THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE ACTIVITY OF BLOOD CELL APOPTOSIS

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Abstract

The activity of apoptosis in blood of residents of large cities is characterized by an imbalance of pro- and anti-apoptotic factors, which may cause a violation of adaptation and metabolic mechanisms. The use of iodine-containing drugs contributes to maintaining the balance of pro- and anti-apoptotic factors in the blood, which has a positive effect on the health status of people living in ecologically unfavorable regions.

Key words: *ecology, public health, city, apoptosis, iodine-containing drugs*

INTRODUCTION

Atyrau region (Atyrau) of Kazakhstan is characterized by difficult climatic conditions and poor infrastructure in rural areas. Active development of oil fields in the region has led to significant changes in the socio-economic profile of the population. Issues of public health and a healthy lifestyle in the areas of oil production are very acute and particularly important. With the increase of the extraction and processing of oil and gas in the region have increased rates of primary morbidity in adults. Such as: diseases of respiratory, nervous and immune systems, the mouth and teeth, the musculoskeletal system, the number of patients with diseases of the blood and blood-forming organs, associated with iron anemia, infectious diseases, often viral "A" hepatitis, acute intestinal infections, tuberculosis [1-3].

According to official data, from 20 settlements of Kazakhstan with environmental monitoring, the city of Almaty for several years ranked first in the level of air pollution [4]. In the context of weak natural ventilation, air pollution has a direct negative impact on public health. Elevated levels of carbon monoxide in the air lead to a disorder of the nervous system: headaches, loss of memory, insomnia. As a result of the constant action of this environmental factors reduced the resistance of the immune system, which often leads to cancer [5]. In 2013, the Ministry of Environment of Kazakhstan gave figures showing that in Almaty the level of harmful emissions into the atmosphere is more than in Russia by 37%, 66% in USA and Germany - 75% [6]. Consequently, in the air of Almaty there is high concentrations of formaldehyde, nitrogen dioxide, phenols, carbon monoxide.

In the scientific literature of the last years, devoted to the rehabilitation of public health, it points out that the reduction of the level of human health is often associated with the combined effect of environment and iodine deficiency in the diet [7-10]. Studies of the Institute have also shown a relationship between the ecology of the regions, the lack of thyroid hormone and low levels of physical health of the working age population [11].

The current problem of iodine deficiency in Kazakhstan, as in many countries of the world, requires the development of new methods of mass iodine prophylaxis, in addition to the already existing - the use of iodized salt. This method against the background of widespread hypertension, does not justify its purpose. Consequently, as the iodine-containing preparation has been investigated the use of biologically active additives to food - balsam "Vozrozhdenie" - the development of Kazakh scientists, officially registered for use on the territory of Kazakhstan since 2010. As has been shown in clinical trials in Russia and Kazakhstan, the balsam has immunostimulant, hypoglycemic, hepatoprotective properties, has an antioxidant, antiparasitic, antiseptic, antiviral and antimicrobial action.

There is no doubt that one of the priority indicators of the vulnerability of the body under the influence of eco factors is activity of the blood apoptosis. Disturbances of apoptosis induction mechanisms

leading to its suppression or, conversely, to excessive activation may be an important step in the pathogenesis of any disease or a marker of effect of environmental factors. In this work showed the importance of diagnostic markers of apoptosis in blood of the urban population of large cities, differ in ecological situations.

METHODS

In total 105 people were screened. In the control group -50 of healthy people living in environmentally safe area of Almaty region, aged between 20 and 50 years. Case - 55 people randomly selected from the total number of the surveyed residents of Almaty and Atyrau (300 pers.). Analysis of blood samples was performed on a flow cytometer FacsCalibur (Becton Dickenson, USA) in the CellQuest program. The following parameters were determined: apoptosis markers (ANNEXIN-V, CD95, bcl-2), immune status (CD3, CD19, CD4, CD8, CD16 + 56, HLA-DR).

RESULTS

Results of immunophenotyping in the case group before and after administration of iodinated balsam "Vozrozhdenie" in comparison with the control group, showed the following. A significant difference in the case group before receiving balsam "Vozrozhdenie " and after its admission (P = 0.01) in the expression of the antigens CD3, CD19, CD4, CD8, CD16 + 56 (Table 1).

Table 1. Immunophenotyping of peripheral blood lymphocytes of persons before and after the adoption of an iodine-containing drug

Group	Amount of people	The content of the relevant lymphocyte populations*, %				The ratio of the content of IRI, CD4 / CD8	The content of the relevant lymphocyte populations*, %	
		CD3+	CD19+	CD4+	CD8+		CD16+56+	HLA-DR
Before	55	71,12±7,1	11,66±4,1	42,53±6,1	26,13±6,4	1,8±0,7	15,51±6,7	13,60±5,1
After	55	73,55±0,8	12,4±0,9	45,15±0,8	26,4±0,4	1,8±0,0	12,3±0,6	14,7±2,3
control	50	68,78±6,1	10,16±3,2	33,75±6,7	35,90±8,3	1,0±0,5	13,6±5,1	15,02±6,1
reference limit of normal (from the manufacturer)		58-85	10-23	35-50	25-35	1,5-2,0	5-10	8-19

There was no significant difference in markers of apoptosis (CD16 antigen expression 56 + and HLA-DR) obtained in the blood of persons who received "Vozrozhdenie" balm and the control group (t <2.0, P = 0.05). Regarding the immunoregulatory index (IRI), ie ratio of CD4 and CD8, in comparing the case group before and after taking balsam "Vozrozhdenie", significant differences were not found (t = 0.0, P = 0.05), whereas in comparison with the control group, this difference was significant (t =

11.8, P = 0.05). It should be noted that all obtained data were within the boundaries of the reference standards, as control group consisted of conditionally healthy people.

A comparison of immunophenotyping parameters of peripheral blood in people of different ages showed no significant difference in expression of the antigens CD3, CD19, CD4, CD8, CD16 + 56, HLA-DR (t <2.2, p = 0.05). At the same time, there was a significant difference in the expression of these antigens with the control group (t> 2.2, p = 0.001, Table 2).

Table 2. Immunophenotyping of peripheral blood lymphocytes according to the age of the subjects before and after administration of the iodide-containing drug

Group	Amount of people	The content of the relevant lymphocyte populations*, %				The ratio of the content of IRI, CD4 /	The content of the relevant lymphocyte populations*, %	
		CD3+	CD19+	CD4+	CD8+		CD16+56+	HLA-DR
20-30	12	80,88±2,1	11,18±0,75	48,46±5,65	28,19±2,37	1,74±0,3	7,08±2,2	22,34±11,3
30-40	13	75,1±4,9	11,75±3,1	46,08±5,8	24,77±4,3	1,8±0,4	11,52±4,0	18,64±8,3
40-50	12	75,02±6,1	12,3±4,0	48,66±3,8	24,19±7,3	2,2±0,8	11,6±4,0	19,02±5,2
>50	13	72,05±6,7	12,82±3,9	43,54±6,6	27,07±7,8	1,7±0,6	13,07±7,3	17,58±6,7
Control		68,78±6,1	10,16±3,2	33,75±6,7	35,90±8,3	1,0±0,5	13,6±5,1	15,02±6,1
reference limit of normal (from the manufacturer)		58-85	10-23	35-50	25-35	1,5-2,0	5-10	8-19

Consequently, the marked changes have undergone all the indicators of cellular immunity after taking balsam "Vozrozhdenie", which certainly reflects the impact of the drug on the performance of “immune status”.

Simultaneous staining of Annexin V-FITC and propidium iodide PI-PE revealed the following: in all individuals the population located in the lower right quadrant of the histogram annexin-FITC / PI-PE - was positive for annexin and negative for propidium iodide. This means that part of the lymphocytes come into early stage of apoptosis. However, in the case group abundance of annexin was significantly (P = 0.001) lower than in the control group (Figure 1).

The mean fluorescence intensity (MEAN) is often used to describe events in the flow cytometry. Each of the values determined via cellular antigens is characterized by the average value of the fluorescence intensity, which depends on the stage of cell differentiation, expression specificity linearly restricted antigens, as well as fluorochrome, compensation and setting of cytometer (Figure 2).

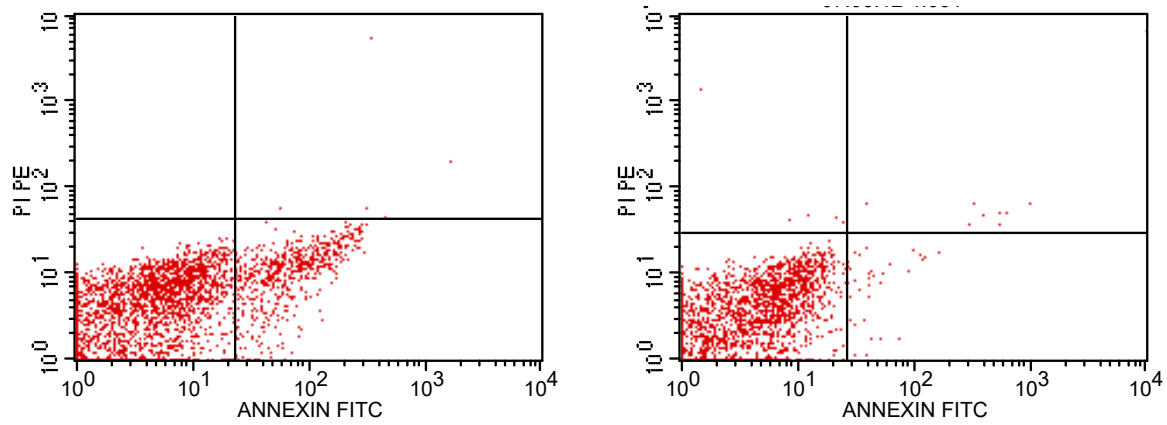


Figure 1. Expression of annexin-FITC in the lower right quadrant of the histogram in the control group and in the case group

The MEAN value is expressed in arbitrary units and is automatically determined by the basic options of cytometer. The value MEAN reflects antigen density.

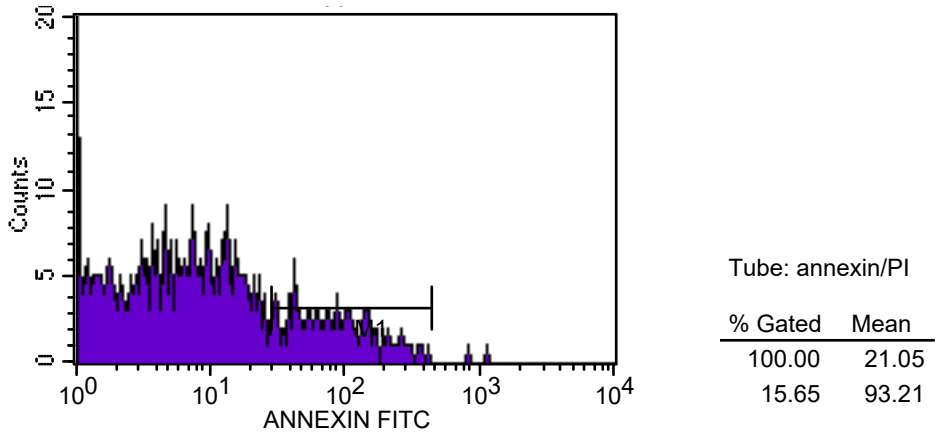


Figure 2. The average fluorescence intensity of Annexin on one-parameter histogram of annexin-FITC / SSC

In general, in the case group after taking balsam "Vozrozhdenie" average value MEAN for annexin was 5,66 ± 2,0. Comparing the percentage of annexin expression and the value of the mean fluorescence intensity in a group of healthy people revealed the following: Student criterion t = 0.651, number of degrees of freedom = 98; P = 0.516. Significant difference between these figures is absent, and therefore, they can be used as a complementary quantity. Obtained values of annexin expression were not significantly different in the group before and after taking balsam " Vozrozhdenie ", as well as among the different age groups. However, as shown in Table 3, a significant difference was found between the expression of annexin in controls (20,17 ± 2,8) and in case groups, both before and after administration of balm (5,57 ± 0,56) and (5,66 ± 2,0), t> 2.2, p = 0.05.

Table 3. The expression of apoptosis markers in people of different age groups after taking balsam "Vozrozhdenie"

group	marker				
	number	ANNEXIN-V	Bcl-2	CD95	CD25
Group before taking balsam	60	5,57±0,56*	13,93±2,02	2,43±0,62**	13,66±3,8*
Group after taking balsam	55	5,66±2,0*	15,83±12,27	15,96±5,88**	40,75±20,12
20-30 years old	2	5,13±0,8	18,12±14,3	12,83±4,8	39,61±21,8
30-40 years old	13	5,8±2,8	17,74±15,3	7,86±3,0	39,13±22,0
40-50 years old	9	5,86±1,7	14,6±9,0	10,34±3,4	37,59±17,2
>50 years old	31	5,57±3,2	12,85±10,5	32,81±12,3**	46,66±19,5
Control	80	20,17±2,8*	50,47±7,8	3,20±0,3**	13,66±3,8
*P≤0.05 annexin, **P≤0.05 CD95					

As can be seen from Table 3, the expression of anti-apoptotic Bcl-2 antigen before (13,93 ± 2,02 standard units) and after (15,83 ± 12,27 standard units) receiving balsam "Vozrozhdenie" had no significant difference (t = 1.08, p> 0.05). The expression of anti-apoptotic Bcl-2 antigen in different age groups showed no significant difference was (t <1.0, p> 0.05). Compared with the control group (50,47 ± 7,8) it was revealed a clear difference in this antigen (13,93 ± 2,02).

Determination of the expression of CD95 ligand on the cell membrane of lymphocytes, showed the significant difference in the case group before and after administration of the balsam "Vozrozhdenie» (2,43 ± 0,62) and (15,96 ± 5,88), as well as with the control group (3,20 ± 0,3). In addition, there was found a significant difference among all age groups, the highest expression was determined in the group older than 50 years (Table 3). Also in the case groups, before and after taking balsam "Vozrozhdenie" there was a significant difference in the expression of the activation marker CD25 in comparison to the control group (13,66 ± 3,8 and 40,75 ± 20,12, respectively). In assessing the expression of this antigen in different age groups, it was significantly higher (46,66 ± 19,5 conv) in the group older than 50 years.

The peripheral blood immunophenotyping parameters in groups of different ages showed no differences in the expression of antigens CD3, CD19, CD4, CD8, CD16 + 56, HLA-DR (t <2.2, p = 0.05), which is consistent with the literature data [12-14]. Whereas in all age group revealed significant difference in the expression of these antigens with the control group (t> 2.2, p = 0.001). Consequently, the parameters of cellular immunity do not depend on the person's age, but are highly dependent on the environmental area of residence. At the same time, it has undergone marked changes in all indicators of cellular immunity after taking balsam «Vozrozhdenie», which certainly reflects the impact of the drug on the immune status indicators. Despite the pronounced changes of immune system, their values did not extend beyond the boundaries of the reference standards. Therefore, the effect of the balsam «Vozrozhdenie» has a beneficial effect on indicators of cellular immunity.

Since apoptosis plays an important role in the elimination of autoreactive cells, controls the duration, intensity of the immune response, the degree of tissue damage and is the mechanism that supports the balance of lymphoid cells in the body, the study of the features of this process has an important prognostic nature. Determination of CD95 molecules on the surface of CD4 + lymphocytes is regarded as an indicator of activated T-helper cells, ready to accept a signal from FasL molecules and start the apoptosis. It is known that the expression of CD95 cells reflects only the readiness of the cells to apoptotic signal reception and it increases with cell activation.

Our studies have shown that during Balsam Vozrozhdenie intake level of CD95 cells tends to increase and shows a willingness to adopt the cells to apoptosis signal. At the same time decrease in Bcl-2 shows the low level of apoptotic activity in peripheral blood cells of persons who received balm. The balance of pro- and anti-apoptotic factors in the blood of the subjects did not change. To proof the fact of a positive role of balsam in maintaining the balance of apoptosis, further studies are needed using flow cytometry.

Thus, the health of the population of large cities with environmental pollution is characterized with changes at the cellular level, requires a deep approach to hematology research, since the majority already has chronic diseases and health disorders associated with impaired adaptive and metabolic mechanisms. The use of iodine-containing drugs contributes to maintaining the balance of pro- and anti-apoptotic factors in the blood, which has a positive effect on the health status of people living in ecologically unfavorable regions.

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