SKIN TEST DIASKINTEST ® IN THE DIAGNOSTICS OF TUBERCULOSIS INFECTION IN CHILDREN AND ADOLESCENTS

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The specifics of conducting antiepidemic work within the foci of tuberculosis infection in the setting of rural extreme northern region are presented. Study of 150 foci of tuberculosis infection showed: remoteness and hard accessibility of rural infection foci to specialized health service delays timely hospitalization of newly identified patients with tuberculosis, complicates examination and isolation of children to sanatoria, and hampers the conduction of antiepidemic measures in new foci of tuberculosis infection by epidemiologists and phthisiologists.

Keywords: tuberculosis, children, tuberculosis infection reservoir, rural area, extreme north, health services accessibility

Introduction. Epidemiologic situation for pediatric tuberculosis (TB) remains intense. In 2010 pediatric TB incidence rate in the Sakha Republic (Yakutia) was 21.8 per 100,000 children, and the number of newly identified children (with latent TB infection) was 1.2% of the total pediatric population, while TB incidence in pediatric contacts of smear-positive patients was 642.8 per 100,000 contacts.

TB develops in 12 children and 100 adolescents per 1000 children and adolescents with newly identified infection. These facts point at late diagnosis of TB in some of the children (especially adolescents), and delayed detection of tuberculin test conversion [10].

Current diagnosis of pediatric primary TB infection is performed mainly by tuberculin skin testing. Yet insufficient specificity of tuberculin test, along with the lack of tools to differentiate between primary TB infection and postvaccinal allergy, prevent adequately effective identification of TB infection. [6]. Insufficient effectiveness of primary infection diagnosis in children and adolescents, with the failure to timely deliver preventive chemotherapy courses, leads to development of the forms of local TB, mostly pulmonary TB.

In this context, the improvement of early detection, diagnosis, and TB prevention efforts is an important goal in phthisiatry.

A new skin test preparation Diaskintest® has been developed and registered for use in Russia (Registration number LSR-006435/08, August 11, 2008, Manufacturer: ZAO (CJSC)

"Pharmaceutical company 'LEKKO' "). Diaskintest is an ESAT-6- and CFP-10-based recombinant tuberculosis allergen, produced by genetically modified *Esherichia coli,* and is intended for use as a skin test providing a delayed type hypersensitivity reaction aimed for better TB infection diagnosis. In BCG-vaccinated persons without *M.tuberculosis* infection, Diaskintest®(DST) causes no skin reaction. [1, 3, 5, 9].

Material and methods. We examined 351 children and adolescents, who have been followed-up in risk groups for TB at the dispensary department of the State Budgetary Institution of the Sakha Republic (Yakutia) "Research-Practice Center 'Phthisiatry' ", and who received the following skin tests: DST and Mantoux test with 2 TU PPD-L. Patients were divided to 4 study groups:

Group 1: children and adolescents with tuberculin skin test conversion (dispensary follow-up group VI A) – 125 patients;

Group 2: children with a history of past infection and with hyperergic reactions to tuberculin (dispensary follow-up group VI B) – 45 patients;

Group 3: children and adolescents who showed gradual increase of sensitivity to tuberculin (dispensary follow-up group VI C) – 114 patients;

Group 4: children exposed to household contacting with active smear-positive or smear-negative TB cases (dispensary follow-up group IV) – 67 patients.

To examine specific reactivity, Mantoux tests were performed using purified liquid tuberculosis allergen in standard dilution: 2 TU PPD-L per 0.1 mL of solution (Order of the Ministry of Health of the Russian Federation of 21 March 2003 no. 109) [7].

This study complied with the following regulations and guidelines:

- Russian Federation Ministry of Health and Social Development order of 29 October 2009 no. 855 "On introduction of amendment to appendix #4 of the Russian Federation Ministry of Health order of 21 March 2003 no. 109 'On improvement of tuberculosis-controlling measures in the Russian Federation'";

- Guides for physicians: "Skin test with DIASKINTEST® preparation (recombinant tuberculosis allergen 0.2 microg in 0.1 mL intracutaneous solution) for identification of tuberculosis infection" and "Skin test with DIASKINTEST® preparation – new opportunities for identification of tuberculosis infection" [2, 4, 8].

DST is performed in the same technique as Mantoux test with 2 TU PPD-L: 0.1 mL were introduced intradermally into inner surface of mid one-third of the forearm.

Both Mantoux test with 2 TU PPD-L and DST were performed before the initiation of

preventive treatment in risk groups for TB (dispensary follow-up groups VI, IV).

Results and discussion. Results of the use of DST in various study groups are presented in Tables 1 and 2.

Group 1 consisted predominantly of preschool children (3-6 years, 85), and included 9 infants (0-2 years), 29 schoolchildren (7-14 years), and 2 adolescents (15-17 years). Most of the children appointed to dispensary follow-up based on their Mantoux test reactions, were aged 3 to 6 years.

Negative results of DST in 31 (24.8%) patients with Mantoux test conversion was found to signify that there was no increased risk of developing active TB. Doubtful reaction to DST was observed in 60 patients (48.0%). Number of children and adolescents who reacted positively to DST (34, 27.2%) was 3.7 times lower, then the number of that to tuberculin skin test, which appears to reflect a lower bacterial burden in a host child. Average sizes of infiltrates (papules) were 11.3±0.3mm for Mantoux test, and 5.8±0.8mm for DST, $p<0.001$. Most of the children, who were appointed to follow-up based on their Mantoux reactions, were aged 3 to 6 years. Positive DST results were seen more often in those aged 7 to 14. This suggests the presence of postvaccinal allergy or parallergy in infants and preschool children.

Group 2 (dispensary follow-up group VI B) included mostly children aged 7 to 14 (25), 3 children aged 0 to 2, and 17 children aged 3 to 6.

Larger proportion of children (n=27, 60.0%) with hyperergic Mantoux reactions tested positive for DST. In this group, average size of the papula following DST (13.3±1.7mm) was reliably larger, than that in a group with tuberculin test conversion ($p<0.05$). Study findings confirm a high risk of active TB in this group of dispensary follow-up.

In group 3 (dispensary follow-up group VI C) most of the children where of school age: 47 children aged 7-14, 18 children aged 15-17, 3 children aged 0-2, 46 children aged 3-6. Positive DST results were seen in 22 (19.3%), doubtful results in 45 (39.5%), and negative results in 47 (41.2%) patients, while average sizes of papules after Mantoux test or DST were almost the same (12.4±0.3mm and 9.9±1.5mm, respectfully, $p>0.05$). Only 22 (19.3%) children and adolescents with gradually increasing sensitivity to tuberculin tested positive for DST. This could possibly mean that the mycobacteria were in the state of dormant persistence – in this case, reaction to DST turns negative.

Group 4 (dispensary follow-up group IV) comprised mostly infants and preschool children: 14 children aged 0-2, 34 aged 3-6, 19 aged 7-14. DST was positive in 26 (38.8%), children, doubtful in 20 (29.9%), and negative in 21 (31.3%) patients in this group. Children in this group showed almost identical average sizes of reactions to Mantoux test and to DST (10.3±0.6 and

11.5±1.4mm respectfully, $p<0.05$). Children from dispensary follow-up group IV had positive results of DST more often, than children and adolescents from follow-up group VI A. This leads to suggestion that children exposed to contacting with TB cases showed high risk of developing active TB.

Conclusions. Use of DST helped to identify groups of patients with the highest risk of developing TB: dispensary follow-up groups VI B and IV.

Findings from the comparison of diagnostic effectiveness between DST and Mantoux test with 2 TU PPD-L permit recommending DST for use in pediatric practice, to diagnose TB infection in children and adolescents. Doubtful and positive reactions to DST are indications for compulsory dispensary follow-up of these patients. Positive reaction to DST may appear later, then a reaction to tuberculin. Therefore, DST must be repeated 3 months later, if the first reaction was either negative, or doubtful. Positive reaction to DST indicates TB infection and requires an array of diagnostic measures, which must include computed tomography as well.

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Table 1

Results using Mantoux test with 2 TU PPD-L and Diaskintest®

Study groups	n=	Mantoux test with 2 TU PPD-L			Diaskintest®		
		Positive abs.n./%	Doubtful abs.n./%	Negative abs.n./%	Positive abs.n./%	Doubtful abs.n./%	Negative abs.n./%
1. Children and adolescents from dispensary follow-up group VI A	125	125/100	-	-	34/27.2	60/48.0	31/24.8
2. Children and adolescents from dispensary follow-up group VI B	45	45/100	-	-	27/60.0	18/40.0	-
3. Children and adolescents from dispensary follow-up group VI C	114	114/100	-	-	22/19.3	45/39.5	47/41.2
4. Children and adolescents from dispensary follow-up group IV	67	53/79.1	6/9.0	8/11.9	26/38.8	20/29.9	21/31.3
Total	351	337/96.0	6/1.7	8/2.3	109/31.0	143/40.8	99/28.2

Table 2

Results by Mantoux test with 2 TU PPD-L and by Diaskintest®

Study groups	Mantoux test with 2 TU PPD-L	Diaskintest®	P
	Papule size (M±m), mm, N=patient number	Papule size (M±m), mm, N=patient number	
1. Children and adolescents from dispensary follow-up group VI A	11.3±0.3, n=125	5.8±0.8, n=34	<0.001
2. Children and adolescents from dispensary follow-up group VI B	16.4±0.6, n=45	13.3±1.5, n=27	<0.05
3. Children and adolescents from dispensary follow-up group VI C	12.4±0.3, n=114	9.9±1.5, n=22	>0.05
4. Children and adolescents from dispensary follow-up group IV	10.3±1.4, n=53	11.5±1.4, n=25	>0.05
p		$P_{1-2.4}$<0.05	

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