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GENETIC AND IMMUNOLOGICAL MARKERS OF SENSITIVITY AND EFFECT IN WORKERS OF POTASSIUM INDUSTRIES EXPOSED TO PRODUCTION RISK FACTORS

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Abstract. An assessment of the immunological and genetic markers in the employees of the potassium industry showed that combined exposure to hazardous production factors (sylvinite dust, noise) can result in higher production of the markers of immune cytokine regulation: tumor necrosis factor (TNFalpha) and vascular epithelial growth factor (VEGF) as well as modified polymorphism of the encoding gene segments in the form of a higher prevalence of variant gene alleles due to minor homozygous (VEGF) and heterozygous (TNFalpha) genotypes. The polymorphism of detoxification genes CYP1A1, CPOX is characterized by specific differences with the comparison group. The TNFaalpha, VEGF, CYP1A1, and CPOX genes were recommended as sensitivity markers, and the encoded cytokine (tumor necrosis factor and endothelial growth factor) – as the effect markers for a health assessment study among the employees of the potassium industry.
Key words: sylvinite dust, noise, gene polymorphism.

The analysis of immunological and genetically mediated defense mechanisms in industrial workers is considered one of the most important areas of research of the adaptive processes under exposure to negative production factors [1,2,3,5,7,8,9,10]. Since deviations in immunological reactivity can appear at relatively low levels of exposure to combined hazardous physical (noise) and chemical (aerosol) production factors, early detection of the markers of the developing pathological changes is essential [4, 6].

The purpose of the study is to analyze the marker indicators of the immune and

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immunogenetic regulation in the employees of the potassium industry.

Materials and methods. The observation group in the conditions of combined exposure to hazardous production factors (sylvinite dust, noise) was made up of 184 employees of the potassium mining industry – men, operators of the mining winning machines, average age – 36.6±1.0 years old, average duration of employment – 7.3±0,9. The workers from the main group experience the impact of a number of dangerous and hazardous production factors including physical, particularly noise, and chemical factors – sylvinite dust in the air. The labor conditions are considered to be hazardous, with 3rd level 3rd class deviations from the hygienic standards (3.3 according to R 2.2.2006 – 05). The comparison group was made up of 55 employees – men who worked above the ground with no exposure to the hazardous factors under study, average age – 40.2±2.7 years old, average duration of employment – 5.8±1.9 years. The groups were comparable in terms of age, gender, and duration of employment.

The following parameters of the immune system were analyzed: the level of serum immunoglobulins by radial immunodiffusion method by Mancini; phagocytic activity indicators when used as the targets of phagocytosis of the formalinized male sheep erythrocytes, markers of intracellular immune regulation (tumor necrosis factor and erythropoietin), and markers of endothelial dysfunction - vascular endothelial growth factor by the method of enzyme immunoassay with the use of test systems. Statistical analysis was performed using descriptive statistics and a two-sample Student t-test. The differences between the groups were considered significant at $p < 0.05$.

The materials for PCR included oral mucosa swabs from which we separated the DNA using the sorbent method based on cell breakage followed by the sorption of nucleic acids on sorbent.

To analyze polymorphisms in genes, we used a PCR procedure based on real-time amplification and detection of the reaction products using fluorescent labels, which are used to mark the pre-amplification reaction primers. Amplification and detection were performed using a thermal cycler CFX96, using the structure of the primers and temperature cycling parameters described in the literature.

Different fluorescent labels and probes (multiplex PCR) are used for simultaneous detection of several reaction products. We used a DNA segment of cytochrome genes P-450 CYP1A1, coproporphyrinogen oxidase (CPOX), tumor necrosis factor (TNFalpha), vascular endothelial growth factor (VEGF), eNOS (G894T), APOE following the guidelines of the "List of the genetic polymorphism markers responsible for the mutagenic activity under technogenic chemical factors "(MP 13 from 4.2.0075- 08.20.2013).

To determine the genotype, we used allelic discrimination: here, the differences between the heterozygous, homozygous of the wild and minor variants were determined based on the differences in the amplification reaction behavior of the corresponding primers.

We processed the genotyping assay data using "Gene Expert" software which can calculate the statistical parameters for the "case-control" studies that use SNP (single nucleotide polymorphisms diagnostics). We used the Hardy–Weinberg principle to estimate the frequencies of alleles and genotypes.

The Results. In the course of the clinical and laboratory studies of the workers' health, we identified functional disorders of the immune system (see Table 1). In the group of patients, we observed significant changes in the level of innate cellular immune response as compared with the physiological norm. In 27.3% of the cases, we revealed inhibited phagocytic immunity indicators by the "absolute phagocytosis" criterion, in 62.9% of the cases – by the "percentage of phagocytosis" criterion, and in 76.5% of the cases – by the "phagocytic number» criterion ($p < 0.05$).

The comparison of the phagocytic segment with the similar indicators of the control group also showed a significant decrease in phagocytosis - absolute and relative number of phagocytes and the phagocytic number in 72.7%, 70.5% and 67.4% of patients, respectively ($p < 0.05$).

Table 1

Indicators of the immune status in the employees of the potassium industry

Indicator	Comparison group (n=53)	Observation group (n=132)
Absolute phagocytosis, $10^9/dm^3$	1,892±0,296	1,417±0,122*
Phagocytosis rate, %	43,189±4,359	33,114±2,001*
Phagocytic number, conditional units	0,733±0,088	0,605±0,052*
Phagocytic index, conditional units	1,668±0,062	1,773±0,046
IgG, r/dm^3	11,016±0,519	11,365±0,332
IgM, r/dm^3	1,392±0,086	1,258±0,061*
IgA, r/dm^3	2,327±0,173	2,296±0,104
Vascular endothelial growth factor, pg/cm^3	298,464±65,881	308,909±40,309
Tumor necrosis factor, pg/cm^3	0,783±0,18	1,773±0,161*
Erythrocyte maturing factor, mME/cm^3	-	19,891±5,429

Note: * – the difference is significant as compared to the comparison group ($p<0.05$).

We have also determined significant bidirectional changes in the level of serum immunoglobulins of the A, M and G classes with IgM and IgG deficit (in 88.5% and 51.1% of samples) and hyperproduction of IgA (in 87% of samples) as compared to the reference range. At the same time, we established a significant decrease in the IgM level as compared to the

comparison group ($p<0.05$).

The level of proapoptotic cytokine of the tumor necrosis factor was within the physiological range, but it was significantly 1.9 times higher as compared to the comparison group ($p <0.05$). Erythropoietin was within the reference level; and although an excess was observed in 13.3% of the employees, no significant differences were detected. The marker of endothelial status, the vascular endothelial growth factor significantly deviated from the age norm by the criterion excess frequency in the absence of significant differences in the comparison group.

The analysis of the results of the study of genetic polymorphisms of cytochrome-450 (CYP1A1), CPOX, VEGF, eNO-synthase, TNFalpha, eNOS, APO revealed that the allelic polymorphism of genes involved in the immune response and apoptosis (TNFalpha) is characterized by increased incidence of minor allele (2 fold) as compared to the comparison group, primarily because of the heterozygous genotype (see Table 2).

VEGF gene polymorphism differed from the comparison group in terms of prevalence of the gene in a mutant homozygous state. Polymorphism of the endothelial dysfunction genes (eNO-synthase) was comparable in the groups under study.

Polymorphism of the detoxification gene CYP1A1, CPOX is characterized by specific differences between the groups under study. The prevalence of abnormal allele CYP1A1 (cytochrome gene) responsible for phase 1 of organic toxicant detoxification is higher than in the comparison group because of homo- and heterozygous genotype.

Table 2

The distribution of gene frequencies of eNO-synthase, TNFalpha, VEGF, CYP1A1, APO, eNOS (G894T), CPOX in the employees of the potash industry

Genotype/allele		Observation group	Comparison group
	n=	184	55
VEGF	GG	52%	54%
	GC	37%	42%
	CC	11%	4%
	G	70%	75%
	C	30%	25%
cyp1A1(2) (A4889G)	AA	88%	91%
	AG	10%	9%
	GG	2%	0%
	A	93%	95%
	G	7%	5%
TNFalpha	GG	78%	89%
	GA	21%	9%
	AA	1%	2%
	G	89%	94%
	A	11%	6%
eNOS(G894T)	GG	57%	56%
	GT	35%	38%

	TT	8%	6%
	G	75%	75%
	T	25%	25%
CPOX	AA	64%	87,5%
	AC	32%	12,5%
	CC	4%	0%
	A	80%	93%
	C	20%	7%
APOE (Cys130Arg)	TT	75%	77%
	TC	24%	20%
	CC	1%	3%
	T	87%	88%
	C	13%	12%

Conclusions

1. In the employees of the potash industry, we revealed significant changes in the immune system, as compared to the normal physiological range and the comparison group, characterized by decreased phagocytosis activity and overproduction of the cytokine regulation markers: tumor necrosis factor (TNFalfa) and vascular endothelial growth factor (VEGF), as well as modified polymorphism of the encoding gene segments in the form of increased prevalence of variant alleles due to minor homozygous (VEGF) and heterozygous (TNFalpha) genotypes. The established modified gene polymorphism of detoxification genes CYP1A1, CPOX describes specific differences with the comparison group.

2. Based on the obtained data, we recommend the identified negative genetic associations (TNFalphaa, VEGF, CYP1A1, CPOX genes) as a marker for susceptibility testing, and their encoded cytokines (tumor necrosis factor and vascular endothelial growth factor) as an effect marker in assessing the health risks in the workers of the potassium industry.

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