Immune System Disfunctions in Children living in Conditions of Organic Compounds Impact

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Abstract

Background: Exposure of anthropogenic origin chemicals on the human organism leads to the stress of adaptive mechanisms, disfunctions of regulatory systems, including the immune system, formation of immunopathological states, which are caused by immunotoxicity of xenobiotics. Chemicals, possessing expressed immunotoxicity, are formaldehyde, phenol and the majority of aromatic hydrocarbons (benzene, toluene, xylene, and styrene).

Aim & Objectives: The purpose of investigation was to define the method of early diagnosis of the immune system disfunctions in children, living in conditions of the impact of organic compounds possessing the immunotoxicity.

Methods/Study Design: Within the research 492 children, living near the oil refining complex, were observed. Comparison group consisted of 165 children living on the territory of relative well-being. Age of children is 3-15 years (median age in groups was 7,32 ± 1,55 and 8,12 ± 0,92 years, respectively). In the represented research the complex of clinical and laboratory examination methods was used: physical (general blood analysis - white blood cells, neutrophils, lymphocytes), immunological methods. Organic compounds (phenol, toluene and formaldehyde) in the blood were determined according to methodological guidelines "Determination of chemical compounds in biological media" (MG 4.1.763-4.1.779-99) and "Determination of hazardous substances in biological media"(MG 4.1 .2102-4.1.2116-06) by paraphase analysis method with various embodiments of sample preparation. All the examined children were subject to the standard examination of spleen on the ultrasound diagnostic system Toshiba APLIO XG SSA-790A (Japan) and evaluation of splenic tissue echostructure using the multifrequency linear array sensor with the frequency of 10 MHz.
For early diagnosis of immune system disfunction it is necessary to develop the screening, reliable noninvasive instrumental methods of investigation, allowing in timely manner to verify the adverse impact of technogenic factors on the child's body. For this purpose all the examined children of the core group have been divided into two groups: 1st Group and 2nd Group. 1st Group

comprised 256 children the spleen echostructure of which was characterized by the presence of multiple hypoechogenic homogeneous roundish inclusions throughout the ultrasonic section of the organ ranging in the size from 0.3 mm to 1 mm, located at a distance about 1.5-2 mm from each other. 2nd group included 236 children that according to ultrasound investigation had no disturbances in echostructure of spleen and it was characterized as homogeneous and fine-gritted.

Results/Findings: The children of the 1st group have the mean group values of toluene, phenol, formaldehyde that are higher ($p \leq 0.05$) than in 2nd group. The results of mathematical modeling, characterizing the relationship between levels of immunological blood parameters and concentration of toxic chemical substances in the blood of children of 1st group has testified about statistically significant effect of investigated organic compounds on the parameters of immunological homeostasis ($p \leq 0,05$). It is possible that in conditions of influence of toxicant load occurs an adaptive change of immune status that involves activation of proliferative processes in lymphoid follicles of the spleen. Eventually it is visualized at carrying out of ultrasound investigation and can be regarded as an increase in the share of white pulp of the spleen. It is established that at increase in concentration of toluene in blood the probability of detection in a blood the high level of CD19 +-lymphocytes is statistically significantly increased. With increasing the concentration of formaldehyde in a blood the T-cellular link of immunity is activated. It was also established that with increasing the level of phenol in the blood it is also registered the decrease in the content of IgG.

Conclusion: Children living in urbanized territories contaminated by chemicals with anthropogenic origin with the developed oil refining industry and having changes in echostructure of spleen in the form of multiple hypoechogenic homogeneous roundish inclusions throughout the ultrasonic section of organ (increasing of white pulp with lymphoid proliferation) having the blood with higher levels of organic compounds (toluene, phenol, formaldehyde) than children with intact spleen. Ultrasound investigation of spleen with the analysis of its echostructure can be recommended as a screening method for assessing of an adverse effect of technogenic environmental factors on the condition of immune system of children living in urbanized territories.

Key words: ultrasound examination, spleen, origin chemicals, immune system.

Introduction

Together with the nervous and endocrine, immune system performs homeostasis sustaining function and is involved in the processes of adaptation to changing environmental conditions. According to the literature data, the level of health of children on 20-25% is determined by the sanitary and hygienic environmental conditions. Its omnipresent contamination by the products of industrial production is one of the main reasons of the high children sickness rate. Exposure of anthropogenic origin chemicals on the human organism leads to the stress of adaptive mechanisms, disfunctions of regulatory systems, including the immune system, formation of immunopathological states, which are caused by immunotoxicity of xenobiotics.[1] Disfunction of

immune processes among the children living in the territories of sanitary and hygienic disadvantage, contributes not only to the development of immunopathological conditions such as allergies, autoimmune diseases, neoplasia, but also to formation of an increased susceptibility to infectious diseases.[2.3.4] According to the data of statistical reports, in the cities with a large, diversified industrial production the clinical features of secondary immune deficiency are detected in 47% of children[5], and the overall child's sickness rate in 1.7 times exceeds the average Russian indicator.

In 2010, the highest level of pathology caused by disorders of immunological reactivity was observed on the territories of sanitary and hygienic disadvantage: immune diseases (D80-84 according to ICD-10) in Perm (6.90 ‰) and Primorskiy (33.8 ‰) Territory, Tyumen (23.8 ‰) and Kurgan (31.5‰) regions were registered in 2.3-6.9 times more frequently than in the Russian Federation (1.0‰).

Chemicals, possessing expressed immunotoxicity, are formaldehyde, phenol and the majority of aromatic hydrocarbons (benzene, toluene, xylene, and styrene). According to research data, formaldehyde can cause the decrease in the resistance of non-specific organism and dysfunction of the immune system[6] ; toluene on the prolonged receipt in an organism at the low doses can reduce the activity of neutrophils as well as the fall of the absolute number of T-lymphocytes, causes the imbalance of immunoglobulins A, D, G, M and interrupt the lysozyme activity[7]; phenols exposure causes the change of quantitative and qualitative cell and lymphocytic link of the immune system and induces the apoptotic death of immunocompetent cells.[8]

On the basis of these pathological processes lies the direct or indirect effect of toxicants on organs of the immune system. It is known that in lymphonoduses, spleen and lymphoid tissue the acquired immune response is formed. In the white and red pulp of the spleen contains substantial amount of immunocompetent cells in different degrees of maturity: T and B lymphocytes (from blast to mature forms), antigen-presenting dendritic cells, macrophages. The spleen is the largest peripheral lymphoid organs, in this connection, the authority in greater extent may reflects the change in the immune status of children. Activation of immune defense mechanisms starts the proliferative processes in the lymphoid tissue and especially in the spleen.[9] In addition, changes in the functional state of the spleen can be caused by disturbance of immunological reactivity and prolonged contact of variously determinate immunologically competent cells with the antigens found in the blood, including anthropogenic origin toxicants. In experimental studies, modeling situations of the impact of different toxic chemicals concentrations on animal's organism, several characteristic of morphological changes in the splenic parenchyma has been established: destructive processes in the white and red pulp of the spleen under the influence of different concentrations of detergents; reducing the number of splenocytes under the influence of potassium dichromate and benzene[10], reducing the number of T and B lymphocytes in the spleen during the benzene vapor inhalation[11]; inhibition of IgM synthesis in the spleen, morphometric changes in the white pulp[12], increasing the thickness of the capsule and significant reduction of lymphoid cell populations, augmentation the number of macrophages and megakaryocytes under the formaldehyde exposition.[13]

According to our own researches of children, living in the urbanized territories, statistically significant increase of the linear dimensions of the spleen against to similar parameters of unexposed children.[14]

Ultrasound investigation of the spleen allows getting an accurate view not only on the size of organ, but also on its structure. Local changes of spleen echostructure among the children may be associated with the limited number of pathological conditions such as tumors, abscesses, cysts, hematomas, infarcts, calcifications.[15] During the standard ultrasound investigation of the spleen the emphasis is upon quantitative characteristics and identification of the above focal formations. However, the modern ultrasonic diagnostic units allow evaluating the anatomy of the organ by its echostructure, which may reflect the active proliferative processes. According to Canadian researchers [15], ultrasonic method in making an assessment of the spleen of children allows to differentiate the lymphoid follicles, which are visualized as hypoechogenic site of damages on the sonographic sections. This conclusion was based on the fact that the distances between the sites identified on the sonograms are correlated with histologic measurements of distances within the white pulp.[16]

During implementation of our own pilot research and development projects we paid attention to the fact that the lymphoid follicles in the spleen in the process of ultrasound investigation in comparable by age and sex groups more often located in children living in conditions of sanitary and hygienic disadvantage. Figures 1 and 2 demonstrate the frequency of ultrasonic verification of normal and reactive changes (with the presence of lymphoid follicles) spleen in children, living on the territory of impact of disadvantage anthropogenic factors and on territory, relatively prosperous in sanitary and hygienic relation. In the first case, parenchymal changes in the spleen have met in 1.4 times more frequently (60.2% and 42.5%, $p = 0.02$; OR = 1.4; DI = 1.1-4.2), which could be due to the specific influence of anthropogenic factors on the structural elements of the immune system.

Consequently on the one hand the parenchymal changes in the spleen may indicate the adverse impact of external exogenous factors on the child's body and on the other side to reflect the immune status disturbances associated with exposure of immunotoxic chemicals.

In connection with this the purpose of investigation was to define the method of early diagnosis of the immune system disfunctions in children, living in conditions of the impact of organic compounds possessing the immunotoxicity.

Methods

Within the research 492 children, living near the oil refining complex, were observed. Comparison group consisted of 165 children living on the territory of relative well-being. Age of children is 3-15 years (median age in groups was 7.32 ± 1.55 and 8.12 ± 0.92 years, respectively). During investigation children were practically healthy.

During the instrumental field study 210 ambient air samples in the investigated territory were selected. Samples were collected during the sampling of blood of the examined children. On the investigated territory there are about 1500 stationary emission sources of 40 companies, including four major enterprises of oil refining and chemical industries. During the investigation of ambient air quality the exceeding of MPC m.s. (maximum permissible concentration maximum single) of toluene more than in 3 times was determined, the multiplicity of formaldehyde and phenol PDK m.s. excess is equal to 1.5 and 1.2 times respectively (Table 1).

On the control area in any of the analyzed samples toluene, phenol and formaldehyde PDK m.s. excesses was not revealed.

In the represented research the complex of clinical and laboratory examination methods was used: physical (general blood analysis - white blood cells, neutrophils, lymphocytes), immunological methods (indicators of nonspecific immunity - phagocytic activity, cellular immunity - CD3 +, CD4 +, CD8 +, CD19 +, CD56 +; humoral immunity - general IgA, IgM, IgG); investigations according to the standard procedures were carried out. Lymphocytes phenotyping was performed on the flow cytometer FACSCalibur of Becton Dickinson (USA) company using universal program CellQuest Pro by means of computer Macintosh. Populations and subpopulations of lymphocytes was determined by membrane immunofluorescence method using labeled monoclonal antibodies to CD-receptors (Becton Dickinson) and concentration of IgA, IgM, IgG using radial immunodiffusion method according to Mancini. Phagocytic activity of blood cells (absolute and relative phagocytosis, phagocytic index, phagocytic number) was investigated using formalinized sheep red blood cells.

Organic compounds (phenol, toluene and formaldehyde) in the blood were determined according to methodological guidelines "Determination of chemical compounds in biological media" (MG 4.1.763-4.1.779-99) and "Determination of hazardous substances in biological media"(MG 4.1 .2102-4.1.2116-06) by paraphase analysis method with various embodiments of sample preparation. Also was used the appropriate equipment: gas chromatograph (models 6890, 6890N, 6850, 7890A; reg. № 15118-07; country of manufacture - USA) liquid chromatograph Agilent 1200 with diode array detector (№ DE 62971887, USA, Germany), hardware -software complex "Chromatec-Crystal5000" (№ FSR 2009/04091; TS 9443-004-12908609-99, Russia).

All the examined children were subject to the standard examination of spleen on the ultrasound diagnostic system Toshiba APLIO XG SSA-790A (Japan) and evaluation of splenic tissue echostructure using the multifrequency linear array sensor with the frequency of 10 MHz.

Statistical analysis of investigation results was carried out by using Microsoft Excel. Quantitative characters presented as the arithmetic mean value and the arithmetic mean error ($M \pm m$). Comparison of quantitative characters was performed using Student's t-test for independent samples, the differences of the obtained results were considered statistically significant at $p \leq 0,05$. Comparative assessment of probabilistic relationship between features in the groups was assessed by OR with the analysis of Preclinical Study, the differences of the obtained results were considered statistically significant at $p \leq 0,05$.

The proof of relation of the immune system analyzed parameters with the level of chemicals content in blood were built by mathematical modeling method. To build this model the information of exposure markers values from the data table and the probability values corresponding to them was used. Mathematical model parameters (b0, b1) were determined by least squares method using software packages on statistical data analysis (Statistica, SPSS, SAS, etc.). Statistical significance of the parameters and the adequacy of the model were evaluated on the basis of one-way ANOVA according to Fisher's ratio test.

Discussion

Outcome analysis of chemical-analytical blood examination of children of both core and control groups allowed to reveal the presence of authentically significant difference in the average concentrations in groups according to substance sampled (Table 2).

Accommodation in conditions of prolonged impact of aromatic hydrocarbons reduces the level of health, formation of chronic somatic disease, which was confirmed by the results of research conducted by FBSI "Federal Scientific Center for Medical and Preventive Health Risk Management Technologies" in the given territory. Higher levels of overall morbidity and, in particular, the greater frequency of respiratory diseases have been demonstrated among the children living in the region of refinery complex enterprises, compared with indicators on the territory of the relative sanitary and hygienic well-being (urban-type settlement). Diseases of the respiratory system took place almost 2 times more frequently (75.3% and 40.4%, respectively, $p = 0.001$) and were presented by chronic inflammatory diseases of oral- and nasopharynx that have been flowed with hypertrophy of lymphoid tissue (65.1% vs. 25.3 % in the comparable area, $p = 0.000$). On the territory with the developed refining industry children with diagnosed secondary immune deficiency was almost 2 times more than in the comparable areas (42.7% and 22.2%, respectively, $p = 0.001$). During epidemiological research has been established the presence of reliable causal relationships between the emergence and development of chronic inflammatory diseases of oral- and nasopharynx, occurring with the hypertrophy of lymphoid tissue, and the impact of phenol (determination coefficient $R2 = 0.57\text{-}0.94$) and formaldehyde ($R2 = 0.68\text{-}0.95$) as well as between the formation of chronic tonsillitis and the toluene influence ($R2 = 0.95$).

Evaluation of immune system cellular link revealed that in child's blood of the core group has been statistically significantly reduced the percentage of CD3 + -, CD4 + -, CD16 +56 +-lymphocytes relative to the values obtained in the control group ($p < 0.05$). It was noted that children with technogenically contaminated territory had significantly increased expression of CD19 +-receptor and CD25 +-receptor on T-lymphocytes (by relative and absolute measure) in comparison with the results stated in the group of children living on the territory of minimal technogenic pollution ($p < 0.05$) (Table 3).

Correlation analysis showed that children of the core group have the significant positive relationship of cells quantity expressing the early activation marker, with the concentration of toluene in biological media ($r = 0.43$; $p < 0.05$).

Consequently, as a result of increased contamination of biological substrates with the toluene, formaldehyde and phenol among the children of the core group have been registered changing in the quantity of immune cells (CD3 +, CD4 +, CD19 +, CD16 +56 +, CD25 +) ($p < 0.05$), responsible for growth, differentiation of organism cells, recognition and destruction of transformed and aged cells. The identified changes of immunogramme indicators testifies the absence of an adequate immune response on the antigenic stimulus (hapten is an organic substance), and the relative depletion of the immune system capabilities.

For early diagnosis of immune system disfunction it is necessary to develop the screening, reliable noninvasive instrumental methods of investigation, allowing in timely manner to verify the adverse impact of technogenic factors on the child's body.

For this purpose all the examined children of the core group have been divided into two groups: 1st Group and 2nd Group. 1st Group comprised 256 children the spleen echostructure of which was characterized by the presence of multiple hypoechogenic homogeneous roundish inclusions throughout the ultrasonic section of the organ ranging in the size from 0.3 mm to 1 mm, located at a distance about 1.5-2 mm from each other (Figure 3).

2nd group included 236 children that according to ultrasound investigation had no disturbances in echostructure of spleen and it was characterized as homogeneous and fine-gritted (Figure 4).

In analyzing the data of chemical and analytical researchers of blood of children with disfunctions of spleen echostructure by cell response type have been obtained statistically significant differences of the number of indicators with immune profile of children without disrupting of echostructure: children of the 1st group have the mean group values of toluene, phenol, formaldehyde that are higher ($p \leq 0.05$) than in 2nd group (Table 4).

Comparative evaluation of clinical and laboratory parameters of examined children allowed to establish an increase of white blood cell count in 55% of samples of 1st group versus 14.5% of samples of the 2nd group ($p < 0.05$). It has been identified the causal relationships between increasing of toluene level and increasing in the absolute quantity of leukocytes ($R2 = 0.60$; $p = 0.005$). Also has been notified the following features of nonspecific resistance of organism and the immune system in the group of children with reactive changes of spleen in comparison with the group of children without disorders of its echostructure: higher mean group values of the absolute phagocytosis ($2.98 \pm 0.17 \times 10^9/dm^3$ and $2.49 \pm 0.16 \times 10^9/dm^3$ respectively; $p = 0.001$) more frequent IgG content reduction (in 33.7% and 11.3% of children respectively) and greater frequency of IgA serum level reduction (in 50.8% and 33.7% of inspected respectively). The 1st group showed higher indicators of relative number of CD3 +-cells than the 2nd group ($72.55 \pm 1.3\%$ vs. $66.32 \pm 0.82\%$; $p = 0.02$), percentage content of CD3 +-CD4 + - lymphocytes ($40.42 \pm 1.74\%$ vs. $36.42 \pm 0.88\%$; $p = 0.04$) and the relative number of CD19 +-cells ($11.25 \pm 1.29\%$ vs. $7.93 \pm 0.53\%$, $p = 0.05$).

The results of mathematical modeling, characterizing the relationship between levels of immunological blood parameters and concentration of toxic chemical substances in the blood of children of 1st group has testified about statistically significant effect of investigated organic compounds on the parameters of immunological homeostasis ($p \leq 0.05$). Models reflecting the possible mechanism of influence of toxicants on the indices of specific (acquired) immunity of children are referred in figures 6-8. It is possible that in conditions of influence of toxicant load occurs an adaptive change of immune status that involves activation of proliferative processes in lymphoid follicles of the spleen. Eventually it is visualized at carrying out of ultrasound investigation and can be regarded as an increase in the share of white pulp of the spleen.

It is established that at increase in concentration of toluene in blood the probability of detection in a blood the high level of CD19 +-lymphocytes (see Figure 5) is statistically significantly increased.

With increasing the concentration of formaldehyde in a blood the T-cellular link of immunity is activated (see Figure 6).

It was also established that with increasing the level of phenol in the blood it is also registered the decrease in the content of IgG (see Figure 7).

Conclusion

1. Children living in urbanized territories contaminated by chemicals with anthropogenic origin with the developed oil refining industry and having changes in echostructure of spleen in the form of multiple hypoechogenic homogeneous roundish inclusions throughout the ultrasonic section of organ (increasing of white pulp with lymphoid proliferation) having the blood with higher levels of organic compounds (toluene, phenol, formaldehyde) than children with intact spleen.

2. At the presence of modified echostructure of spleen in the form of proliferative changes of white pulp the indicators of immunologic status of children are in statistically significant dependence on the levels of investigated toxicants. By the method of mathematical modeling has been established the phenomena of increasing the content of CD19 +- lymphocytes at the increasing in blood the concentration of toluene, expression of CD3 +- lymphocytes at the increasing in blood the concentration of formaldehyde and decreasing the content of IgG at the increasing in blood the level of phenol.

3. Ultrasound investigation of spleen with the analysis of its echostructure can be recommended as a screening method for assessing of an adverse effect of technogenic environmental factors on the condition of immune system of children living in urbanized territories.

4. For children living in the urbanized territories with the detected changes in echostructure of spleen in the form of proliferative changes it is necessary to conduct immunologic research of the 1 and 2 levels for identification of disturbances of the immune status and chemical and analytical research of biological media for the purpose of an exception of technogenic toxicants exposition as the reasons of available immunologic deviations.

Conflict of Interest: None declared.

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Table 1: Laboratory data on admission

Variable	Mean Values	Reference range
White blood cell count ($x10^3/mm^3$)	**11**	**4-9**
Erythrocytes sedimentation rate (mm/h)	**108**	**2-13**
C-reactive protein (mg/l)	**77**	**<12**
Fibrinogen (mg/dl)	**866**	**200-400**
Hemoglobin (g/dl)	**9.8**	**12-15.5**

The results of investigations of ambient air quality on the territory of surveillance

Index	Toluene	Formaldehyde	Phenol
Maximum single MPC(mg/m^3)	0.6	0.035	0.01
Maximal of single concentrations (mg/m^3)	1.95	0.053	0.012
Shares of MPC	3.3	1.5	1.2

Table 2: Contamination level of low molecular weight chemical compounds in the blood of examined children (M ± m)

Indices	Core group	Control group
Toluene (mg/m^3)	0.00031±0.0002 (*n*=165)	0.0047±0.0033* (*n*=134)
Formaldehyde, (mg/m^3)	0.00432±0.00052 (*n*=121)	0.0355±0.0127* (*n*=154)
Phenol, (mg/m^3)	0.0369±0.0118 (*n*=40)	0.373 0.0055* (n =55)

*Note: * - difference is significant in comparison with the core group (p <0.05).*

Table 3: Indicators of immunogramme of children, living in the different sanitary and hygienic conditions

Indices	Control group (*n*=100)	Core group
CD3+,(%)	72.58±0.74	66.33±0.41*
CD4+, (%)	40.60±0.99	36.31±0.44*
$CD19_+$, (10^9/l)	0.35±0.02	0.45±0.01*
$CD16^+56_+$, (%)	11.56±0.75	7.92±0.26*
$CD25_+$, (%)	6.29±0.30	7.00±0.17*
$CD25_+$, (10^9/l)	0.16±0.01	0.20±0.01*

*Note: * - difference is significant in comparison with the core group (p <0.05).*

Table 4: Content of contaminants in the blood of children in the 1st (with disorders of spleen echostructure) and 2nd (with normal spleen echostructure) groups, (M ± m)

Index	Normal range	1st group, n = 256	2nd group, n = 236	Intergroup differences in average values (p)
Toluene (mg/dm^3)	0	0.0241 ± 0.011	0.014 ± 0.006	≤ 0.05
Phenol (mg/dm^3)	0.010 ± 0.012	0.052 ± 0.011	0.021 ± 0.008	≤ 0.05
Formaldehyde (mg/dm^3)	0.0019 ± 0.005	0.033 ± 0.007	0.014 ± 0.003	≤ 0.05

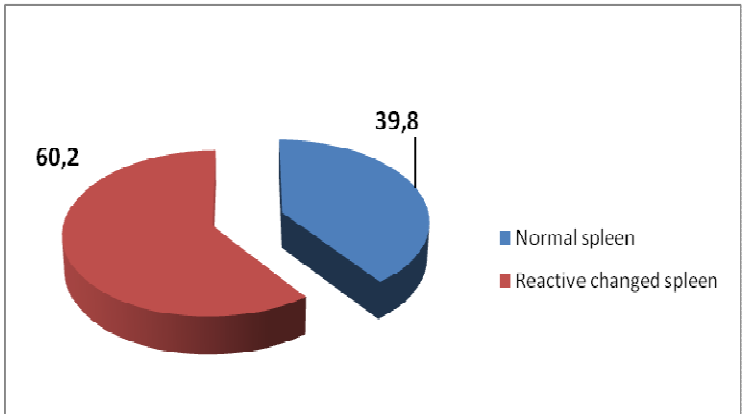

Figure 1: Frequency of ultrasonic verification of normal and reactive changed spleen in children living on the territory with high environmental footprint

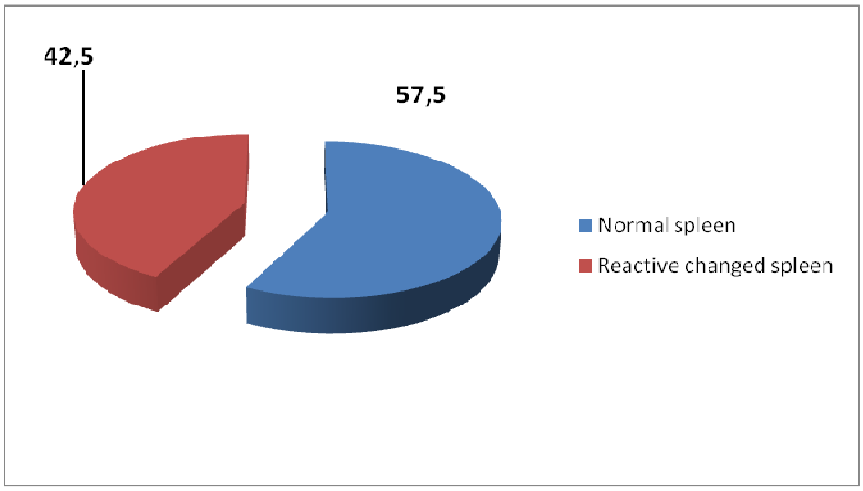

Figure 2: Frequency of ultrasonic verification of normal and reactive changed spleen of children living on the territory with relative sanitary and hygienic well-being,

Figure 3: Reactive changes in spleen

Figure 4: Normal echostructure of spleen. Photos of authors

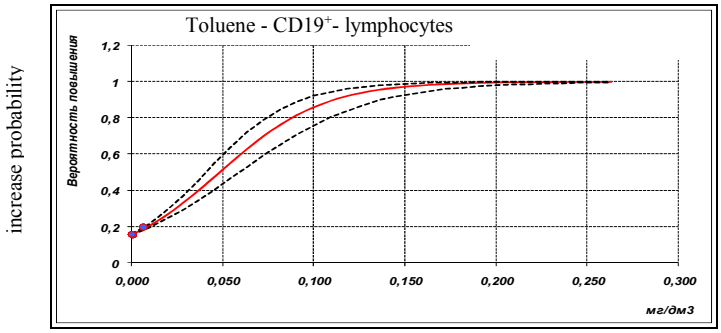

Figure 5: Models of dependence of the probability of $CD19^{+}$- lymphocytes level increase from the toluene concentration in the blood

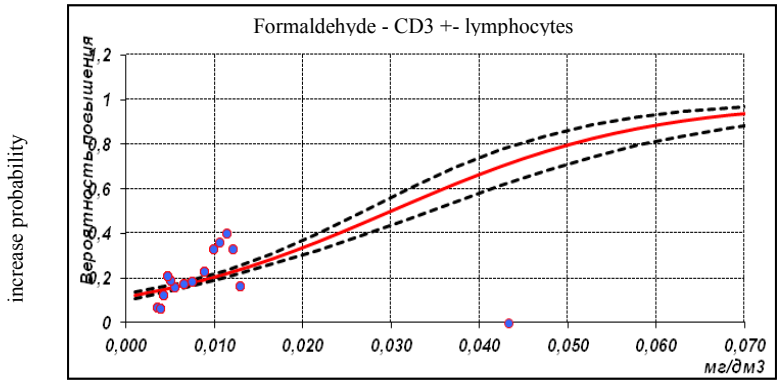

Figure 6: Models of dependence the probability of CD3 +- lymphocytes level increase from the formaldehyde concentration in the blood

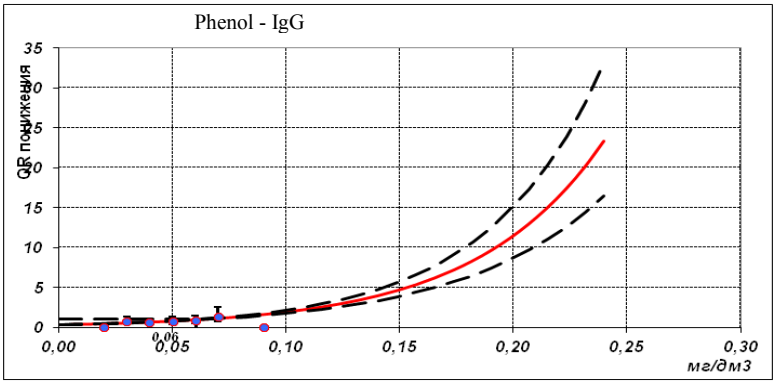

Figure 7: Models of dependence of immunoglobulin G reduction risk from phenol content in the blood