Immunological Parameters in Children with Tuberculosis Disease and Latent Tuberculosis Infection

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Abstract

It is necessary to identify immunologic parameters in children with latent tuberculosis infection. Immunological profile in children with various manifestations of tuberculosis infection isdescribedin literature not sufficiently that requires further investigation. The article presents results of the survey of 155 children aged 3 to 14 years with various manifestations of tuberculosis (TB) and positive results ofDiaskintest® (DST) and QuantiFERON-TV Gold (QFT). Diagnostic complex included the data of clinical, radiological (multislice computed tomography) and immunological examination methods. All children were assigned to two groups: I group - 126 patients with tuberculosis disease (TB), II group - 29 patients with latent tuberculosis infection (LTBI). Based on results of the research it was found out a number of peculiaritiesof immune response in children with latent TB infection and active TB disease of the respiratory organs. In LTBI marked trend of elevation in induced production of cytokines (IL-2, IL-4, IFN-g) and changes of some indicators of phagocytic activity of neutrophils (FC and PHI) were revealed. While in development of TB activation of the humoral response was a prevailing feature(increased titers of PTOT in the complex serological reactions and level TB antibodies of Ig A and Gclassesbyanda-tb ELISA). This data can serve as additional diagnostic criterion for the diagnosis of LTBI in children.

KEYWORDS: children, diagnostics, Diaskintest®, immunity, Quantiferon TB-Gold, tuberculosis

Introduction.

The relevance of wide application of immunological tests for the early diagnosis of tuberculosis in children is justified by minimal clinical manifestations of the disease in these patients [1,2,3].

Used in Russia immunological tests (Diaskintest® (DST) and QuantiFERON -TB (QFT) help to identify patients with high risk of tuberculosis disease, since their positive results associated with the process of active multiplication of M. tuberculosis. Diaskintest® (is used in the Russian Federation since 2009) is a skin test with proven high diagnostic sensitivity and specificity, is based on recombinant tuberculosis allergen, including peptides ESAT-6 (early-secreted antigenic target) and CFP-10 (culture filtrate protein, which is presented in M. tuberculosis and absent in all strains of Mycobacterium bovis BCG (Bacillus Calmette-Guerin) and most non-tuberculous mycobacteria (except M. kansasii, M. marinum, and M. szulgai) [4,6].QuantiFERON -TB Gold test is based on the quantification of interferon gamma (IFN-g) enzyme-

linked immunosorbent assay in detecting in vitro cell response after stimulation specific to M. tuberculosis peptide antigens ESAT-6 and CFP-10 and TB7.7. [5,6,7,8]. Having a high diagnostic efficiency in determining of tuberculosis infection,these methods do not allow however to distinguish between latent tuberculosis infection (LTBI) and active TB. Thus, the final answer to the question of differential diagnosis provides only a multisession computer tomography. Actual problem of nowadays is identification of new immunological criteria for differential diagnosis of LTBI and active TB in children.Immunological profile in children with various manifestations of tuberculosis infection is described in literature not sufficiently that requires further investigation.

The objectiveis to determine the characteristics of immune response in children with latent TB infection.

Material and methods.

Atpediatric department of Research Institute of Phthisiopulmonologythe cohort prospective studywas conducted in 2011-2013.The study was based on analysis of clinical, immunological and radiation survey of 155 children admitted for examination.The inclusion criteria were age from 3 to 14 years, positivereaction for tuberculin skin test (TST). The age distribution: patients preschool age (3-6 years) was 28.9% (45), patients of younger school age (7 to 11 years) prevailed – 49.3% (76), school age (12-14 years) - 21.8% (34); by sex: girls - 56.1% (87), boys – 43.9% (68).

The diagnostic complex included standard clinical, bacteriological, immunological and x-ray methods of examination. Immunological methods: TST; defining titers of TB antibodies (PTAT) in the complex of serological reactions: consumption compliment (PKK), passive hemolysis (RPG) and enzyme-linked immunosorbent assay (IFA); assessment of the subpopulation composition of lymphocytes in peripheral blood (CD3+, CD4+, CD8+, CD4+/CD8+, CD16+, CD20+, CD25+, CD95+, HLAII); assessment of antimycobacterial antibodies Ig A, Ig G, Ig Mclasses in the blood through a set anda-tb ELISA(BioXimMak); determination oftuberculin induced production of cytokines (IL-2, IL-4, TNFα, INF-γ); phagocytic activity of neutrophils (phagocytic index (PI), phagocytic number Wright (FC) and the index of completion of phagocytosis. Radiation diagnosis was based on multislice spiral CT scanner Aquilion-32" (Toshiba) by the standard method.The research presents analysis of results of the above mentioned immunologic assessments in children with LTBI and active tuberculosis of respiratory organs.

After complex examination all the children were divided into 2 groups: I group (126) - children with TB, II group (29) - children with LTBI (no specific changes in CT, positive results of DST and QFT).

The results of immunological methods were compared between groups.

All the data was processed employing the variation statistics methods using the software SPSS. 16.0. andGraphPad Prism 6. P value less than 0.05 was considered significant.Unpaired Student's criterion (t), Chi-square (C2), and Spearman's rank correlationwereused.Differences of $p<0.05$ were considered significant.Quantitative data is represented in the form M (SD), where M is the sample mean, SD - standard deviation. For all types of statistical analysis critical significance level was 0.05

Results and discussion.

In comparing the results of TST significant differences in the level of sensitivity to tuberculin between groups were identified: the first group has significantly higher proportion of hyperergic test (>15 mm) - (46.0% (I) against 27.6 % (II), $p<0.05$;in

group II normoergic test dominated (10-15mm) (65.5 % (II) against 46,8% (I), $p<0.05$).

The average size of papules in response to the TST in the I group – 14.1±3.1 mm was significantly higher vs. II group – 12.3 ±2.8 mm (p=0.0060) (Fig.1).

Titers of specific antibodies in patients of the first group washigher than in the II group. Statistically significant differences were established only on the results RPK (15.34±13.86 (I) against 9.75±7.6 (II), p=0.04)(Fig.2).

Byanda-tbELISA statistically significant differences in titers ofantimycobacterial antibodies Ig G, Ig M classes in the blood were observed. In the I group level of Ig Gwas significantly higher, in IIgroup level of Ig M was higher (table 1).

As can be seen from table 1, differences in levels of Ig A was not significant between groups, but the tendency of increase in patients with active TB was revealed, which indicates the humoral response to long antigenic stimulation. Subpopulation composition of lymphocytes in the peripheral blood was examined in 109 patients.

As seen from table 2, patients of the first group were characterized by significantly high values of relative contents of CD3+, CD4+ and CD95+;in group II significant increase CD25+ and HLA II + were observed.

The level of induced production of cytokines was studied in 60 children. Statistically significant differences were not found, however, a tendency of increase of level of all studied cytokines in patients with LTBI (group II) was noted: IL-2 (323±244.9 pg/ml vs 274.5±203.6 pg/ml); IL-4 (2.30±1.05 pg/ml vs 1.65±1.02 pg/ml); INF-γ(22856±10800 pg/ml vs. 20800±11055 pg/ml); TNFα (1111±681.5 pg/ml vs 954.9±732.1 pg/ml).

Indicators phagocytic activity of neutrophils in patients of both groups were comparable with a slight increase of phagocytic number (71.1±3.05 vs. 66.88 ±3.36) and phagocytic index (7.34±1.23 vs. 4.85±0.49) in IIgroup.

Index of completion of phagocytosis in the groups did not differ (0.92±0.14 (I) vs. 0.94±0.06(II) and was lower than average normal range that indicates the restrictions of phagocytic opportunities.

Conclusions:

1) The detected features of the immune response in children with LTBI in contrast to active TB can serve as additional diagnostic criteria for differential diagnosis in children.
2) In the I-st.group (active tuberculosis) high results of TST prevailed. The titers of PTT were higher in comparison with II group in the full range of serological reactions;however, significant differences are marked only by results of the PKK. When determining antimycobacterial antibodies (anda-tb ELISA) significant increase of Ig G and the tendency of increase Ig Awas found.Cellular immunity was characterized by the increase of CD3+, CD4+ and CD95+, while the level of induced cytokines was lower than in group II. Marked inhibition of phagocytic activity of neutrophils, phagocytosis indices in both groups were comparable, both at the stage of LTBI and progression to active TB.
3) For the II-nd group (latent TB infection) the following features were observed: the average size of the infiltrate for the TST was significantly smaller in comparison with the first group. Marked trend of the elevation in induced production of cytokines was revealed, increase of the level of antibodies class

Ig M byanda-tb ELISA; subpopulation composition of lymphocytes showed a significant increase in the relative number of CD25+ and HLA II +.

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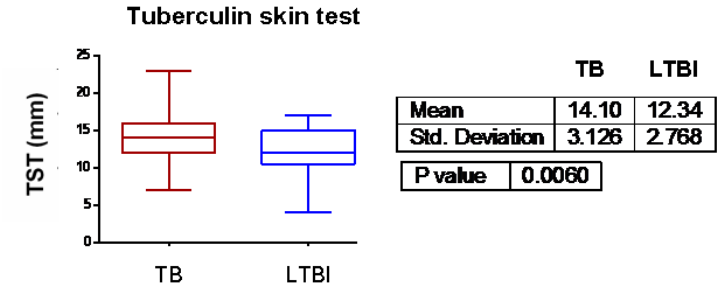

Figure 1. The sensitivity of TST in groups of observations (mm).

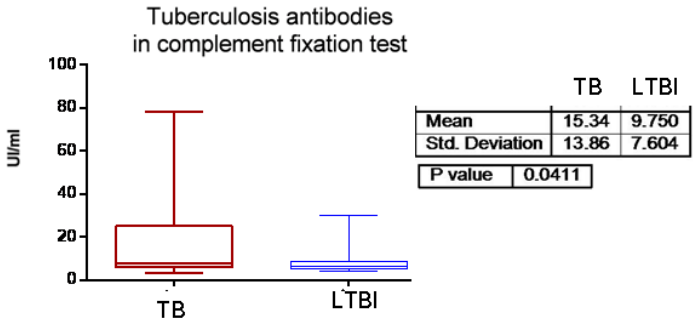

Figure 2. The titers of anti-tuberculosis antibodies in reaction consumption of complement (PKK) in the comparison group.

Table 1.
The level antimycobacterial antibodies Ig A, Ig G, Ig M classes in blood according to anda-tb ELISA.

	Ig Au/ml		Ig G u/ml		Ig M u/ml	
	M±SD	Diagnostic titre Abs(%)	M±SD	Diag-nostictitre abs.(%)	M±SD	Diagno-stictitre abs.(%)
Igroup n=126	53.2±43.9	2 (1.6%)	**80.4±78.3**	15 (11.7%)	**0.84±0.29**	20 (15.9%)
IIgroup n=29	49.4±36.4	-	58.8±17.7	-	**1.06±0.35**	9 (31.0%)
p	-	-	p<0.01	-	p<0.05	-

Table 2.
Subpopulation composition of lymphocytes in peripheral blood of the patients.

Indicatorsofcellularimmunity(%)	**Igroup (n=94)**			**IIgroup (n=15)**			**p**
	M	**SD**	**CI95%**	**M**	**SD**	**CI95%**	
CD3+	**59.3***	11.8	56.9-61.7	49.6	8.8	44.8-54.5	**0.003**
CD4+	**35.9***	7.0	34.5-37.3	31.7	5.,3	28,7-34.6	**0.02**
CD25+	12.7	6.0	11.4-13.9	**17.1***	6.4	13.6-20.7	**0.009**
HLA II +	19.5	11.7	17.1-21.9	**24.2***	5.2	21.3-27.1	**0.01**
CD95+	**22.0***	10.1	20.0-24.1	19.1	4.5	16.6-21.6	**0.01**