UDC:616.24-002.5-097-053.2

ROLE OF QUANTIFERON TB GOLD TEST IN DIAGNOSIS OF LATENT TUBERCULOSIS INFECTION (LTBI) IN CHILDHOOD AND ITS CORRELATION WITH TUBERCULIN SKIN TEST

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ABSTRACT

Tuberculosis is a significant health problem among children population worldwide. Timely diagnosis and treatment of the disease are the basis for prevention of its further spreading. However, the diagnosis of latent tuberculosis infection is a challenge because there is no gold standard. The aim of this study was to evaluate the importance of the diagnostic Quantiferon TB gold test in the diagnosis of latent TB infection and to correlate it with Tuberculin skin test according to the Mantoux method.

For the realization of this study we analyzed 32 patients examined for possible M. tuberculosis infection at the Institute of Respiratory Diseases in Children, Kozle, Skopje. The study included 16 girls and 16 boys, aged 9 months to 17 years, with an average age of 6.96 ± 4.49 years. In all children basic biochemical analyses were made: acid-alcohol-resistant bacilli in a direct sample of sputum, Levenstein Jensen cultures, chest X-ray, tuberculin skin test according to the Mantoux method and Quantiferon TB gold test.

The results showed that 24 patients had a BCG scar. All participants in this study had normal radiographic findings of the lungs. In 4 cases Quantiferon TB gold test was positive, while in 28 patients the test was negative. Tuberculin skin test was positive in 13 subjects. In children with negative Quantiferon TB gold test LTBI was excluded and drug prevention with Isoniazid was not started or it was interrupted.

Determination of IFN-γ contributes to better diagnosis of LTBI and in reducing the unnecessary drug use. Using Quantiferon TB gold test may be an alternative tool for Tuberculin skin test in the diagnosis of tuberculosis in countries where vaccination with BCG is widespread.

Keywords: children, latent tuberculosis infection, tuberculin skin test, Quantiferon TB gold test

INTRODUCTION

World Health Organization defines tuberculosis (TB) as an infectious bacterial disease caused by Mycobacterium tuberculosis (M. tuberculosis). Patients with lung tuberculosis from whose sputum M. tuberculosis bacilli are isolated are the main source of the infection. M. tuberculosis, which was discovered in 1882 by Robert Koch, is an aerobic, facultative intracellular slow-growing acidophilic bacillus, naturally pathogenic only in humans [1, 2]. In children TB usually develops as a result of close contact with sputum of a family member positive for lung TB.

According to WHO in 2010 about 8 million people fell ill with TB, whereas about 1 million died from TB [3]. Tuberculosis is contagious/infectious disease which is characterized by a high rate of morbidity and mortality in the world. Children account for 5 to 15% of the cases with tuberculosis worldwide; they are more often infected and have more severe forms of the disease [4]. In the Republic of Macedonia in 2005 the prevalence was 53/100,000, and in 2012 it was 26/100,000. In 2014, 285 new cases of tuberculosis were discovered, the rate being 13.8 per 100.000 population, which is 38 cases less in comparison with 2013 when the number of newly discovered cases amounted to 325 persons.

Diagnosis of tuberculosis in children is based on data about the history of the disease, epidemiologic data, clinical signs, laboratory analyses, x-ray examinations and immunologic examinations, tuberculin skin test (TST) and Interferon-Gamma Release Assays (IGRA) tests, while the unique secure proof for correct diagnosis is isolation of the causer from biologic material [5, 6]. Establishing the diagnosis in children may be difficult because the symptoms are often very discreet; there is not self-recognition/self-awareness of the disease; direct microscopic sputum smears are positive only in 10 to 15%; positive cultures are obtained in 30 to 50% of children, whereas in smaller age groups even less than in 20% [5, 6].

The latent tuberculosis infection (LTBI) is an infection caused by M. tuberculosis bacilli, without signs of a disease, radiographic changes and bacteriological confirmation of TB. Common features for both TB and LTBI are positive TST and IGRA tests. LTBI is defined as an infection with M. tuberculosis inside the granuloma where it remains in non-replicating condition but later on it can be transformed into an active TB. However, recent experimental data support the dynamic model of LTBI presenting with continual endogenous reactivation and inflammatory response [7]. This has been supported by a Norwegian study from 2010 demonstrating that reactivation of tuberculosis is decreased over time [8]. The dynamic model offers explanation for the influence of isoniazid, a drug which influences the actively replicating bacilli only. As isoniazid prevents the episodes of reinfections with bacteria released from the resting phase, together with the delayed drainage and damaging of non-replicating bacteria in the stomach, latent infection weakens gradually [7].

The aim of the study was to present the diagnostic importance of the released IFN-γ from T lymphocytes for confirming and excluding LTBI in children.

MATERIALS AND METHODS

In this study we have analyzed 32 patients who have been referred to examination for potential infection with M. tuberculosis at the Institute for Respiratory Diseases in Children – Kozle, Skopje, Republic of Macedonia, in the period from September 2014 to March 2015. The study included 16 boys and 16 girls. The age of the patients ranged from 9 months to 17 years, with an average age of 6.96 ± 4.49. In all patients included in the study the following parameters were analyzed: demographic characteristics, history of previous exposure to active TB, presence of BCG scar, lung X-ray findings, direct samples of acid-alcohol-resistant bacilli of sputum, Löwenstein Jensen cultures, tuberculin skin test by the Mantoux method and the value of γ-INF according to the Quantiferon TB gold test. Informed parental consent was obtained for each child included in the study.

Exclusion criteria

Patients with cardiopulmonary diseases, a history of a severe allergic reaction to purified protein derivative, a history of active tuberculosis, lack of immunity, and malnutrition were excluded from the study. Also, children whose parents did not give permission for participation in the study were excluded.

In vivo methods

Tuberculin skin test

Tuberculin skin test was carried out on the volar aspect of the left forearm by injecting 0.1 ml purified protein derivative (PPD) of 5 tuberculin units (TU) intracutaneously. Measuring of induration diameter was made after 72 hours with the help of a ruler.

In patients who did not have a BCG vaccination scar, the value of TST diameter ≥6 mm was considered as borderline for a positive skin test. In patients who had a BCG scar, the value of TST diameter ≥15 mm was considered as borderline for a positive skin test. In patients who had a BCG scar, the value of TST test < 15 mm was considered as negative.

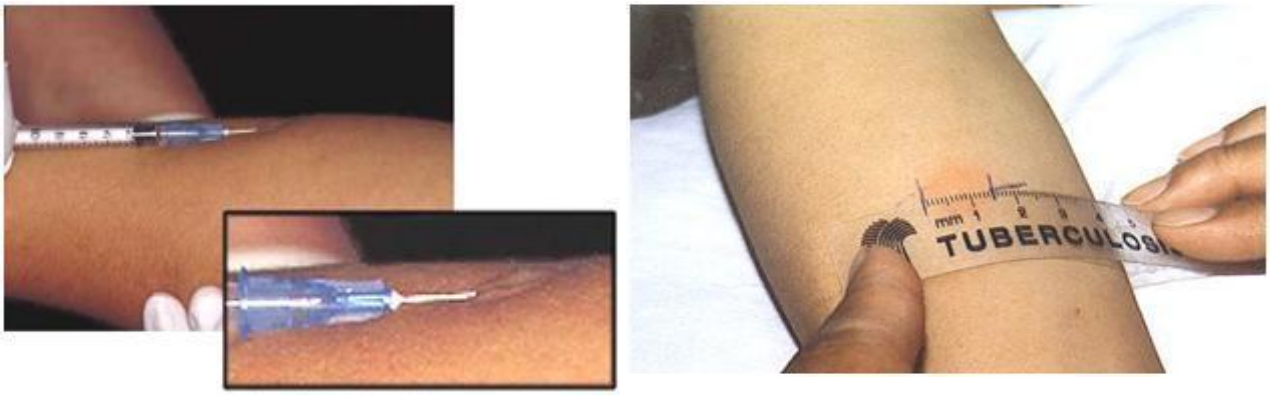

Fig. 1. Description of performing and measuring induration in tuberculin skin test

Ex vivo methods

Quantiferon TB gold test

Quantiferon TB gold analysis was conducted at the Institute for Respiratory Diseases and TB in line with the guidelines of the manufacturer. The examination was performed in two phases: the incubation of the whole blood with antigens was made in the first phase and in the second phase the measuring of IFN-γ was made by the ELISA method. For realization of the test 1 ml vein blood was taken from patients in three test-tubes that contained:

1. Specific antigens of M. tuberculosis (ESAT-6, CFP10 and TB7.7)
2. Mitogen phytohemagglutinin (positive control) and
3. Does not contain mitogen or specific antigens (negative control).

In 2-6 hours after blood taking, the test-tubes with blood were placed for incubation at 37ºC. 24 hours after incubation the test-tubes had been centrifuged and the plasma was separated and frozen at -70ºC. The concentration of γ-INF was measured by the ELISA method (Enzyme-Linked Immunosorbent Assay) using the commercial test Quantiferon TB gold (Cellestis, QIAGEN Company). The values of γ-INF were expressed in international units on millimeter and γ-INF ≥ 0.35 IU/ml was taken as a reference value for a positive test.

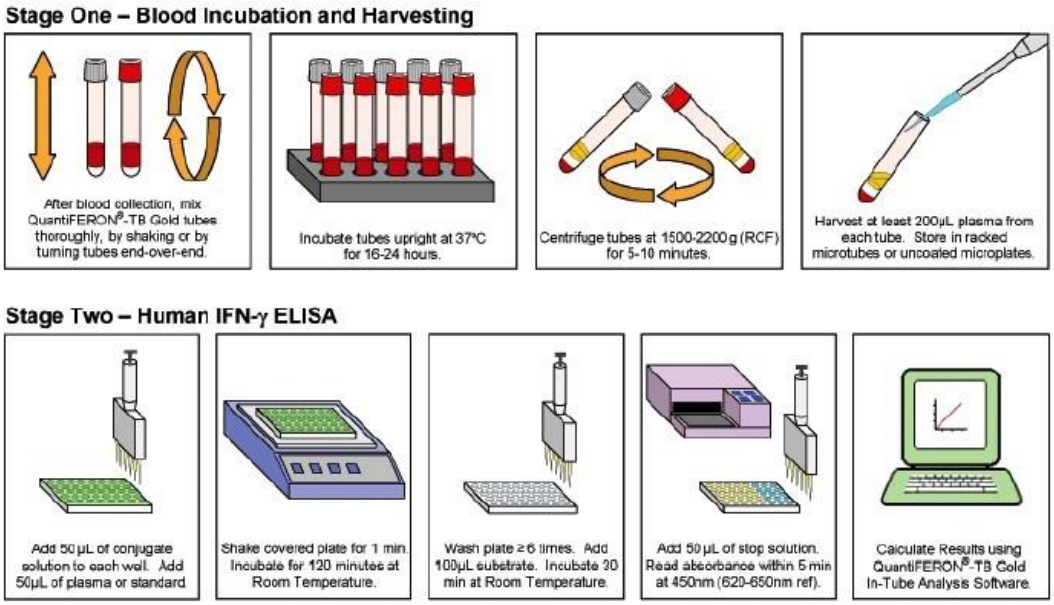

Fig. 2. Overview of Quantiferon TB gold test

RESULTS

A total of thirty-two children were analyzed. Both sexes were equally represented. The age ranged from 9 months to 17 years with average age of 6.96±4.49 years. Eighteen of the patients (56.25%) were from urban area, while 14 (43.75%) from rural area.

The results obtained about BCG showed that all 32 children had BCG vaccine at their birth. Twenty-four of them (75%) had scar from the vaccination while in 8 (25%) children the BCG scar could not be seen. Twenty-six (81.25%) children had a positive contact with a patient infected with active TB.

Tuberculin skin test was positive in a total number of 13 (40.62%) children; 10 children had a BCG scar while three children had no visible scar. Three of the positive children were tuberculin hyper-reactors after regular testing with PPD, while 10 had a positive contact with a patient infected with active TB. In the remaining 19 (59.37%) children tuberculin skin test was negative.

Radiography of the lungs was made in all children and it showed normal findings. Direct specimens for acid-alcohol-resistant bacilli from sputum as well as Löwenstein Jensen cultures in all children were negative.

In 4 (12.5%) children Quantiferon TB gold test was positive with titer ≥ 0.35 IU/ml. In one patient there was disagreement between TST and Quantiferon TB gold test results, in terms of negative tuberculin skin test and positive interferon gamma test. In three children there was agreement between tuberculin skin test and interferon gamma test, that is, both tests were positive by which TB infection was confirmed. Ten (31.25%) patients had positive tuberculin skin test and negative interferon-gamma test. In 18 (56.25%) patients TST test and IGRA test were negative.

DISCUSSION

The chance LTBI to develop into active tuberculosis during lifetime in infants amounts to 43%, in children at the age from 1 to 5 years it amounts to 29%, while in children aged from 11 to 15 years it is 15%. In children with LTBI younger than five years the risk of TB development two years after the infection is 20-40% [9,10].

Children with LTBI are a reservoir of future infected individuals, hence when we speak about eradication of TB it is not sufficient only to treat patients with active tuberculosis but to diagnose them and to treat adequately those with LTBI.

Mainly, there are two groups of children in whom LTBI is looked for: children in contact with a patient with active TB and children who are tuberculin hyper-reactors after regular testing with PPD. The most contagious are those TB patients who are in the phase of having a positive finding in the sputum on direct microscopy, and the biggest threat comes from a close contact with a family member.

Diagnosis of LTBI is a challenge since there is no gold standard. The unique ascertained fact is the risk of development of active tuberculosis. It partially depends on virulence of bacilli, while mostly it depends on the condition of the host such as nutrition, immunologic system etc. Certain clinical conditions and therapeutic procedures contribute to development of active TB (AIDS, chronic renal insufficiency, diabetes mellitus, chemotherapy, immunosuppressive therapy).

Until recently, tuberculin skin test by the Mantoux method has been the uniquely available immunologic test for diagnosis of LTBI. It is an *in vivo* test that is based on measurement of the reaction of postponed hypersensitivity after injecting mixture of mycobacterial antigens, PPD subcutaneously on the forearm [11]. PPD of 5 tuberculin units (TU) is used in our country. The size of induration on the site of injection is proportional to the strength of the immunologic response to competent cells. Induration diameter is read after 72 hours [12].

Positive outcome can be expected if two to eight weeks have passed after the infection with M. tuberculosis. Since the solution of PPD contains more than 200 protein components that are common for most of mycobacteria, tuberculin test may give false-positive results in persons vaccinated with BCG or who were in contact with nontuberculous mycobacteria [13].

False-negative results may be found in persons with damaged or immature cell immunity such as patients infected with HIV, patients with iatrogenic-caused immunosuppression, children in younger age due to weak reactivity on skin or if the result is falsely read as negative [14].

According to the above said it is clear that TST is not a secure test for detecting LTBI especially in countries where there is BCG vaccination such as in our country.

Knowledge about the immunologic response of the organism to the infection with M. tuberculosis has been used in the field of laboratory diagnostic of LTBI over the past ten years. The new approach is based on *in vitro* tests from full blood that serves to determine the concentration of IFN-γ released from T lymphocytes after incubation with specific antigens for M. tuberculosis. These tests are known as IGRA tests. The concentration of IFN-γ is measured by the Enzyme-Linked Immunosorbent Assay-ELISA.

Since 2004 the Quantiferon TB gold test has been used and it has largely contributed to the diagnosis of LTBI and TB. The specific antigens that are used in this test are early secreted antigenic target-6KD (ESAT-6), culture filtrate protein-10KD (CFR-10) and TB 7.7. These antigens do not exist in BCG (Bacillus Calmette-Guerin) and in most of nontuberculous mycobacteria, except in M. kansaii, M. szulgai, M. marinum, M. flavescens and M. gastrii [14]. Therefore, the chance of having false-positive results of IGRA tests is very small since T lymphocytes in healthy BCG-vaccinated uninfected persons as well as in those infected by nontuberculous mycobacteria do not secrete gamma interferon after stimulation with the mixture of antigens ESAT-6, CFP-10 and TB 7.7 [16].

The big advantage of this test is the possibility for determination of negative and positive control. The negative control gives insight in the quantity of circular IFN-γ that is present independently of the irritations *ex vivo*. The positive control on the other side is used for checking the capability of T lymphocytes to release IFN-γ under adequate irritation as well as for control of the correctness of the procedure with the sample by which false-negative results would be avoided.

The positive features of IGRA tests are their high diagnostic sensitivity and specificity, reproducibility and possible standardization. The research of Pai et al. (2008) showed 99% specificity of Quantiferon TB gold test in persons who were not BCG-vaccinated and 96% in BCG-vaccinated persons, while the sensitivity reached 78% [17].

Determining IFN-γ contributes to more precise diagnosis of LTBI, especially when there is disagreement between IFN-γ and TST results. When there is doubt about LTBI it is necessary to apply *in vivo* and *in vitro* tests. Interpretation of the results is the simplest when both tests are positive and negative, that is, the diagnosis of LTBI is either confirmed or excluded. There were TST and IGRA negative tests in 18 patients in our study, by which LTBI was excluded, and resulted in discontinuation of drug therapy with isoniazid. Three patients had positive TST and IGRA tests, that is, LTBI was confirmed and drug preventive therapy was continued. When there is TST positive/IGRA negative disagreement, TST test can be false positive although IGRA test might be false negative. In that case, it is necessary to repeat IGRA test in 8-10 weeks. According to Veerapathran et al. there is conversion of IFN-γ if two criteria are met: change of the value from negative to positive and increase of concentration for at least 30% from the starting one [18]. We had TST positive test in 10 patients, while IGRA test was negative. Three of them did not have contact with TB infected individual and the diagnosis of LTBI was excluded. In the remaining seven patients the control Mantoux test was made after two months, which was negative, and hence LTBI diagnosis was excluded and the unnecessary drug prevention with isoniazid was discontinued. In TST negative/IGRA positive disagreement LTBI is indicated, while tuberculin test is false negative. In our study, one patient had TST negative and IGRA positive test, indicating LTBI. The investigations of LTBI in children showed that IGRA tests had advantage over TST test due to higher specificity, good negative predictive value, and good correlation with the grade of exposure to infection. That is why IGRA tests are the first choice in children who had BCG vaccine and hence are useful in reducing preventive drug therapy due to possible false- positive results of TST tests [19].

CONCLUSION

Determination of IFN-γ contributes to better diagnosis of LTBI by which unnecessary drug prevention is decreased. Using Quantiferon-TB gold test may be an alternative tool for tuberculin skin test in diagnosis of TBC in countries where BCG vaccination is widely spread.

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