

The Peculiarities of Immune Regulation and the Polymorphic Variants of Candidate Genes Associated With Them in Oil Production Workers With Vegetative Regulation Disorders

Nikonoshina N.A.* Dolgikh O.V.

Federal Scientific Research Center for Medical and Preventive Health Risk Management Technologies, Perm, Russia

*Corresponding author. Email: nat08.11@yandex.ru

ABSTRACT

The analysis of the peculiarities of the immune status and the associated polymorphic variants of candidate genes in workers of an oil-extracting industry with established disorders of autonomic regulation – vegetative dystonia syndrome – was carried out. Pathology of the autonomic nervous system in the examined group of persons exposed to a complex of harmful production factors (excessive concentrations of aromatic hydrocarbons, noise intensity, vibration, unfavorable climatic conditions) is associated with suppression of the cellular link of the immune response according to the criterion of reducing the number of activated CD3 + CD4 + lymphocytes relative to the group comparison ($p < 0.05$) with a simultaneous increase in the content of regulatory CD3 + C25 + CD127-lymphocytes in relation to the reference values ($p < 0.05$). Signs of inhibition of apoptosis processes (CD95 + deficiency) are observed in the examined individuals, regardless of the presence of autonomic regulation disorders. The humoral immune status of the surveyed contingent is characterized by an increased level of serum IgM production relative to the comparison group. A decrease in the phagocytic activity of leukocytes was established according to the criteria of the percentage of phagocytosis and absolute phagocytosis, as well as the level of expression of the neurotransmitter – dopamine – relative to the comparison group. The revealed changes in immune and humoral regulation in oil production workers with vegetative dystonia syndrome are associated with the variant allele of the G>A CYP2C19*2 gene and the wild-type allele of the C>T FAS gene.

Keywords: harmful production factors, vegetative dystonia syndrome, immune status, apoptosis, genetic polymorphism

1. INTRODUCTION

The preservation and strengthening of the health of the working-age population, involving the prevention and early diagnosis of occupational diseases, is the most important task of modern medical science. Harmful and hazardous chemical (high concentrations of aromatic hydrocarbons), physical (noise intensity, vibration, unfavorable climatic conditions), as well as psychophysiological production factors of an oil production enterprise negatively affect the health of persons involved in oil production [1–3]. At the same time, the exposure of professional groups to these factors in the workplace is more pronounced compared to the environment, which can provoke early disturbances in adaptive reactions, imbalance in immune, humoral, and nervous regulation of physiological functions of the body [4–6].

Purpose of the work: the analysis of the characteristics of immune regulation and associated polymorphic variants of candidate genes in workers of an oil production enterprise with disorders of autonomic regulation of physiological functions of the body.

2. METHODS AND MATERIALS

The study of indicators of the immune status and polymorphism of candidate genes in male workers of an oil production enterprise at the age of 28–59 years with established disorders of autonomic regulation of physiological functions of the body (syndrome of vegetative dystonia) was carried out. A total of 170 people were examined. The observation group consisted of 91 men with an established diagnosis of vegetative dystonia syndrome. The comparison group consisted of 79 men without clinical manifestations of this pathology.

The groups are comparable in age, social status and ethnicity.

The study of populations and subpopulations of lymphocytes (CD3+CD4+, CD127-, CD3+CD25+, CD3+CD95+) was carried out by flow cytometry on a FACSCalibur device (Becton Dickinson, USA) using the CellQuestPro universal program.

The study of the phagocytic activity of venous blood leukocytes (percentage of phagocytosis, absolute phagocytosis) was carried out using formalized sheep erythrocytes.

Dopamine content was determined by enzyme-linked immunosorbent assay (ELISA).

In the subjects, SNP (single-nucleotide polymorphisms) of 2 genes was studied using real-time PCR on a CFX96 Real Time System C1000 Thermal Cycler (BioRAD, Singapore). Genetic material was isolated from buccal scrapings using a set of reagents for DNA extraction from clinical material "AmpliPrime DNA-sorbB Form 2 variant 100" (LLC "NextBio", Russia) using the sorbent method. To determine the genetic polymorphism of the studied genes, we used the test system (Sintol, Russia) – reagent kits for determining polymorphisms: G861A (rs4244285) of the *CYP2C19**2 gene of the 2C19 isoenzyme (S-mephenitoin hydroxylase) of cytochrome P450; C14405T (rs1159120) of the *FAS* membrane receptor gene for *FAS* lymphocytes. To determine the human genotype, we used the method of allelic discrimination in the specialized TaqMan program when the differences between heterozygotes, homozygotes of the wild and minor variants were established by the differences in the course of amplification reactions of the corresponding primers.

Methods of mathematical statistics were used for statistical processing of the research results using the Statistica 6.0 applied software package (StatSoft, USA). To assess the level of reliability of the data obtained, the parametric Student's test was used, taking into account the normal distribution of variables in the compared groups. In case of deviation from the normal distribution, the nonparametric Mann-Whitney U test was used to compare the data. The research results are presented as the mean (M) and the error of the mean (m) of the studied indicators. Differences between groups were considered statistically significant at $p < 0.05$.

The distribution of the frequencies of genotypes and alleles, as well as the odds ratio OR and its 95 % confidence interval (CI) in the observation and comparison groups were calculated using the unified SNPStat program used to calculate statistical parameters for case-control studies using SNP (single nucleotide polymorphism diagnostics).

3. RESULTS

As a result of the clinical and laboratory study of the blood samples from the workers in the oil refining industry with revealed disorders of autonomic regulation – vegetative dystonia syndrome – changes in the parameters of immune

regulation of physiological processes in the body (TABLE I) were established.

52.4 % of the persons included in the observation group revealed a statistically significant ($p < 0.05$) decrease in both absolute and relative content of CD3+CD4+ lymphocytes relative to the values of these indicators in the comparison group.

It was also found that the absolute and relative content of regulatory T-lymphocytes with the CD127- phenotype in the observation group is statistically significantly ($p < 0.05$) higher than the same value in the comparison group, which also indicates signs of suppression of the cellular immune response in oil production workers with the vegetative dystonia syndrome. In addition, the average values of the relative content of CD127-lymphocytes significantly exceed the reference level, regardless of the presence of autonomic regulation disorders. Signs of inhibition ($p < 0.05$) of the phagocytic activity of peripheral venous blood leukocytes were found in 51.6 % of workers in the oil industry with vegetative dystonia syndrome according to the criteria of the percentage of phagocytosis and absolute phagocytosis relative to the comparison group.

The detected production of antibodies of the IgM class in the blood serum of oil production workers with disorders of autonomic regulation significantly exceeds the same indicator in the comparison group.

In addition, the observation group was distinguished by a significantly reduced level of the neurotransmitter dopamine relative to the comparison group. According to the literature, dopamine may be involved in the mechanisms of development of pathologies of the nervous system, which may be directly related to impaired immunoregulation. It has been shown that dopamine can participate in modulating the immune response through specific receptors of immunocytes (Th17, B-lymphocytes and NK-cells), which are also actively involved in immunoregulation [7, 8]. Consequently, suppression of the characteristics of the immune response, a decrease in the level of dopamine and the level of expression of its receptors may be associated with the development of disorders of autonomic regulation of physiological functions of the body [9].

The revealed changes in the immune regulation in the workers with established disorders of autonomic regulation are reliably associated with polymorphic variants of candidate genes for detoxification and apoptosis (TABLE II).

The gene pool of the observation group is distinguished by an increased frequency of the variant A-allele of the cytochrome P450 gene *CYP2C19**2 (rs4244285) due to an increase in the proportion of variant homozygotes and heterozygotes for this gene in the surveyed sample (OR = 2.69; CI: 1.05–8.45). According to the literature, the replacement of G681A in the *CYP2C19* gene determines the expression of an enzyme with low activity and a decrease in the metabolic rate of xenobiotics and drugs in carriers of the variant genotype [12].

Table 1 Peculiarities of immune and humoral regulation in employees of the oil production enterprise with the syndrome of vegetative dystonia

Indicator	Reference interval	Observation group (n = 91)	Comparison group (n = 79)
Bah, %	5–9	18.808±2.159*/**	12.198±2.064**
CD3+CD25+ lymphocytes, abs., 10 ⁹ /л	0.19–0.56	0.327±0.066*	0.561±0.142
CD3+CD25+ lymphocytes, rel., %	13–24	14.571±3.442*	23.75±4.667
CD3+CD95+ lymphocytes, abs., 10 ⁹ /л	0.63–0.97	0.827±0.193*	0.488±0.083
CD3+CD95+ lymphocytes, rel., %	39–49	34.000±5.494*	22.286±3.584
CD127- lymphocytes, abs, 10 ⁹ /cubic dm	0.015–0.04	0.177±0.126**	0.157±0.038**
CD127- lymphocytes, rel., %	0.8–1.2	7.423±5.096**	6.550±2.244**
IgA, g/cubic dm	1.1–3	2.548±0.095*	3.091±0.155
IL-1β, pg/ml	0 –11	1.762±0.237*	1.173±0.216

Note: * differences are statistically significant relative to the comparison group ($p < 0.05$).

** differences are statistically significant relative to the reference level ($p < 0.05$).

Table 2 Peculiarities of immune and humoral regulation in employees of the oil production enterprise with a syndrome of vegetative dystonia. Distribution of the frequencies of alleles and genotypes of candidate genes in employees of the oil production enterprise with the syndrome of vegetative dystonia

Gene/allele		Observation group (n = 91)	Comparison group (n = 79)
<i>CYP2C19</i> *2 G861A rs4244285	G/G	55.6	75.0
	G/A	33.1	25.0
	A/A	11.3	0
	G	72.8	87.5
	A	28.2	12.5
<i>FAS</i> C14405T rs1159120	C/C	63.0	41.2
	C/T	37.0	51.0
	T/T	0	7.8
	C	81.5	66.7
	T	18.5	33.3

The polymorphism of the gene for the regulator of apoptosis FAS (rs1159120) is characterized by a significant ($p < 0.05$) increase in the frequency of the C-allele of the wild type due to the significant prevalence of wild-type homozygotes in the group of oil production workers with disorders of autonomic regulation relative to the comparison group. According to the available literature data, the predominance of the C-allele of the wild-type FAS gene is associated with an increase in the expression level of the membrane receptor of lymphocytes, but the surveyed population is characterized by only a slight increase in the content of CD3+CD95+ lymphocytes. At the same time, the level of CD3+CD95+ lymphocytes is statistically significantly ($p < 0.05$) decreased relative to the reference level, regardless of the presence of violations of autonomic regulation of physiological functions of the body in oil workers, which indicates the suppression of apoptotic processes in the examined individuals [11, 12].

4. CONCLUSION

Thus, a group of employees of an oil production enterprise with established disorders of autonomic regulation of physiological functions of the body – vegetative dystonia syndrome – is characterized by changes in immune regulation indicators, indicating signs of suppression ($p < 0.05$) of the cellular immune response (CD3+CD4+, CD127-) and apoptosis processes (CD3+CD95+) relative to similar values in the comparison group and the reference interval. A decrease in the phagocytic activity of leukocytes was established according to the criteria of the percentage of phagocytosis and absolute phagocytosis relative to the comparison group. The humoral immune status of the surveyed contingent is characterized by an increased level of serum IgM production relative to the comparison group. Oil production workers with autonomic dystonia syndrome have reduced dopamine levels in relation to the comparison group. The revealed changes in immune and humoral regulation in oil production workers are associated with the variant allele of the CYP2C19*2 gene and the wild-type allele of the FAS gene. The established features of the parameters of immune (CD3+CD4+, CD127-, CD95+, IgM, percentage of phagocytosis, absolute phagocytosis) and humoral regulation associated with polymorphic variants of candidate genes CYP2C19*2 and FAS form a complex of features of the immune status of oil production workers associated with development of pathology of the autonomic nervous system, under the influence of a complex of harmful production factors.

REFERENCES

- [1] G.G. Gimranova, L.K. Karimova, T.M. Zotova et al., Occupational health risks of workers involved in oil refining, Occupational health and human ecology 11 (2009) 9–12.
- [2] O.V. Dolgikh, A.V. Krivtsov, K.G. Starkova et al., Immune and genetic changes in workers exposed to industrial noise and dust, Occupational health and human ecology 9(i.2) (2015) 551–553.
- [3] L.M. Karamova, G.R. Basharova, E.T. Valeyeva et al., Health status of healthy oil and petrochemical workers, Occupational health and human ecology 4 (2015) 270–275.
- [4] M.Yu. Kuzmina, M.V. Shubin, Professional risks health of workers of the oil refining industry, Bull. of Kazan technolog. Univer. 19 (2011) 287–290.
- [5] R.R. Zakharova, G.N. Kalimullina, V.S. Romanov, Working conditions and health status of oil refining workers, Occupational health and human ecology 4 (2015) 120–122.
- [6] E.M. Vlasova, A.E. Nosov, T.A. Ponomareva, Yu.A. Ivashova, Autonomic dysfunctions in hazardous industries workers, Zdorov'e Naseleniya i Sreda Obitaniya i.12 (2014) 12–14.
- [7] K. Nakano, T. Higashi, K. Hashimoto et al., Antagonizing dopamine D1-like receptor inhibits Th17 cell differentiation: preventive and therapeutic effects on experimental autoimmune encephalomyelitis, Biochem. Biophys. Res. Commun. 22(i.373) (2008) 286–291.
- [8] A.K. Patanella, M. Zinno, D. Quaranta et al., Correlations between peripheral blood mononuclear cell production of BDNF, TNF-alpha, IL-6, IL-10 and cognitive performances in multiple sclerosis patients, J. Neurosci. Res. 88 (2010) 1106–1112.
- [9] E.V. Orlova, M.V. Pashchenkov, M.V. Davydovskaia et al., The role of dopamine in the regulation of the interaction between nervous and immune systems in multiple sclerosis, Zh. Nevrol. Psikiatr. Im. S.S. Korsakova 112(i.2) (2012) 34–40.
- [10] D. Rothenbacher, M. Hoffmann, L. Breitling et al., Cytochrome P450 2C19*2 polymorphism in patients with stable coronary heart disease and risk for secondary cardiovascular disease events: results of a long-term follow-up study in routine clinical care, BMC Cardiovascular Disorders 13 (2013) 61.
- [11] S. Sakaguchi, M. Miyara, C.M. Hafler, D.A. Costantino, FOXP3+ regulatory T cells in the human immune system, Nat. Rev. Immunol. 10 (2010) 490–500.
- [12] Z. Islam, I. Jahan, R.U. Ahammad et al., FAS promoter polymorphisms and serum sFas level are associated with increased risk of nerve damage in Bangladeshi patients with Guillain-Barré syndrome, PLoS ONE 13(i.2) (2018) e0192703.