THE EFFICIENCY OF IMMUNE MODULATOR BIVEL IN THE PREVENTION OF PULMONARY TUBERCULOSIS IN EXPOSED CHILDREN FROM THE MULTIDRUG-RESISTANT TUBERCULOSIS SITES

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Summary.

120 exposed children/adolescents (75 children and 45 adolescents) from the multi-drug resistant tuberculosis sites underwent the complex clinical radiological and immunological examination.

Insignificant functional disorders of cellular response (immunoregulatory processes) caused by the prevalence of suppressor and cytotoxic reactions by 1.3 times and by the prevalence of pro-inflammatory cytokines in the regulatory system (2.0 times above the norm, TNF-α/IL 10.0. p<0.01) were revealed in the infected children/adolescents from the multi-drug resistant tuberculosis sites, while their CD3+. CD3+CD4+. CD3+CD8+ were within norm. The evident disorders of the regulatory system and cell immune system were eliminated after the completion of the autumn-spring BI-V course. The non-specific immune regulator BI-V is efficient for the prevention of multi-drug resistant tuberculosis for the exposed children/adolescents from the multi-drug tuberculosis sites. Consequently, the latent TB infection grew into the active form by 2.8 times less often in the children that took BI-V as compared to the infected children who did not take the drug.

Key words: BIVEL immunomodulator, pulmonary tuberculosis in children, cellular immunity, tuberculosis.

Резюме. Эффективность иммуномодулятора БИВЕЛ для профилактики туберкулеза легких у детей из очагов мультирезистентного туберкулеза.

Проведено комплексное клинико-рентгенологическое и иммунологическое обследование 120 облученных детей/подростков (75 детей и 45 подростков) из очагов туберкулеза с множественной лекарственной устойчивостью.

Незначительные функциональные нарушения клеточного ответа (иммунорегуляторных процессов), обусловленные преобладанием супрессорных и цитотоксических реакций в 1,3 раза и преобладанием провоспалительных цитокинов в системе регуляции (в 2,0 раза выше нормы, TNF-α/IL 10.0. p<0.01)выявлены у инфицированных детей/подростков из очагов МЛУ-ТБ, а у них CD3+. CD3+CD4+. CD3+CD8+ были в пределах нормы.

Выраженные нарушения регуляторной системы и клеточного иммунитета были устранены после прохождения осенне-весеннего курса BI-V. Неспецифический иммунорегулятор BI-V эффективен для профилактики туберкулеза с множественной лекарственной устойчивостью у детей/подростков, контактировавших с полирезистентными очагами туберкулеза. Следовательно, латентная туберкулезная инфекция перешла в активную форму в 2,8 раза реже у детей, принимавших BI-V, по сравнению с инфицированными детьми, не принимавшими препарат.

Ключевые слова: иммуномодулятор БИВЕЛ, туберкулез легких у детей, клеточный иммунитет, туберкулез.

Rezumat. Eficiența imunomodulatorului BIVEL pentru prevenirea tuberculozei pulmonare la copiii din focarele de tuberculoză multidrogrezistentă.

A fost efectuată o examinare clinică, radiologică și imunologică cuprinzătoare a 120 de copii/adolescenti (75 de copii și 45 de adolescenți) din focarele de tuberculoză multidrogrezistentă.

Tulburări funcționale minore ale răspunsului celular (procese imunoreglatoare) datorită predominării reacțiilor supresoare și citotoxice de 1,3 ori și predominării a citokinelor proinflamatorii în sistemul de reglare (de 2,0 ori mai mare față de normă, TNF-α/IL 10,0. p<0,01) au fost determinate la copii/adolescenti infectați, din focarele de TB-MDR și CD3+CD3+CD4+. CD3+CD8+ au fost în limite normale.

Dereglările pronunțate ale sistemului de reglare și a imunității celulare au fost eliminate după urmarea cursului de toamnă-primăvară de BI-V. Imunoregulatorul nespecific BI-V este eficient pentru prevenirea tuberculozei multidrogrezistente la copiii/adolescenții din focarele de tuberculoză multidrogrezistentă. Prin urmare, infecția TB latentă a trecut în activă de 2,8 ori mai rar la copiii tratați cu BI-V decât la copiii infectați care nu au urmat medicamentul.

Cuvinte cheie: imunomodulatorul BIVEL, tuberculoza pulmonară la copii, imunitate celulară, tuberculoză.

Introduction.

Tuberculosis is one of the crucial issues of modern medicine directly affecting the social and economic aspects of society. This is due to the increase in the newly diagnosed people with the specific process caused by the mycobacterium tuberculosis strains resistant to antimycobacterial drugs [9, 15,18].

Under the increased occurrence in the drug resistant pulmonary tuberculosis among the adult population, special attention should be paid to children and adolescents from the multi-drug resistant tuberculosis sites [1, 2, 6-8, 11]. In particular, preemptive measures to prevent the dangerous disease should be taken [3, 12, 13]. The prevention of the specific process and chemoprophylaxis of tuberculosis play the main role [5, 6].

Tuberculosis belongs to the group of diseases expressed as the chronic granulomatous inflammation of immune genesis caused by the long-lasting effect of tuberculosis bacterium persisting in the organs and tissues. Immunocompetent cells play the main role in fighting against tuberculosis by developing a specific granuloma, which isolates the infection and is affected by the latter. The immunological diagnostic techniques are important for defining the etiology and flow of the process as well as for predicting it for the adults and children/adolescents [6, 14].

According to the last regulatory documents, the chemoprophylaxis of tuberculosis implies isoniazid or isoniazid and rifampicin for the children and adolescents with the first positive responses to tuberculin test (virage), with hyperergic reactions to tuberculin (17 mm and more) and for the infected children and adolescents from the sites of tuberculosis at the time when their contacts with bacillary patients may be the most unfavorable.

However, chemoprophylaxis is reasonable only for the exposed children residing in the drug-susceptible tuberculosis sites [16]. The first-line drugs are not reasonable for the children residing in the multi-drug resistant tuberculosis sites [16]. If this is the case, chemoprophylaxis shall include drugs based on the infection source having affected the children/adolescents. In such cases, the choice of the second-line preventive drugs for the exposed children and adolescents is often challenging. For this reason, the selection of preventive drugs for the children and adolescents exposed to the multi-drug resistant pulmonary tuberculosis (MDR-TB) is required.

Objective.

To study the expediency of using the natural immunomodulator BIVEL (BI-V) as a non-specific immunoprophylaxis of TB in contact children from foci of multidrug-resistant tuberculosis infection.

Materials and methods.

120 exposed children/adolescents (75 children and 45 adolescents) from the multi-drug tuberculosis sites (MDR-TB sites) were the research objects. The exposed children underwent the complex clinical, radiologic and laboratory examination.

The microbiological study of the infection source among the adults included the reveal of the mycobacterium tuberculosis in sputum by applying the sputum smear microscopy, cultivation in the Lowenstein-Jensen media, typing of excreted mycobacteria to define BACTEC MGIT 960, testing mycobacterium tuberculosis (MTB) susceptibility to the first and second lines antimycobacterial drugs, the molecular-genetic sputum testing by means of GeneXpert MTB/RIF and the line-probe assay (Jain Lifescience) aimed at defining the MBT susceptibility to the first-line antimycobacterial drugs (isoniazid and rifampicin) by applying the GenoTyp MTBDRplus hybridization techniques, and to the second-line drugs (fluoroquinolones and aminoglycosides) by means of GenoTypMTBDRsl [4].

The lymphocyte population and subpopulation patterns (CD3+. CD3+CD56+. CD3+HLA–DR+. CD3+CD4+. CD4+45RA+. CD3+CD8+. CD4+/CD8+. CD19+. CD16/56+. CD16/56+CD8+) of 25 (before immunoprophylaxis) and 22 (after immunoprophylaxis), exposed children from the MDR-TB sites were evaluated. The evaluation was performed in the medical laboratory DILA by applying the direct immunofluorescence technique with the use of anti-CD-monoclonal antibodies and further identification of the lymphocyte structures with the flow cytometry FACScan BD Bioscience (the USA).

The function of B-cells was evaluated based on the serum concentration of immunoglobulins (Ig) (classes A. M. G) by applying the ELISA technique and KHEMA-MEDICA (Ukraine) test system. The results were fixed with the help of μQuant spectrophotometer (BioTek, the USA). The measurement range was 200-999 nm, inaccuracy ± 1.0%.

The concentration of circulating immune complexes was evaluated by performing precipitation tests with polyethylene glycol and the KHEMA-MEDICA test-system (Ukraine). The results were fixed with the μQuant spectrophotometer (BioTek, the USA). The measurement range was 200-999 nm, inaccuracy ± 1.0%.

Serum cytokines (IL-1β. IL-2. IL-10. TNF-α) levels were tested by applying the ELISA technique (analyzer MR-96A) and Vector-Best (Russia) kits.

To improve the efficiency in the non-specific immunoprophylaxis of tuberculosis in the exposed children and adolescents from the MDR-TB sites, we made a decision to boost the immunity by using the combined immune modulator BIVEL (BI-V)

consisting of dry yeast fermentation Saccharomyces cerevisiae (EpiCor®), vitamin C, zinc (Zn) and vitamin D3. This is a 120 ml suspension for internal use produced by ErgoPharma Ltd (Slovenia). The immune modulator BI-V was given to the children aged 3 and more to take 5.0 ml once a day for 24 days twice a year (in autumn and spring).

The children and adolescents from the MDR-TB sites were divided into two groups. The first group consisted of 95 patients who did not receive BI-V. The second group consisted of 25 infected children/adolescents who were given BI-V. 10 of them had hyperergic reactions and 15 had virage tuberculin test. The tuberculosis risk factors were evident in the past medical history of all the exposed patients. According to the requirements, the immune modulator BI-V was prescribed for children aged 3 and more to take 5 ml once a day for 24 days. The non-specific immunoprophylaxis course was repeated twice a year (in autumn and spring) at the time when the risk of the disease was the highest.

The results and discussion.

The adults that were in contact with the children/ adolescents, excreted mycobacterium tuberculosis with the resistant profiles specified below: multi-drug resistance (MDR) was revealed in 11 cases (22.0%); resistance to rifampicin (Rif) was evident in 23 patients (46.0%); 5 (10.0%) and 11 (22.0%) patients demonstrated the extensive drug-resistance and high drug-resistance correspondingly. 30% of the adults (the infection source) who resided in the multi-drug resistant tuberculosis sites died because of the specific process progression.

The most part of the exposed children/adolescents with pulmonary tuberculosis from the multi-drug resistant tuberculosis sites had comorbidities: acute respiratory viral infections (33.0 %), chronic non-specific respiratory diseases (22.0%) and other pathologies (spasms, mental diseases, allergies, diabetes mellitus) leading to the lower reactivity of the body (Fig. 1).

Social factors had a considerable impact on tuberculosis flow in children and adolescents from the multi-drug resistant tuberculosis sites (Fig. 2). According to the research, most exposed patients infected by tuberculosis were the children/adolescents from the low-income families (33.0%), large families (27.7%), asocial families (27.7%) and migrated families (11.3%). Their amount has increased in the wartime.

Consequently, tuberculosis occurrence among the exposed children is closely related to the medical and social factors lowering protective immune response and causing the specific inflammation.

The protective barrier of the immune system may be insufficient due to other factors depending on the antigenic impact, the causative agent type and virulence, massiveness, the macro organism affected by the external and internal factors (nervous and endocrine systems, metabolic processes), the primary and secondary immune deficiency, etc. Thus, it is very important to work in the tuberculosis infection sites and reveal infected people including those with immune system suppression, conduct preemptive measures, consider the need for immune therapy and control its efficiency. The selected and implemented preemptive measures must be efficient and prevent the disease.

The reveal of the infected people is the important stage of work in the site of any infection. The subcutaneous tuberculin skin Mantoux test with 2 TU PPD-L is the most widely spread and cheap technique used to reveal the mycobacterium tuberculosis. QuantiFERON-TB Gold ELISA has been recently applied in Ukraine for in vitro diagnostics. This is an enzyme immunoassay for testing the cell response to the peptide antigens ESAT-6 and CFP-10 based on the whole blood interferon level. During the research, the infection in the exposed children/adolescents was revealed by applying both the Mantoux test with 2 TU PPD-L and QuantiFERON-TB test in parallel. According to the research results, the Mantoux skin test revealed the tuberculin-type hypersensitivity in 82.5% (99) patients. 33.3% or 40 patients of all the examined people (120) had a slightly positive tuberculin reaction with the papule diameter of 5-11 mm. 49.2% (59) children/adolescents showed the hypersensitive and hyperergic reactions to tuberculin (Table.1). The Mantoux skin test with 2 TU PPD-L virage was evident in 33.3% cases (40 people).

The QuantiFERON-TB test was found positive and confirmed the latent tuberculosis infection in 86.7% (104) cases. It was negative in 13.3% (16) cases.

Consequently, according to the specific diagnostics results, in average, 84% of the children and adolescents from the tuberculosis infection sites were infected by the mycobacterium tuberculosis. The infection was confirmed by the Mantoux skin test with 2 TU PPD-L in 82.5% cases and by the QuantiFERON-TB test in 86.7% cases.

Table 1.

Tuberculin sensitivity of children/adolescents from the MDR-TB sites

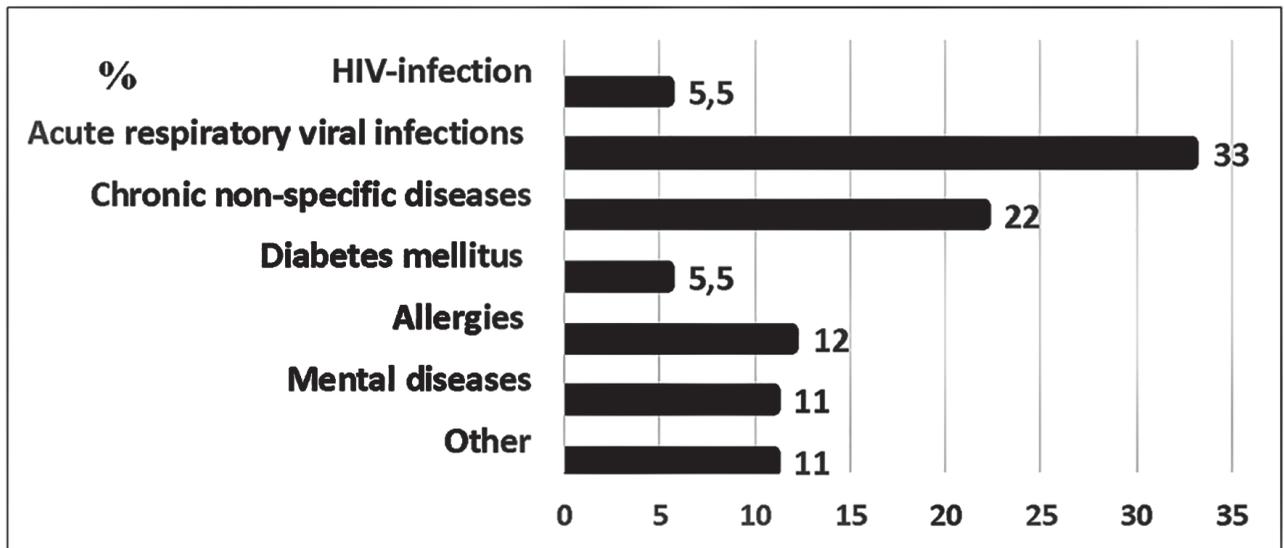| Papule size. Mm	The examined groups of children (n=120)	
	Absolute amount	%
0 – 4	21	17.5
5 – 11	40	33.3
12 – 16	30	25.0
17 and more	29	24.2
The average papule size, mm	15.4 ± 0.3	

Figure 1. The frequency of medical risk factors causing tuberculosis in children/adolescents from the multi-drug resistant tuberculosis sites.
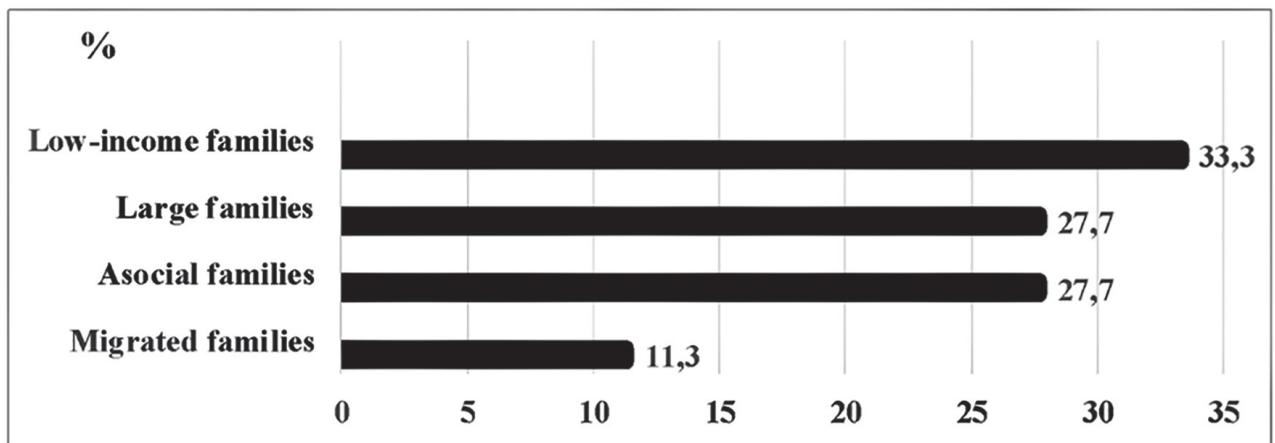

Figure 2. The frequency of social risk factors causing tuberculosis in the exposed children/adolescents from the MDR-TB sites.
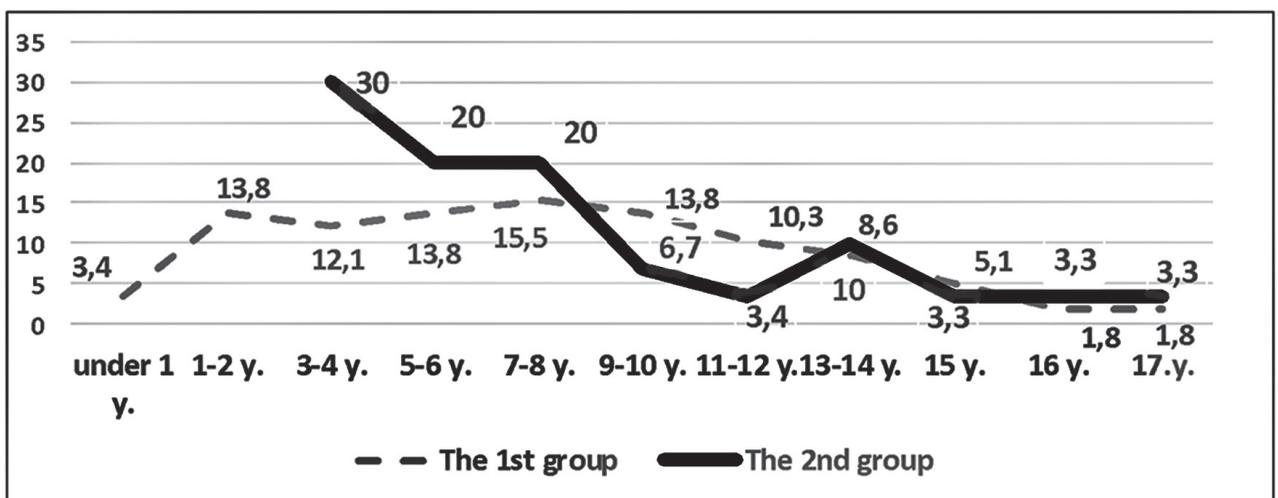

Figure 3. The age structure of the exposed children from the multi-drug resistant tuberculosis sites. The children from the first group were not given BI-V; the children from the second group were given BI-V.

The reveal of the infected people in the tuberculosis infection sites excludes the possibility of unreasonable chemoprophylaxis for children/adolescents considered as non-infected according to certain clinical and laboratory indicators. Based on the clinical protocol [16], levofloxacin and prothionamide are recommended to the exposed children from the multi-drug resistant tuberculosis sites for the sake of chemoprophylaxis. At the same time, levofloxacin causes a lot of adverse effects like nausea, vomit, diarrhea, hyper-bilirubinemia, skin rash, itch, headache, fatigability, loss of sleep, hallucinations, lethargy, faintness, motor excitement, psychosis, leukopenia, agranulocytosis, thrombocytopenia, nephrotic syndrome, sometimes acute renal failure, edematization of face and vocal folds, arthralgia, myalgia, vision disorders, photosensibilization, etc. The possible adverse effects of prothionamide are stomatitis, metallic taste in the mouth, nausea, vomit, diarrhea, hepatic impairment, anorexia, neuritis, headache, faintness, poor sleep, neurosis, mental depression, tachycardia, arterial hypotension.

In practice, the chemoprophylaxis of MDR-TB with the use of levofloxacin and prothionamide among the exposed children and adolescents has not been widely applied due to high toxicity of the drugs. In most cases, patients refuse to take the drugs or the chemoprophylaxis time limits are violated decreasing its efficiency. The use of additional pathogenic means to reduce the adverse effects of anti-tuberculosis drugs is one of the solutions. In case of the unconditional refuse to take the drugs, immune boosting modulators serve as the alternative infection resisting solutions [5, 17]. The seasonal catarrhal diseases occur most often in winter and spring when there is vitamin D deficiency.

The research conducted by R.M. Daly et al. [19] proves that vitamin D deficiency is by 3 or 4 times more severe during winter and spring as compared to summer, and is twice as severe for people residing at 35^0 of latitude along the parallel. A. Fares examined 12 research papers on the disease seasonal occurrence during 1971-2006 in 11 countries [20]. He believes that the variability of vitamin D level, the seasonal changes in the immune system and nutrition, etc. are significant factors that may affect the disease seasonal occurrence. Unlike respiratory diseases, more often revealed in winter, tuberculosis is not affected by seasons. According to L.I. Mykolyshyn, Z.I. Piskur [10], J.H. MacLachlan [21], C.M. Parrinello [22], T. Wingfield et al. [23], susceptibility to tuberculosis may be caused by the lack of vitamin D. L.I. Mykolyshyn and Z.I. Piskur [10] state that vitamin D deficiency was evident in 43.8% of the

children diagnosed with respiratory tuberculosis. 37.5% children suffered from the vitamin deficiency, and the vitamin level was within the norm only in 18.8% cases. Thus, considering the decrease in the immune responsiveness and vitamin D deficiency (both seasonal and alimentary) typical for children, we preferred BI-V.

The efficiency of BI-V in the prevention of multi-drug resistant pulmonary tuberculosis for the exposed children and adolescents residing in the drug resistant tuberculosis sites was the objective of our research paper. The total amount of people involved in the research was 120. 75 (62.5%) of them were children aged 0-14 and 45 (37.5%) were adolescents aged 15-18. The age structure of the exposed children and adolescents is available at Figure 3. According to Figure 3, the largest group consisted of the exposed children aged 9 and less. The first group (0-9 years) made 72.4%, 14.2% of which were aged 0-3. The second group (3-9 years) made 76.7%. Children aged under 3 were not included in the second group because of BI-V age restrictions.

The frequency of acute respiratory viral infections and the aggravation of non-specific bronchopulmonary diseases lowering the immunity of the children/adolescents and exposing them to different infections is higher during the cold months of the year. Consequently, the prophylaxis of catarrhal diseases among the exposed people is one of the factors boosting the body's resistance and decreasing the risk of tuberculosis.

Our research revealed that the incidence rate of the acute respiratory viral infections was 55.5% in the first group of children without BI-V, the aggravation of non-specific bronchopulmonary diseases made 33.3%. Immune modulator BI-V was prescribed twice a year (in autumn and spring) apparently decreasing the incidence rate of the diseases to 27.8% and 16.7% accordingly. p<0.05. The children's appetite and memory improved, their physical activity increased. The children's academic performance went up. Due to the unique combination of vitamins C and D, zinc and dry yeast fermentation, EpiCor® Bi-V boosts the body resistance, provides more energy during seasonal catarrhal diseases and protects cells from the oxidative stress.

The evaluation of the immunity status, the cellular immune response and the pro-inflammatory and anti-inflammatory cytokines of the children/adolescents was made in the second group. The evaluation revealed that the cellular immune response disorders in the infected children/adolescents from the multi-drug resistant tuberculosis sites were more apparently caused by the intracellular off-

Table 2.

Immune response of the exposed children/adolescents from the multi-drug resistant tuberculosis sites before and after the BI-V preventive course

Indicators	Examined groups		
	Donors	Exposed children/adolescents	
	(n=17)	before the preventive course (n=25)	after the preventive course (n=22)
T-cells CD3+ (%)	69.5 ± 2.0	63.8 ±3.1	73.6 ± 3.3#
T-helper cells CD3+CD4+ (%)	39.0 ± 1.6	34.2±2.7	38.3±2.4
T-suppressor cells/ cytotoxic cells CD3+CD8+ (%)	28.5 ± 1.4	33.5 ± 2.3	29.1 ± 1.8
The correlation of CD3+CD4+/ CD3+CD8+ (IRI – Th/Ts)	1.30 ± 0.08	1.02 ± 0.10*	1.32 ± 0.10#
TNF-α pg/ml	1.47±0.12	3.50±0.32*	2.13±0.28*#
IL-6 pg/ml	1.72±0.14	2.25±0.20*	1.84±0.16#
IL-10 pg/ml	5.14±0.26	5.23±0.42	5.30±0.29
TNF-α/IL 10	0.33±0.07	0.67±0.05*	0.40±0.08#
IL 6/IL 10	0.29±0.05	0.43±0.07	0.35±0.04

Notes: *the apparent difference with the group of donors is $p<0.05$; # the apparent difference before the BI-V preventive course is $p<0.05$.

Table 3.

The dynamics of the Mantoux test with 2 TU PPD-L before and after the preemptive measures for the exposed children from the multi-drug resistant tuberculosis sites

The groups of children	Infiltrate size, mm	
	Before the preventive measures	After the preventive measures
Virage tuberculin reaction (n=15)	12.2±0.4	10.7±0.5*
Hyperergic reaction to tuberculin (n=10)	18.6±1.0	14.2±0.7*

Note. * – the apparent difference with the indicators before the BIVEL (BI-V) preventive course. $p < 0.05$-0.01.

Table 4.

Tuberculosis occurrence among the children/adolescents from the multi-drug resistant tuberculosis sites during 4 years of observation

The years of observation	Groups				p
	The first (p = 95)		The second (p = 25)		
	Absolute amount	%	Absolute amount	%	
For 4 years	21	22.1	2	8	<0.05
The first year	8	38.2	1	50	>0.05
The second year	9	42.8	1	50	>0.05
The third year	2	9.5	-	-	>0.05
The fourth year	2	9.5	-	-	>0.05
Total	21	100	2	100	

Note. *- the apparent difference with the first group. $p < 0.05$

balance of certain lymphocyte pools, as well as the off-balance of the pro-inflammatory and anti-inflammatory cytokines in the regulatory system, rather than by the quantitative changes (decrease/increase) of certain lymphocytes. This is confirmed by the results of the cell populations research: general T-cells CD3+, T-helper cells CD3+CD4+, suppressor T-cells, cytotoxic T-cells CD3+CD8+. The tendency towards the insignificant decrease in CD3+ ($p > 0.05$) as compared to the donors, CD3+CD4+($p > 0.05$), and the increase in T-CD3+CD8+ ($p > 0.05$) was revealed in the infected children/adolescents from the multi-drug resistant tuberculosis sites. The values never exceeded the confidence intervals and never clearly specified the immune responses of the body infected by mycobacterium tuberculosis (Table 7.2). Taking into consideration the immune system complexity and multicomponent nature, the immunoregulatory indices have often been used in practice. They describe the interrelation of different lymphocyte subpopulations and specify the immune response. The most widely applied immunoregulatory index (IRI) is CD4+/CD8. It evaluates the imbalance of T-helper cells, suppressor and cytotoxic T-cells and specifies the immunoregulatory response (inflammatory, allergic or combined).

The correlation of CD3+CD4+ with CD3+CD8+ in the infected children/adolescents from the multi-drug resistant tuberculosis sites complied with index (1.02 ± 0.10) versus healthy people (1.30 ± 0.08). ($p < 0.05$). The difference was apparent and pointed at the specific immune response (inflammation) and the prevalence of suppressor T-cells and cytotoxic T-cells, which was impossible to prove based only on CD3+CD4+ and CD3+CD8+.

The increase in the pro-inflammatory interleukins TNF-α ($p < 0.05$), IL-6 ($p < 0.05$) and cytokine ratio TNF-α/IL 10 ($p < 0.05$) (as compared to the donors) proved the evidence of the mycobacterium tuberculosis infection in the children/adolescents from the multi-drug resistant sites. The cytokine ratio pointed at the prevalence of pro-inflammatory mediators in the immune response (table 2).

The examination of the children/adolescents after the tuberculosis preventive course of BI-V showed the increase in T-cells CD3+ ($p < 0.05$) by 1.2 times as compared to the baseline data, the restore of balance between CD3+CD4+ and CD3+CD8+ lymphocyte pools and the normalization of IRI ($p < 0.05$).

After the completion of the preventive BI-V course, the level of pro-inflammatory cytokines TNF-α in the immune response was decreased by 1.6 times – from (3.50 ± 0.32) pg/ml to (2.13 ± 0.28) pg/ml ($p < 0.05$) at the norm of (1.47 ± 0.12) pg/ml. $p < 0.0$;

IL-6 was by 1.2 times less – from (2.25 ± 0.20) pg/ml to (1.84 ± 0.16) pg/ml ($p < 0.05$) at the norm of (1.72 ± 0.14) pg/ml $p > 0.05$; cytokines TNF-α/IL 10 decreased by 1.7 times – from (0.67 ± 0.05) to (0.40 ± 0.08). $p < 0.05$ at the norm of (0.33 ± 0.07) $p > 0.05$.

Thus, the insignificant immune response disorders were revealed in the infected children/adolescents from the multi-drug tuberculosis resistant sites. Immunoregulatory disorders were caused by the prevalence of suppressor and cytotoxic immune responses and the imbalance between the pro-inflammatory and anti-inflammatory cytokines in the regulatory system along with the production of the pro-inflammatory cytokines. The immune response disorders were eliminated after the completion of the preventive BI-V course.

The evaluation of the specific process and the efficiency of tuberculosis preventive or treatment measures requires retesting, in particular, tuberculin skin testing with 2 TU PPD-L. The decrease in skin sensitivity to tuberculin is one of the efficiency indicators. As mentioned above, 15 children/adolescents that were given BI-V, had virage tuberculin skin test, and 10 children/adolescents had hyperergic reactions. The autumn-spring preventive course of BI-V resulted in the decrease in skin sensitivity to tuberculin. In particular, the intensity of Mantoux test with 2 TU PPD-L reaction lowered from (12.2 ± 0.4) mm to (10.7 ± 0.5) mm $p < 0.05$ in the virage children, and from (18.6 ± 1.0) mm to (14.2 ± 0.7) mm. $p < 0.01$ in the children with the hyperergic reaction (table 3).

The summary of the 4-years' observations of the specific process evident in the exposed children/adolescents from the multi-drug resistant tuberculosis sites was probably the most important final stage of the research.

Tuberculosis indicators for 4 years of observation are available in Table 4.

According to the 4-years' observation of the children/adolescents from the multi-drug resistant tuberculosis sites, tuberculosis reached its peak during the first 2 years of observation. During the first year, the active process was diagnosed in 38.2% (8) and 50.0% (1) cases in the first and second groups accordingly. During the second year, it was diagnosed in 42.8% (9) and 50.0% (1) cases accordingly. Within the next years, 19.0% (4) of the exposed patients who did not take BI-V, got infected: 9.5% (2) and 9.5% (2) during the third and fourth years accordingly.

Consequently, 22 (22.1%) exposed patients from the first group who did not take the immune modulator BI-V, and 2 (8.0%) people from the second group got infected with different forms of primary pulmonary tuberculosis. $p < 0.05$.

Conclusions

1. Non-specific immune modulator BI-V is efficient for the prevention of the multi-drug resistant tuberculosis in the exposed children/adolescents from the drug resistant infection sites. The latent TB infection grew into the active form by 2.8 times less often in children that took BI-V as compared to the infected children who did not take the drug.

2. According to the research results, in average, 84.0% of the children and adolescents from the multi-drug resistant tuberculosis sites were affected by mycobacterium tuberculosis as confirmed by diagnostic testing of specificity. The mycobacterium tuberculosis infection was confirmed by the Mantoux skin test with 2 TU PPD-L and QuantiFERON-TB test in 82.5% and 86.7% cases correspondingly. The intensive and hyperergic reactions to tuberculin were evident in 49.2% cases, virage of tuberculin test was revealed in 33.3% cases.

3. Along with the normal findings of CD3+, CD3+CD4+, CD3+CD8+, the insignificant cell response functional disorders (immunoregulatory) were revealed in the infected children/adolescents from the multi-drug resistant tuberculosis sites. The disorders were caused by the prevalence of the suppressor and cytotoxic reactions (IPI CD3+CD4+/CD3+CD8+, $p<0.05$ was by 1.3 times less as compared to the donors) and the prevalence of the pro-inflammatory cytokines in the regulatory system (by 2.0 times above the norm, TNF-α/IL 10.0. $p<0.01$). The disorders of the regulatory system and cell response disappeared after the completion of BI-V course in spring and autumn.

4. Immune modulator BI-V preventively prescribed for the children/adolescents from the multi-drug resistant tuberculosis sites reduced the incidence rate of acute respiratory viral infections and the aggravation of non-specific bronchopulmonary diseases by 2.0 times. The latent TB infection grew into the active form by 2.6 times less often, appetite got revived, memory and academic performance improved, physical activity increased.

5. According to the-years' observation results, 8% of the children from the multi-drug resistant tuberculosis sites who took BI-V preventatively and 22.1% of the children who did not take BI-V, $p<0.05$, were affected by different forms of primary pulmonary tuberculosis. In both groups, the disease reached its peak during the first 2 years of observation.

6. Immune modulator BI-V is efficient for the prevention of certain bronchopulmonary diseases including the drug-resistant tuberculosis for the exposed children/adolescents from the drug resistant infection sites.

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