

# Features of Immunological Indicators in Workers Under the Influence of Factors of the Production Environment

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## ABSTRACT

An assessment of the immune regulation, its cellular and humoral components was carried out for diagnosing early health disorders in potassium production workers affected by excessive contamination of biological media with formaldehyde. The purpose of the article is to study the influence of chemical factors of the working environment on the parameters of immune regulation in workers of the mining industry. The observation group included 157 people aged  $43.2 \pm 1.3$  years working in hazardous working conditions. The control group included 78 patients aged  $44.5 \pm 1.2$  years working as managers of the company. Phagocytosis was assessed using formalized sheep erythrocytes. Serum immunoglobulins A, M, G were determined by the method of radial immunodiffusion according to Mancini. Specific IgE to formaldehyde was determined by the allergosorbent test with an enzyme label. Total IgE, interleukin-1 $\beta$ , tumor necrosis factor were determined using enzyme-linked immunosorbent assay. CD lymphocytes were phenotyped by flow cytometry. Populations and subpopulations of lymphocytes (CD16 +, CD19 +, CD25 +, CD95 +) were determined by the method of membrane immunofluorescence. The significance of differences between the groups was considered significant at  $p < 0.05$ . According to the results of chemical analytical studies, an excess of the permissible level of formaldehyde in the blood was observed both in the observation group and in the comparison group. There was a significant decrease in the phagocytic activity according to the criterion of absolute phagocytosis ( $p \leq 0.05$ ). A significantly increased content of serum immunoglobulins G, A, M was established. At the same time, an increased level of general sensitization and an increased level of specific sensitization to formaldehyde were revealed in 21.8 % of workers ( $p < 0.05$ ). An increased expression of the killer lymphocyte receptors CD16 + CD56 + and CD19 + was revealed ( $p < 0.05$ ). Activation of cell death receptors CD95 + and CD25 + was also observed ( $p < 0.05$ ). An excess of the level of IL-1 $\beta$  and a decrease in the level of TNF relative to the control group ( $p < 0.05$ ) were observed. The content of the mediator of catecholamine regulation of serotonin was lower in the observation group, and the hormone of the adrenal cortex cortisol was higher. According to the results of an immunological study of mining workers, deviations in the system of humoral and cellular immunity in the form of a decrease in the phagocytic activity, an increase in the content of serum immunoglobulins, the level of general and specific sensitization, increased expression of killer lymphocytes CD19 + and CD16 + CD56 + and cell death receptors CD25 + and CD95 + were revealed. An increase in the content of IL-1 $\beta$  and inhibition of TNF were observed. A decrease in the level of expression of serotonin and an increase in cortisol were observed as well. It is necessary to use high-tech research methods in the sanitary and epidemiological surveillance and immunological monitoring of workers exposed to man-made pollutants.

**Keywords:** immunological indicators, environment, immune regulation, monitoring of workers

## 1. INTRODUCTION

Employees of enterprises extracting and processing potash salts work under difficult conditions due to specific microclimatic conditions (noise, vibration, high humidity and temperature), harmful chemicals used in production, and psycho-emotional stress. The combined effect of the factors that form the working environment contributes to the development of pathologies; the lack of measures aimed at monitoring the health status and preventing diseases leads to the development of chronic diseases that affect the quality of life and work capacity of employees [1, 2]. Oncological diseases are not the leading ones, but they exceed the population level, which is associated with the direct contact of workers with oncogenic substances used in production [3].

Formaldehyde has toxic (irritation of the mucous membranes of the upper respiratory tract, throat, eyes, causes nausea and headache), and carcinogenic effects, causing the development of oncopathology and precancerous conditions [4, 5]. Smulevich, who has been dealing with professional cancer, describes formaldehyde as an increased risk of developing skin cancer (disinfectants, embalmers), tumors of the upper respiratory tract – the nasal cavity, paranasal sinuses and nasopharynx (furniture production), oral cavity, pharynx, stomach, large intestine, prostate, lungs, leukemia (formaldehyde production) [6, 7].

The immune system is highly sensitive to harmful factors [8]. Due to this feature, quantitative disorders can serve as an indicator of the impact of harmful factors. In real conditions, production factors do not act in isolation, and with their various combinations, it is not always possible to establish the leading factor. The constant influence of chemical compounds causes the development of secondary immunodeficiencies, and the assessment of the immune status is a reliable method reflecting the unfavorable effect of the working environment on the human body [9, 10].

The preservation of professional health and longevity of working citizens is the main government task, which should be reflected in the social policy. An assessment of the state of the immune system, its cellular and humoral parts should be used to diagnose early health disorders in industrial workers in order to prevent a reduction in the working period, disability, and deaths [11, 12].

The purpose of the article is to study the influence of chemical factors of the working environment on the parameters of immune regulation in workers of the mining industry.

## 2. MATERIALS AND METHODS

An in-depth study of the health status of 195 patients (men and women) aged 23 to 63 years, working at a

potassium enterprise was carried out. The main harmful chemical factor is formaldehyde.

The patients were divided into those working directly with mineral fertilizers and those working in the management sector. The observation group included 157 people (125 women, 32 men) aged  $43.2 \pm 1.3$  years, working in hazardous conditions. The control group included 78 patients (61 women, 17 men) aged  $44.5 \pm 1.2$  years, working in the management sector.

The blood test for formaldehyde content was conducted by high performance liquid chromatography using the Agilent liquid chromatograph with a diode array detector in accordance with MUK 4.1.2111-06 "Measurement of the mass concentration of formaldehyde in blood samples by high performance liquid chromatography" [13].

The phagocytic activity of cells was assessed using formalinized sheep erythrocytes.

Serum immunoglobulins A, M, G were determined by the method of radial immunodiffusion according to Mancini.

The change in the content of immunoglobulin E (Ig E) specific to the pollutant (formaldehyde) was determined by the allergosorbent test with an enzyme label.

E immunoglobulin and cytokines (interleukin- $1\beta$  (IL- $1\beta$ ), tumor necrosis factor (TNF)) in blood serum were determined using the enzyme-linked immunosorbent assay (test system of the "Vector-Best" company, Novosibirsk) on the analyzer "Elx808IU".

Phenotyping of CD lymphocytes was performed on the Becton Dickinson FACSCalibur flow cytometer in CellQuest.Pro. Populations and subpopulations of lymphocytes (CD16 +, CD19 +, CD25 +, CD95 +) were determined by membrane immunofluorescence using a panel of labeled monoclonal antibodies to membrane CD receptors (Becton Dickinson, USA), and a total of at least 10,000 events were recorded.

For the qualitative implementation of the statistical analysis, the material was processed in Microsoft® Office Excel 2003 and Statistica 6.0. The data were processed by the method of variation statistics with the calculation of the arithmetic mean, its standard error. The significance of differences was determined by the Student's t-test, the dependencies were assessed using the correlation-regression analysis, Fisher's test and the coefficient of determination ( $R^2$ ). Qualitative data are presented as absolute or relative (%) frequencies, quantitative features are presented as  $M \pm m$  (arithmetic mean  $\pm$  mean error). The differences between the groups were considered significant at  $p < 0.05$ .

### 3. RESULTS

According to the results of chemical and analytical studies in the observation group, an increased content of formaldehyde was established, while for all the substances under study, an excess of the permissible level ( $N = 0.0169\text{--}0.0191 \mu\text{g} / \text{cm}^3$ ) of formaldehyde in the blood was observed in the observation group ( $0.0457 \pm 0.0039 \mu\text{g}/\text{cm}^3$ ) and in the control group ( $0.0374 \pm 0.0076 \mu\text{g}/\text{cm}^3$ ) ( $p \leq 0.05$ ).

The clinical and laboratory studies of the health status of workers indicate the presence of disorders in immune regulation (Table 1).

The comparative analysis established that in the observation group there were no significant changes in innate cellular immunity by phagocytosis, while relative to the control group there was a significant decrease in the phagocytic activity by absolute phagocytosis (lower by 1.08 times) ( $p \leq 0.05$ ).

The use of the technique for assessing the odds ratio of changes in the phagocytic activity of cells with an increase in the concentration of contaminants in biological media made it possible to establish a significant ( $p < 0.05$ ) decrease in absolute phagocytosis with an increase in the concentration of formaldehyde ( $R^2 = 0.25$  at  $p < 0.05$ ).

A significantly increased content of serum immunoglobulins G, A, M was found: an excess of the content of Ig G by 1.05 times, Ig M – by 1.03 times and Ig A – by 1.08 times ( $p < 0.05$ ), which reflects probable violations in the immunoregulation.

An analysis of the odds ratio of changes in humoral immunity indicators with an increase in the concentration of contaminants in biological media made it possible to establish a significant ( $p < 0.05$ ) increase in the IgG content with an increase in the concentration of formaldehyde ( $R^2 = 0.58$  at  $p < 0.05$ ).

The similar trend was described by Gromova E.N. who conducted a survey of residents living under the combined chemical load of phenol and formaldehyde for more than 10 years and found a decrease in the content of T-lymphocytes, T-helpers, T-cytotoxic, A immunoglobulin and significantly lower phagocytic activity [14]. [14].

21.8 % of workers showed a 1.05-fold increased level of general sensitization (total IgE) compared to the control group ( $p < 0.05$ ).

There was also a significantly increased (3.42 times) level of specific sensitization to formaldehyde according to the Ig E criterion compared to the control group ( $p < 0.05$ ).

There were no changes in general and specific sensitization in relation to the reference level.

An analysis of the odds ratio of hypersensitivity increase with an increase in the concentration of contaminants in biological media made it possible to establish a significant ( $p < 0.05$ ) increase in total IgE and IgE to formaldehyde with an increase in the concentration of formaldehyde in the blood ( $R^2 = 0.14\text{--}0.30$  at  $p < 0.05$ ).

The workers of the observation group had significant deviations in the indicators of the CD-immunogram. The increased expression of killer lymphocyte receptors CD16 + CD56 + (absolute – 1.13 times, relative – 1.12 times) and CD19 + (absolute – 1.04 times, relative – 1.03 times) ( $p < 0.05$ ). Activation of cell death receptors CD95 + (absolute – 1.17 times, relative – 1.21 times) and CD25 + (absolute – 1.05 times, relative – 1.04 times) was also observed ( $p < 0.05$ ).

An analysis of the odds ratio of changes in the parameters of cellular immunity with an increase in the concentration of contaminants in biological media made it possible to establish a significant ( $p < 0.05$ ) increase in the expression of the receptor for cell death CD95 + with an increase in the concentration of formaldehyde in the blood ( $R^2 = 0.14\text{--}0.73$  at  $p < 0.05$ ).

At the same time, there were no changes in the indicators of the CD-immunogram.

The data obtained indicate an excessive activation of the rate of the life cycle of immune cells and the receptor activity of immunocompetent cells. The observed cell rearrangement can be accompanied by processes associated with viral and oncoproliferative conditions.

Ye X., et. al. revealed an increase in B-lymphocytes, but no decrease in T-lymphocytes, T-helpers, and T-suppressors [15].

An analysis of the cytokine regulation of IL-1 $\beta$  and TNF made it possible to establish that these indicators are within the reference level, but there is an excess of the IL-1 $\beta$  level (1.34 times) and a decrease in the TNF indicator (1.81 times) relative to the control group ( $p < 0.05$ ) which indicates an excessive activation of the inflammatory process and a decrease in the antitumor cytokine status (Table 1).

The frequency of occurrence of work-related pathology tends to increase depending on the harmfulness of working conditions, while a person working in harmful conditions can simultaneously develop several occupational diseases that have a similar etiology with the inclusion of both the immune and hormonal systems. The immune system, being a defense for the body, is the first to take on the impact of production factors and starts a cascade of reactions that involves all the related systems, thereby forming a breakdown in the reaction leading to the onset of pathology.

**Table 1.** Immunological indicators in mining industry workers

Indicator	Reference level	Observation group (n = 157) M ± m	Comparison group (n = 78) M ± m
Absolute phagocytosis, 10 <sup>9</sup> / dm <sup>3</sup>	0.964–2.988	2.340±0.145**	2.174±0.216
IgG, g/ dm <sup>3</sup>	10–18	14.190±0.681**	13.572±0.505
IgM, g/ dm <sup>3</sup>	1.1–2.5	1.637±0.079**	1.588±0.119
IgA, g/ dm <sup>3</sup>	1.1–3	2.649±0.181**	2.445±0.122
CD16+CD56+- lymphocytes, rel., %	5–27	14.840±1.535**	13.308±1.083
CD16+CD56+- lymphocytes, abs., 0 <sup>9</sup> / dm <sup>3</sup>	0.09–0.59	0.322±0.036**	0.286±0.027
CD3+CD19+- lymphocytes, rel., %	6–25	8.108±0.585**	7.840±0.784
CD3+CD19+- lymphocytes, abs., 0 <sup>9</sup> / dm <sup>3</sup>	0.09–0.66	0.178±0.020**	0.171±0.020
CD3+CD25+- lymphocytes, rel., %	13–24	15.636±1.511**	14.987±1.131
CD3+CD25+- lymphocytes, abs., 0 <sup>9</sup> / dm <sup>3</sup>	0.19–0.56	0.339±0.039**	0.324±0.029
CD3+CD95+- lymphocytes, rel., %	39–49	41.510±2.231**	34.267±2.355
CD3+CD95+- lymphocytes, abs., 0 <sup>9</sup> / dm <sup>3</sup>	0.63–0.97	0.904±0.076**	0.774±0.079
IgE total, IU/cc	0–149.9	62.666±18.956**	59.532±19.553
IgE spec. formaldehyde, IU/ml	0–1.5	0.188±0.033**	0.055±0.031
Interleukin-1beta, pg/ml	0–11	1.782±0.171**	1.329±0.195
Tumor necrosis factor, pg/ml	0–6	2.010±0.284**	3.636±1.108

Note: \* – the difference is significant relative to the reference interval (p <0.05), \*\* – the difference is significant relative to the comparison group (p <0.05). The content of the mediator of catecholamine regulation of serotonin and the hormone of the adrenal cortex, cortisol, does not go beyond the reference limits; however, compared to the values in the control group, the content of serotonin was 1.71 times lower, and the level of cortisol was 1.15 times higher (p <0.05), which indicates a decrease in the protective mechanisms of stress resistance (table 2).

**Table 2.** Indicators of hormonal status of mining industry workers

Indicator	Reference level	Observation group (n = 157) M ± m	Comparison group (n = 78) M ± m
Serotonin, ng/ml	40–400	176.389±18.597**	301.397±37.065
Cortisol, nmol/cc	140–600	337.107±49.513**	294.126±18.740

Note: \* – the difference is significant relative to the reference interval (p <0.05), \*\* – the difference is significant relative to the comparison group (p <0.05).

#### 4. CONCLUSION

The results of an immunological study of mining workers, revealed deviations in the systems of cellular and humoral immunity in the form of an imbalance in the phagocytic activity (decrease) and the content of serum immunoglobulins G, A, M (hyperproduction), verified by the mathematical models (reliable dependence "IgG content – formaldehyde in the blood", p <0.05).

By the IgE criterion specific to formaldehyde, the level of specific haptenic sensitization exceeded the control level by more than 3 times and was characterized by a significant dependence on the concentration of formaldehyde (p <0.05).

Overexpression of killer lymphocytes CD16 + CD56 + and activation of cell death receptors CD25 + and CD95 + were established, and the model revealed a direct dependence of CD markers on the level of blood contamination with formaldehyde.

An imbalance of the cytokine profile with excessive activation of the pro-inflammatory cytokine IL-1β with

a simultaneous inhibition of TNF expression was established, which indicates a decrease in the antitumor protection.

A decrease in the level of serotonin and overproduction of cortisol indicate the inhibition of stress resistance mechanisms under the excessive formaldehyde contamination.

The results obtained using the high-tech research methods make it possible to recommend a set of indicators (IgE specific to formaldehyde, the expression level of killer and apoptotic membrane receptors, the expression of IL-1β and TNF alpha cytokines, imbalance of serotonin and cortisol) as markers for the early diagnosis of the influence of chemical factors of the production environment on the immune regulation in workers exposed to man-made pollutants (for example, formaldehyde).

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