0.04		0.05		0.02	

In 35 (50 %) patients with pathospermia we have identified local mutations of the gene CAT, and

about 2/3 of the patients were registered with the heterozygous genotype (262CT), and 7 (10 %) men showed the genotype homozygous for the defective allele (262TT). It is interesting to note that in 1/3 of men with normozoospermia, we also found local CAT gene mutations in both heterozygous and homozygous types. Consequently, these patients may be at risk for male infertility. We found a direct correlation between the level of reactive oxygen species and the frequency of –262 C>T polymorphisms of the CAT gene in infertile men ($r=0.7$).

Catalase is an endogenous antioxidant enzyme, and genetic changes in the regulatory regions of the CAT gene can alter the activity of the CAT enzyme and can subsequently alter the risk of disease associated with oxidative stress [9].

Several major studies have shown that single nucleotide polymorphism (SNP) in the CAT gene may be associated with many diseases [5, 10, 11]. According to Tefik T. et al., men with the TT CAT C262T genotype are twice as likely to have prostate cancer [10].

The results of the work of V. C. Sousa et al. patients with chronic hepatitis C were shown to have a higher risk of developing liver fibrosis or hepatocellular carcinoma ($p=0.009$; OR 2.40) in the CT+TT genotype of CAT C–262T (rs1001179) [11].

Rodriguez A. G. revealed statistically significant differences between fertile and infertile males for STP CAT C–262T. the Authors found that allele carriers of 262T (C+T genotypes) had a twice higher risk of male infertility compared to healthy individuals ($p=0.001$; OR 2.26) [5]. Our study showed that an increased amount of reactive oxygen species in the ejaculate in men with pathospermia is associated with CAT C–262T (rs1001179) gene polymorphism. In half of the infertile men, oxidative stress is associated with polymorphism of the antioxidant enzyme catalase. The results of our research are comparable with the studies of foreign authors [5]. We believe that further work on the study of the Association of polymorphism C–262T (rs1001179) of the CAT gene in infertile men is relevant because studies of this nature have not been conducted in the Russian Federation so far.

Conclusions. The catalase (CAT) gene C–262T (rs1001179) polymorphism occurs in 50 % of cases in infertile men of the Moscow region. The allele –262T of the CAT catalase gene can be considered not only as a genetic risk factor for oxidative stress and pathospermia, but also as a prognostic factor for the successful treatment of male infertility factor.

Disclosures:

The authors declare no conflict of interest.

References

1. Vinogradov I. V., Zhivulko A. R., Vinogradova L. M., Korolev S. V. Docosahexaenoic acid in the treatment of male infertility. *Andrology and Genital Surgery*. 2018;19(4):21-27. <https://doi.org/10.17650/2070-9781-2018-19-4-21-27>
2. Agarwal A., Virk G., Ong C., du Plessis S. S. Effect of oxidative stress on male reproduction. *The World Journal of Men's Health*. 2014;32:1-17.
3. Kulchenko N. G. Oxidative stress in the development of non-obstructive azoospermia. *Difficult patient*. 2017;15(4-5):44-46.
4. Aitken R. J., Baker M. A., De Iulius G. N., Nixon B. New insights into sperm physiology and pathology. *Handb. Exp. Pharmacol.* 2010;(198):99-115.
5. Rodriguez A. G., de la Casa M., Johnston S., Gosalvez J., Roy R. Association of polymorphisms in genes coding for antioxidant enzymes and human male infertility. *Annals of Human Genetics*. 2019;83(1):63-72. <https://doi.org/10.1111/ahg.12286>
6. Kulchenko N. G., Myandina G. I., Alhedjoj H. Association-genetic study of polymorphism G-105A SEPS1 gene in male infertility. *Research and Practical Medicine Journal*. 2018;5(2):65-71. <https://doi.org/10.17709/2409-2231-2018-5-2-7>
7. Kaprin A. D., Kostin A. A., Kulchenko N. G., Fomin D. K., Aliev A. R. O. Diagnosis of idiopathic infertility. What's new? *Vestnik Rossijskogo nauchnogo centra rentgenologii Minzdrava Rossii*. 2014;2:3.
8. Sabouhi S., Salehi Z., Bahadori M. H., Mahdavi M. Human catalase gene polymorphism (CAT C-262T) and risk of male infertility. *Andrologia*. 2014;47:97–101.
9. Saify K. Genetic polymorphisms in the promoter region of catalase gene, creates new potential PAX-6 and STAT4 response elements. *Mol. Biol. Res. Commun.* 2016;5(2):97-100.
10. Tefik T., Kucukgergin C., Sanli O., Oktar T., Seckin S. [et al.] Manganese superoxide dismutase Ile58Thr, catalase C–262T and myeloperoxidase G-463A gene polymor-

phisms in patients with prostate cancer: relation to advanced and metastatic disease. *BJU Int.* 2013;112(4):406-414.

11. Sousa V. C., Carmo R. F., Vasconcelos L. R., Aroucha D. C., Pereira L. M. [et al.] Association of Catalase

and Glutathione Peroxidase 1 Polymorphisms with Chronic Hepatitis C Outcome. *Ann. Hum. Genet.* 2016;80(3):145-53. <https://doi.org/10.1111/ahg.12152>

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EFFECTIVENESS OF ANTI-VEGF AGENTS IN PATIENTS WITH RETINAL VEIN OCCLUSION

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ЭФФЕКТИВНОСТЬ АНТИ-VEGF ТЕРАПИИ У ПАЦИЕНТОВ С ОККЛЮЗИЕЙ ВЕН СЕТЧАТКИ

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Retinal vein occlusion is a significant medical problem that requires urgent correction. We conducted a prospective observational study (n=124 patients) to investigate the efficacy of anti-vascular endothelial growth factor therapy (intravitreal injections of aflibercept 2 mg in 0.05 mL) compared with standard treatment in patients with retinal vein occlusion. When post-thrombotic neovascular glaucoma occurred, the concentrations of transforming growth factor α and β in the lacrimal fluid in both groups (with and without aflibercept) increased by 1.7-fold and 28-fold, respectively. When aflibercept was administered, the blood flow rates in ophthalmic and central retinal arteries, as well as the maximum velocity in the superior ophthalmic and central retinal veins, were improved compared with patients who received standard therapy. Thus, aflibercept therapy for retinal vein occlusion leads to the normalization of blood flow in retinal vasculature, preventing neovascularization and providing a better clinical outcome for patients.

Keywords: central retinal vein occlusion, anti-angiogenic therapy, optical coherence tomography, angiography

В современной офтальмологии окклюзия вен сетчатки является одной из наиболее важных медицинских проблем, требующей поиска оптимальных методов коррекции. Нами выполнено проспективное наблюдательное исследование (n=124) по изучению эффективности антиангиогенной терапии (интравитреальные инъекции афлиберцепта 2 мг в 0,05 мл) по сравнению со стандартной терапией у пациентов с окклюзией вен сетчатки. Установлено, что при развитии посттромботической неоваскулярной глаукомы концентрации трансформирующих факторов роста α и β в слезной жидкости в обеих группах (с применением афлиберцепта и без) увеличивались в 1,7 и 28 раз соответственно. При введении афлиберцепта скорость кровотока в центральной артерии сетчатки и максимальная скорость в центральной вене сетчатки улучшались по сравнению с пациентами, получавшими стандартную терапию. Таким об-