

## LONG-TERM IMMUNITY ALTERATIONS IN THE EMPLOYEES OF THE HIGH HYDROGEN SULFIDE CONTENT GAS CONDENSATE PROCESSING FACILITY

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The current measures for protection of the gas processing plant employees cannot fully prevent the impact of pollutants. Evaluation of the immune system is one of the methods for monitoring of the employees' health, and testing the system of measures used to improve the working conditions. The study was aimed to identify alterations in the immune status of the employees at the gas processing and high hydrogen sulfide content condensate processing facility depending on their working experience. The working environment and the employees' immune system were evaluated by standard methods. Pollutants were detected with the Bruel & Kjaer 1302 Multi-Gas Monitor, and the Tsvet-550 gas chromatographer. A total of 160 employees and 81 controls (blood donors of the regional blood transfusion station) were surveyed. The immune system was evaluated using the System 9000 Plus hematological analyser, Cyto FLEX LX flow cytometer, UNICO 2100UV spectrophotometer, and KFK-3-03-ZOM3 photometer. It was concluded that the existing complex of occupational and industrial hazards affects the immune status of the main production unit employees, which is reflected in the decreased CD20 levels and increased CD8 levels along with the constant levels of CD4. Correlations were revealed between the immunoglobuline level alterations, decrease in the phagocytic index and phagocytic number, as well in lysozyme activity, and the working experience. Pollutant exposure results in altered immunity of the employees, which could be considered the adaptation mechanism.

**Keywords:** production factors, pollutants, immunological indicators

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## СТАЖЕВЫЕ ИЗМЕНЕНИЯ ИММУНИТЕТА РАБОЧИХ ПРЕДПРИЯТИЯ ПО ПЕРЕРАБОТКЕ ГАЗОКОНДЕНСАТА С ВЫСОКИМ СОДЕРЖАНИЕМ СЕРОВОДОРОДА

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Существующие меры защиты сотрудников газоперерабатывающих заводов не могут полностью предотвратить воздействие на них поллютантов. Одним из методов мониторинга здоровья рабочих и системы мероприятий по нормализации условий труда является исследование иммунной системы. Целью работы было выявить изменения в иммунном статусе рабочих, занятых на предприятии по переработке газа и конденсата с высоким содержанием сероводорода в зависимости от их стажа. Использовали стандартные методы для характеристики производственной среды и оценки состояния иммунной системы работающих. Для индикации поллютантов применяли универсальный газовый монитор 1302 Bruel & Kjaer, газовый хроматограф Цвет-550. Были обследованы 160 рабочих, а также 81 человек контрольной группы (доноры областной станции переливания крови). Исследования иммунной системы проводили на гематологическом анализаторе Sistem 9000 Plus, цитофлуориметре Cyto FLEX LX, спектрофотометре UNICO 2100UV, фотометре фотоэлектрическом КФК-3-03-ЗОМЗ. Сделаны выводы, что комплекс существующих профессионально-производственных вредностей оказывает влияние на состояние иммунитета рабочих основных производств, которое проявляется в снижении содержания CD20 и увеличении содержания CD8 при почти неизменном содержании CD4. Выявлена взаимосвязь изменения концентрации иммуноглобулинов, снижения фагоцитарного индекса и фагоцитарного числа, а также активности лизоцима с увеличением производственного стажа. Воздействие поллютантов вызывает изменения состояния иммунитета рабочих, что может быть расценено как приспособительный механизм.

**Ключевые слова:** производственные факторы, поллютанты, иммунологические показатели

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Air pollution with harmful chemicals inside the gas processing plants remains the major hygiene factor that might have some influence on the employees' health [1–6]. Gas from the Astrakhan field, having the unique natural composition, has a certain impact both on the environment and the human body. It is distinguished by high hydrogen sulfide content (up to 25%), along with hydrocarbons (2.84%), carbon oxides (14–20%), nitrogen oxides (2.45%), mercaptans (0.03–0.22%), and

carbonyl sulphide (0.02–0.42%) [3]. The current measures for protection of the gas processing plant employees cannot fully prevent the impact of harmful factors.

In this regard, there is a scientific and practical interest in the qualitative and quantitative assessment of the changing immunological parameters, which reflect the essence of the employee's body alteration and make it possible to monitor the employees' health in a timely manner in order to prevent

the risk of developing a disease [7–8]. Immune system is one of the body systems, being the most responsive to pollutant exposure; immunological tests may be regarded as the most reliable tests for establishing the causal relationship between the disease and the hazardous working conditions. However, there are just a few papers on this issue [8–11]. The data on the immune system sensitivity to the long-term exposure to production factors is also insufficiently represented in literature [9–16].

## METHODS

The workplace hygiene certification involved assessing the concentrations of air pollutants in the working area based on legislative requirements.

Determination of  $\text{SO}_2$  in air was performed using the Bruel & Kjaer 1302 Multi-Gas Monitor (Bruel & Kjaer; Denmark). The  $\text{H}_2\text{S}$  concentrations were assessed by photometric method based on the interaction of hydrogen sulfide with sodium arsenite and silver nitrate. Alkanes (C1–C10), alkenes (C2–C5), and aromatic hydrocarbons (benzene, toluene, xylenes and ethylbenzene) were assessed by gas chromatography. These substances were detected using the Tsvet-550 gas chromatographer (Rospribor; Russia). Along with the listed above methods, hydrocarbons (total) were assessed with the Bruel & Kjaer 1302 Multi-Gas Monitor. The concentration of nitrogen oxides was measured by photometry. Determination of methanol in air in the working areas was performed by gas chromatography, and carbon monoxide was assessed by the reaction gas chromatography. The Bruel & Kjaer 1302 Multi-Gas Monitor was also used for this purpose.

A total of 160 employees (operators, engine drivers) of the main and auxiliary facilities of the gas processing plant were surveyed. Inclusion criteria: males; age 30–40 years (the average age was 36.4 years). A total of 81 controls (blood donors of the regional blood transfusion station) were also surveyed. The control group inclusion criteria were as follows: individuals matching the facility employees in gender and age (comparing the groups by age revealed no significant differences ( $p > 0.05$ )); exclusion criteria: professional experience in the gas processing, petroleum or chemical industry; exposure to any chemical process hazard.

The studied group was surveyed during the routine medical examination with the use of the standard assessment methods. All the participants were divided into groups based on their length of service with the plant: 1–3 years, 3–5 years, 5–10 years, 10 years or more.

Hematological tests were performed using the System 9000 Plus automatic hematological analyser (Serono; Switzerland). Samples were analyzed with the Cyto FLEX LX flow cytometer (Beckman Coulter; USA) in order to determine the lymphocyte subpopulations. Phagocytic cells were examined by the direct morphological method. Classes of immunoglobulins were determined by a turbidimetric assay; concentrations of the circulating immune complexes (CIC) were defined by precipitation with polyethylene glycol (PEG-6000) and registered at 280 nm with the UNICO 2100UV spectrophotometer (United Products & Instruments, Inc.; USA). Lysozyme activity was detected by turbidity assay based on measuring the changes in the turbidity of the *Micrococcus lysodeikticus* suspension with the KFK-3-03-ZOM3 photometer (Zagorsk Optical-Mechanical Plant; Russia).

Statistical analysis of the results was performed using the Statistica 12 software (StatSoft; USA) and the analysis of variance. Previously the descriptive statistics data were

assessed: number of observations that constituted the sample ( $n$ ), arithmetic mean of the data obtained ( $M$ ), standard deviation ( $m$ ), standard error of the mean ( $\tau$ ), minimum ( $\min$ ) and maximum ( $\max$ ) values of the studied parameter, as well as the relative values (%) and the corresponding errors. The quantitative data distribution was assessed using the Shapiro–Wilk test. In case of compliance with the normal distribution law, the method of statistical analysis was selected (parametric or nonparametric). When the quantitative variables were distributed normally, central tendencies and dispersion were described using mean values ( $M$ ) and standard deviations ( $m$ ). Significance of the differences was defined with the use of the Wilcoxon test and the Mann–Whitney U test; the differences were considered significant when  $p < 0.05$ .

## RESULTS

The findings demonstrate the presence of significant air concentrations of numerous harmful substances in the working area. Despite the fact that almost all of these substances are involved in the technological processes and are almost completely converted to sulfur compounds (particularly, to sulfur dioxide) or removed through the chimneys of the facility (carbon oxides), the constant presence of pollutants in the working area is observed. The presence of those is characteristic not only of the purely production areas (pump rooms, engine rooms), but also of the soundproof compartments with no production machinery installed (Table 1).

The impact of production factors on the employees' health is confirmed by the correlation between the detected changes in immune status and the working experience. It was found that with an increase in the working experience, there were trends towards the increase in white blood cell and lymphocyte counts, decrease in the levels of CD20, and progressive increase in the levels of CD8 along with the constant levels of CD4 (Table 2).

Correlations between the alterations of the cellular and humoral factors and the employees' working experience. The indicators of phagocytosis were characterized by progressive decrease in the phagocytic index and phagocytic number with the increase in the working experience, which was to some extent offset by the increase in the total number of phagocytic cells. However, such a compensatory capacity started to decline in individuals with working experience exceeding 10 years.

The humoral factors of nonspecific resistance demonstrate a greater diversity of the correlations with working experience. Thus, lysozyme activity in employees progressively decreases with the increase in their working experience, and the classes of immunoglobulins show a variety of responses. The trend towards the decrease in concentrations is characteristic of IgG, and the trend towards the increase is characteristic of IgA and IgM.

Thus, the correlation between the changes in the majority of the immune status indicators and the employees' working experience and, therefore, their working conditions, has been shown, which confirms the professional etiology of these alterations. However, the changes are phase-type, which makes it possible to treat the alterations as different stages of the adaptation process.

To identify the possible correlations between the serum and saliva levels of certain humoral resistance factors, we assessed lysozyme activity in the saliva obtained from different groups of employees. It was found that the changes of this indicator in blood and saliva of the employees were almost exactly the same both in terms of tendencies and intensity. Moreover, the discovered similarities have been found in all groups of employees, which makes it possible to use lysozyme levels for

**Table 1.** Concentrations of harmful substances in the air of the working areas

Air sampling site	Pollutants	Number of tests	Pollutant concentrations, mg/m <sup>3</sup>			MPC, mg/m <sup>3</sup>
			min	max	mean $M \pm m$	
Engine rooms	Hydrogen sulfide	25	1.3	8.1	$5.4 \pm 0.8$	3.0
	Sulphur dioxide	25	1.3	45.3	$23.1 \pm 2.6$	10.0
	Nitrogen dioxide	22	0.9	4.1	$2.1 \pm 0.3$	2.0
	Carbon monoxide	16	3.9	53.1	$29.1 \pm 6.0$	20.0
	Hydrocarbons	25	1.5	80.0	$43.1 \pm 4.6$	300.0
	Mercaptans	15	0.3	2.2	$1.24 \pm 0.2$	0.8
Pump rooms	Hydrogen sulfide	25	1.1	7.7	$5.1 \pm 0.8$	3.0
	Sulphur dioxide	25	2.5	54.6	$27.4 \pm 4.5$	10.0
	Nitrogen oxides	22	1.4	3.7	$3.0 \pm 0.6$	2.0
	Carbon monoxide	16	3.2	49.7	$25.1 \pm 5.6$	20.0
	Hydrocarbons	25	2.7	63.8	$38.3 \pm 3.9$	300.0
	Mercaptans	15	0.2	2.1	$1.07 \pm 0.25$	0.8
Soundproof compartments in engine rooms	Hydrogen sulfide	23	1.0	7.4	$5.0 \pm 0.7$	3.0
	Sulphur dioxide	25	0.6	32.2	$12.6 \pm 3.4$	10.0
	Nitrogen dioxide	22	1.7	3.8	$3.01 \pm 0.09$	2.0
	Carbon monoxide	16	3.6	49.4	$25.3 \pm 6.0$	20.0
	Hydrocarbons	25	1.8	72.8	$30.6 \pm 2.6$	300.0
	Mercaptans	15	0.2	2.0	$1.7 \pm 0.3$	0.8
Soundproof compartments in pump rooms	Hydrogen sulfide	23	1.0	6.2	$4.8 \pm 0.6$	3.0
	Sulphur dioxide	25	3.3	31.4	$14.3 \pm 5.9$	10.0
	Nitrogen oxides	22	1.3	3.0	$1.9 \pm 0.2$	2.0
	Carbon monoxide	16	3.1	41.6	$24.3 \pm 5.9$	20.0
	Hydrocarbons	25	18.2	88.6	$45.2 \pm 9.2$	300.0
	Mercaptans	15	0.2	1.4	$0.7 \pm 0.21$	0.8
Compressor house	Hydrogen sulfide	24	0.6	1.8	$1.1 \pm 0.2$	3.0
	Sulphur dioxide	24	3.8	22.6	$16.6 \pm 2.4$	10.0
	Nitrogen oxides	23	0.8	3.8	$2.2 \pm 0.2$	2.0
	Carbon monoxide	15	3.3	33.3	$17.2 \pm 1.6$	20.0
	Hydrocarbons	25	3.0	38.6	$23.2 \pm 5.8$	300.0
	Mercaptans	14	0.2	1.4	$0.8 \pm 0.31$	0.8
Rack for manual loading of sulfur and trucking area for solid and granulated sulfur	Hydrogen sulfide	50	1.3	66.5	$29.8 \pm 6.8$	3.0
	Sulphur dioxide	53	7.4	360.0	$57.2 \pm 9.6$	10.0
	Nitrogen oxides	28	0.6	3.4	$1.8 \pm 0.2$	2.0
	Carbon monoxide	62	3.8	47.5	$22.9 \pm 5.1$	20.0
	Hydrocarbons	54	1.7	12.2	$7.9 \pm 0.9$	300.0
	Sulphur dust	42	8.4	21.4	$13.7 \pm 1.4$	6.0

noninvasive diagnosis. Serum lysozyme levels in employees reached  $5.43 \pm 0.29$  µg/mL, and serum lysozyme levels in controls were  $6.48 \pm 1.42$  µg/mL. Saliva lysozyme levels in employees were  $8.82 \pm 0.49$  µg/mL, and in controls these were  $10.41 \pm 0.65$  µg/mL.

## DISCUSSION

Research has shown that the complex of occupational and industrial hazards specific to the enterprises that process gas condensate with high hydrogen sulfide content has a certain impact on the immune system of the employees. The accompanying bodily processes are directly related to the duration of exposure to the production factors, i.e. depend on the working experience in the gas industry.

The decrease in the phagocytic activity of peripheral blood neutrophils we have identified is very much in line with the

existing literature data, and can be interpreted as a consequence of the employees' intoxication with air pollutants present in the factory premises. The groundwork is thus being laid for inefficient elimination of the infectious causative agents, and, consequently, for chronic infections, to the extent of becoming the resident bacteria carrier. This assumption could be supported by the elevated levels of IgA found in the factory employees. It is known that it is IgA which is responsible for the mucous membrane resistance to pathogens, and the IgA concentration increase is associated with inflammation in the area of the entrance gate of infection. Taking into account the decrease in lysozyme activity observed both in blood serum and saliva of the employees compared to controls, there is a theoretical possibility of the chronic infectious disease.

The long-term persistence of an infectious agent in the human body may, among other things, result in the autoimmune disorder development. An example is the autoantibody against the TSH receptor gangliosid region, which is responsible for

**Table 2.** Immune status indicators in employees of the Astrakhan Gas Processing Plant with different working experience

Indicators	Mean values by groups, M ± m				
	Group 1	Group 2	Group 3	Group 4	Control group
White blood cells, × 10 <sup>9</sup>	7.35 ± 0.37	7.88 ± 0.33	8.35 ± 0.26**	7.35 ± 0.32	5.27 ± 0.36
Lymphocytes, %	36.8 ± 1.87	37.2 ± 0.83	38.9 ± 1.46	38.3 ± 1.42	34.23 ± 1.88
CD3, %	56.6 ± 1.54	58.0 ± 1.76	57.5 ± 1.76	57.3 ± 1.6	56.38 ± 2.12
CD4, %	36.1 ± 1.31	36.1 ± 1.49	38.0 ± 0.67	38.7 ± 1.6	38.69 ± 1.98
CD8, %	19.5 ± 0.73	20.5 ± 1.37	21.9 ± 1.35	18.6 ± 1.8	17.69 ± 0.88
CD4/CD8	2.32 ± 0.12	2.06 ± 0.19	1.83 ± 0.15**	2.42 ± 0.3	2.25 ± 0.17
CD20, %	14.7 ± 0.72	13.9 ± 0.38	13.5 ± 0.69	14.2 ± 0.6	14.64 ± 0.85
IgG, g/L	9.21 ± 0.12	9.04 ± 0.19	9.03 ± 0.2	9.11 ± 0.2	9.45 ± 0.33
IgA, g/L	1.81 ± 0.07	1.84 ± 0.08	1.84 ± 0.04	1.86 ± 0.06	1.87 ± 0.08
IgM, g/L	1.24 ± 0.07	1.28 ± 0.06	1.28 ± 0.04	1.29 ± 0.07	1.33 ± 0.11
CIC, AU	4.77 ± 0.41	5.01 ± 0.44	5.83 ± 0.27	5.45 ± 0.47	2.49 ± 0.5
Phagocytic index, %	72.1 ± 2.51	70.7 ± 0.75*	66.4 ± 0.16**	67.1 ± 3.0	73.64 ± 2.3
Phagocytic number,	5.73 ± 0.27	5.65 ± 0.2*	5.08 ± 0.11**	5.36 ± 0.32	6.98 ± 0.39
Phagocytic activity per 1 µL	3033 ± 239	2961 ± 231	3183 ± 143	2609 ± 202	2345 ± 232
Lysozyme, µg/mL	5.67 ± 0.48	4.86 ± 0.64	4.17 ± 0.54**	6.16 ± 1.29	6.48 ± 1.42

**Note.** \* — significant differences ( $p < 0.05$ ) between groups 2 and 3; \*\* — significant differences ( $p < 0.05$ ) between groups 1 and 3; group 1 — working experience 1–3 years, group 2 — working experience 3–5 years, group 3 — working experience 5–10 years, group 4 — working experience exceeding 10 years.

the development of hyperthyroidism associated with Graves' disease. Certain bacterial infections trigger the development of such autoimmune disorders, since glycosphingolipids of bacterial antigens often cause cross-reactivity. In Graves' disease, this role is played by *Yersinia enterocolitica*.

Considering the fact that IgM is capable of complement activation and playing a part of the mediator in the cytotoxic reactions, that we and other researchers define as the increased IgM levels in the employees of the chemical production facilities, IgM is capable of facilitating the employees' predisposition to autoimmune disorders.

## CONCLUSIONS

Maximum consumption of raw material and all intermediate products used in the the natural sulfur containing gas and condensate processing, achieved by highly efficient operation of the major production objects (Claus process and Sulfreen), is of primary importance in terms of improving working conditions in the studied industries. This would make it possible to minimize

the overall air pollution and the pollution of air specifically inside the premises of the Astrakhan Gas Processing Plant. High level of sealing for the technological equipment, installed in the engine room (pumps, compressors, valves), is required, as well as organization of automated control of the components in the flow. Both direct and indirect impact of the Astrakhan Gas Processing Plant production factors causes the noticeable changes in the immune status of the employees, which could be considered the adaptation mechanism. The identified changes in the immune status could be one of the factors, contributing to the rising morbidity rate in the facility employees, being the most susceptible to the damaging effects of specific factors of the working environment. This suggests that it is appropriate to include immunological tests in the routine hygienic assessment of the working conditions at the gas production facilities. Taking into account the strength of the used laboratory methods, these methods can be recommended for the inclusive health check-ups of employees. The results obtained may be used as criteria for distinguishing the risk groups for rehabilitation, as well as for occupational selection of new personnel.

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