Molecular Genetic Methods in Diagnosis of Tuberculosis in Children

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Abstract

Tuberculosis in children characterized by such as oligosymptomatic onset of the disease, frequently no bacterial excretion or poor bacterial excretion, reduce the informative value of conventional microbiological methods of detection of the causative pathogen and etiological verification. In comparison the positive result of polymerase chain reaction (PCR) was significantly higher than the results of bacterial methods. Positive result of PCR was ascertained to exhibit the highest sensitivity in detecting Mycobacterium tuberculosis (MBT) in children with primary generalized tuberculosis (62.5%) and in those with a disseminated specific process (55.6%), it is significantly higher in the results of bacterial methods (25.0% and 44.0% accordingly). By taking into account the findings, the RT-PCR detection of M. tuberculosis may be considered as a substantial criterion for evaluating the magnitude of specific changes and the degree of tuberculosis infection activity in children.

KEYWORDS: molecular biological methods, real-time polymerase chain reaction, tuberculosis, generalized tuberculosis, children.

Introduction.

Tuberculosis in children characterized such as oligosymptomatic onset of the disease, frequently no bacterial excretion or poor bacterial excretion, reduce the informative value of conventional microbiological methods of detection of the causative pathogen and etiological verification of the diagnosis [3,4].

Molecular genetic methods for mycobacteria identification and typing; these methods are highly sensitive, specific and allow for rapid detection of *M. tuberculosis* complex (MTB) in the sputum in case of pulmonary tuberculosis and in liquor, urine or exudates in case of extrapulmonary forms of the disease [2,3,6]. These benefits enable to detect MTB at early stages of the disease and in various diagnostic materials [2,5,6]. Taking into account difficulties in MTB detection in children, a study of the informative value of molecular genetic methods of MTB detection is very actual.

The research objective is to study the characteristics of clinical manifestations and course of tuberculosis (TB) in children and to assess the informative value of the method of real-time polymerase chain reaction (RT-PCR) for the detection of MTB in patients with several of TB.

Materials and methods

236 children at the age of 3-14 stayed for examination and treatment: 99 (41.9%) patients at the age of 3-6 and 137 (58,1%) – at the age of 7-14 was conducted in the Department of Pediatric Phthisiology from 2011 and 2013 years.

Diagnosis complex included: tuberculin skin tests (TST), Diaskintest (DST), X-ray

method diagnosis (multislice computed tomography and CT-angiography) and laboratory methods.

Diaskintest (Generium) uses recombinant tuberculosis allergen based on M. tuberculosis specific proteins: ESAT-6 and CFP-10) – and by its nature represents immunologic skin-test.

Laboratory methods included bacteriologic examination of bronchial washings and sputum, luminescent bacterioscopy, examination of cultures on solid Löwenstein–Jensen medium and on liquid medium using the automated Bactec MGIT 960 system, detection of MTB by means of RT-PCR. In the course of PCR diagnostic was purified using the system “Amplitube PB” and the nucleotide sequence IS6110, which is a marker of M. tuberculosis complex, was amplified using the test system of ZAO “Sintol” (Russia) “AmpliTube-PB-Screen” on the analyzer iCycler iQ5, BioRad (USA). Bronchial washings received during bronchoscopy and purulent discharge from the fistula of the peripheral lymph node were used as test material.

Apart from these X-ray method diagnosis ((multislice spiral computed tomography (MSCT) and MSCT angiography (MSCT-AG)) and X-ray examination were done using a spiral CT scanner with a multirow detector (multislice) «Aquilion-32» (Toshiba Medical Systems Corporation, Japan), with intravenous bolus contrast administration using an automatic injector "CT 9000 ADV» (Liebel-Flarshein (Mallincrodt Inc.).

All the data was processed employing the variation statistics methods using the software Statistica 8. P value less than 0.05 was considered significant. Spearman's rank correlation coefficient was also used. Used unpaired Student's t test (t), chi-square test ($\chi 2$).

The predominant clinical form was intrathoracic lymph node tuberculosis (ILNT) in 89.4% of cases (211 patients), in 6.8% (16 patients) primary generalized tuberculosis has been diagnosed, in 8 patients infiltrative and in 1 child disseminated pulmonary tuberculosis developed. Positive results of MBT detection with RT-PCR in 15.3% of cases (36 patients): in 91,7% (33) thereof the MBT was found in bronchial washings received during bronchoscopy; in 1 patient with primary generalized tuberculosis – in venous blood; in 2 patients the MBT was detected in different diagnostic materials (bronchial washings and purulent discharge from the fistulous passage of the affected peripheral lymph node against the background of tuberculosis generalization) at the same time.

According to the findings received during the examination, the children were divided into 3 groups: group I – patients with ILNT (n = 211); group II – with primary generalized tuberculosis (n = 16), and group III – with pulmonary TB (n = 9).

Statistical processing of the results was carried out with the help of Microsoft Office Word/Excel 2007 using nonparametric methods and statistical significance of differences according to Mann–Whitney U test. Differences of $p < 0.05$ were considered significant.

Results and discussion

In the group I group patients in 19.4% (41) showed moderate intoxication syndrome, peripheral micropolyadeny, moderate signs of toxic syndrome in 34.6% (73) were of a mixed character due to concomitant diseases. Changes in intrathoracic lymph nodes involved the affection of 1-2 groups, in 41.7% of cases (88 patients) – unilateral process, in 24.7% (52) – changes in 2 or more groups were revealed in one in three children with bilateral localization. Bronchoscopy (n=121) showed no abnormalities

in the tracheobronchial tree. In all patients from this group microscopy was negative and culture was not determined; however, in 2.9% of cases (4 patients) positive PCR results were obtained.

Bacteriological methods showed MTB only in 2.8% of cases (2 patients) whereas according to the PCR in the group II with primary generalized tuberculosis [associated with intrathoracic lymph node lesion and peripheral lymph node involvement (9), affection of eyes (3), central nervous system (3), liver (2), spleen (1), skin (1)] in 81.3% of cases the disease was accompanied by evident toxic syndrome, peripheral polyadeny, involvement of 2-3 and more groups of lymph nodes, in 3 children with peripheral lymph node lesions lymph node abscesses were formed with a fistula and purulent discharge.

Bronchoscopy revealed changes in 5 (31.3%) patients in the form of elevation of the slopes of the tracheal bifurcation and bulging of the bronchial walls due to the pressure of underlying lymph nodes. In 3 (18.6%) patients scars on the mucous membrane of the bronchus were found, one patient was diagnosed with infiltrative exudative bronchial tuberculosis. Bacterial excretion was revealed in one in four children (25%; 4) whereas PCR detected MBT in 62.5% (10 cases) ($\chi^2 = 4.57$, $p < 0.05$) (table 1).

The course of the specific process in group III as well as in group II was characterized by evident toxic syndrome (88.9%), complaints in one in three children with exudative inflammation type and tuberculosis cavities (55.6%; 5). In 8 of 9 patients bronchoscopy showed changes including active bronchial tuberculosis in 3 cases (33.3%). With the help of bacteriological methods MTB was detected in the half of the patients (44.4%; 4) which is comparable to the results of PCR diagnostic (55.6%; 5).

The comparison of the results obtained by the means of PCR diagnostic in I group showed significantly more MTB-positive results in II group (62.5% vs. 10.1%, $\chi^2 = 23.4$, $p < 0.001$) (Fig 1.). In comparison I and III group (55.6% vs. 10.1%; $\chi^2 = 41.4$, $p < 0.001$) that corresponds to the severity and extent of the specific process. No significant differences in the positive PCR findings were found between the groups II and III ($\chi^2 = 0.12$, $p < 0.1$).

Therefore, the highest sensitivity of PCR for MTB DNA (62.5%) was found in case of primary generalized tuberculosis and disseminated forms of the disease (55.6%) in comparison with bacteriological methods. These results are several times better than those of bacteriological methods of MTB detection of generalized tuberculosis (62.5 versus 18.6%, $\chi^2 = 6.3$, $p < 0.05$).

Conclusion

The positive result of PCR is closely correlated with the severity and extent of the specific process. RT-PCR showed the highest sensitivity in detecting MTB in children with primary generalized tuberculosis (62.5%) and in those with a disseminated specific process (55.6%) which was much higher than using the conventional bacteriological study of diagnostic materials. Taking into account the findings, the RT-PCR detection of MTB may be considered a substantial criterion for evaluating the magnitude of specific changes and the degree of tuberculosis infection activity in children.

References

1. Vladimirskyi M. A., Shipina M.K., Mordovskaya L.I. New methods of immunological and molecular-genetic diagnostics of tuberculosis infection// J. Tub.

and lung disease -2011.-№4- P.88.
3. Marenina E.A. Diagnosis of tuberculosis in children using PCR method : abstracts disser candidate of medical Sciences.- Moscow, 2003 –P.23.
4. Pozdnyakova A.S. Tuberculosis in children and adolescents: the specifics of the disease, new technologies of diagnostics and specific prevention. Abstracts disser DM .-M.,2011-47
5. Draft guidelines. Concept of chemotherapy and etiological (microbiology and molecular biology) diagnosis of tuberculosis in the Russian Federation at the present stage. Medical alliance.-2012-№1-5-37
6. Salina T.U., Morozova T.I. Modern technology laboratory diagnostics of tuberculosis. (Efficiency of use in clinical practice)// Probl tub.-2008.-№11-42-44

Table 1.

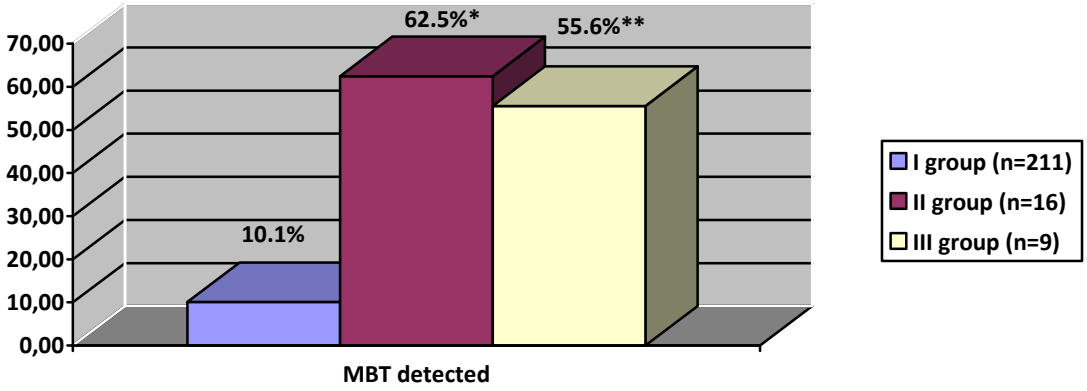
Comparison of the findings received by means of bacteriological and PCR methods of diagnostic material examination

Groups	Positive result	
	RT-PCR	Bacteriological examination
I (n = 211)	9.9% (21)	0.9% (2)
II (n = 16)	62.5% (10) **	25.0% (4)
III (n = 9)	55.6% (5)	44.4% (4)
Total	15.3% (36)	10% (4.2)

Notes: * - p < 0.001 for comparison of RT-PCR and bacteriological results in the children of the I group; ** - p < 0.05 in comparison of RT-PCR and bacteriological results in the children of the II group .

Fig 1.

Positive results in RT-PCR method of MBT detection in children with tuberculosis



Notes: ** - p < 0.001 in comparison of I and II groups; ** - p < 0.001 in comparison I and III groups.