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RESEARCHARTICLE

DIAGNOSTIC POSSIBILITIES OF QFT IN TUBE IN CHILDREN WITH DIFFERENT FORMS OF TUBERCULOSIS

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ABSTRACT

97 children with different forms of TB and latent TB infection were examined at the University children hospital for pulmonary diseases – Sofia. The children were tested with TST and QFT. Only 9,3% of the children had negative TST and 72,2% reacted with sizes of the infiltrate above 15mm. With QFT 55,7% had positive results. We discovered that the largest share of the children who reacted to both TST и QFT is in the highest age groups. TST sensitivity is 77,7%, while forQFTis 88,9%, which shows greater diagnostic possibilities of QFTGIT. WithQFT 21(55,3%) of the children without BCG scar reacted positively and 33(55,9%) of the children with BCG scar reacted positively, which supports the statement for the efficacy ofQFTintube in cases with compulsory BCG vaccination.In conclusion, we think that QFT GIT together with TST increases the diagnostic possibilities in children with suspected TB disease, as well as for therapy control

INTRODUCTION

Tuberculosis is a disease that undergoes a renaissance in recent years. One third of the world population is infected. More than 8 million people are affected every year and between 2-3 million die from tuberculosis.There is a constant search for new diagnostic methods for early diagnosis of tuberculosis. IGRA / Interferon gamma release assay / tests give new optimism not only for early detection of the disease but also for monitosring the therapeutic effect. The production of bacilli in children with tuberculosis is 14-18%, therefore the diagnosis of TB in childhood usually is a clinical one, as different clinical methods are used as well as chest X-ray (CXR) and positive Tuberculin skin test (TST). Until recently the Tuberculin skin test (TST) was the only method for diagnosis of tuberculosis infection (TB). Recent advances in genomics and immunology lead to development of two new analysis methods based on release of gamma interferon (IFN-γ) that use highly specific peptides for Mycobacterium tuberculosis, absent in Bacillus Calmette - Guerin (BCG) vaccine and in nontuberculous mycobacteria (WHO 2006). QFT relies on *M. tuberculosis* (MTB) specific T-cell responses as it measures the interferon gamma (IFN-γ) levels in whole blood in response to stimulation with specific MTB antigens : ESAT-6, CFP-10 and TB7.7 and the results show past or present infection. IGRAs as a whole are more concrete than TST because there is no cross-reactivity with BCG vaccine, M.avium or most of the other nontuberculous mycobacteria (NTM). IGRAs are increasingly used worldwide,separately or together withTST,for diagnosis of latent tuberculosis and diagnosis of active TB, IGRAsare often used by clinicians as an additional method for diagnosis of active TB. (Mazurek *et al.,* 2010; Lewinsohn *et al.,* 2010; Marais *et al.,* 2006) Nevertheless there are still not enough such studies in children. In its recommendations for test usage to prove the validity of IGRAs in children, the Center for disease control and prevention (CDC) recommends comparing these tests with TST. (http://www.cdc.gov/tb/publications/newsletters/notes /TBN_2_11/images%5Ctbn211.pdf)

The basic purpose of this scientific paper is to study Quanti FERON-TB GoldIn-Tube (QFT) (Cellestis Limited Cha dstone, Australia) as a reliable method in children with TB or latent TB infection /LTBI/ and the related changes in IFN - γ production in younger age population. The study was supported by the "National TB program for disease control in Bulgaria" who supplied the QFT tests.

MATERIALS AND METHODS

Participants in the study

The study was conducted at the University children hospital for pulmonary diseases - Sofia, from January to December 2013, in children with active form of TB and latent TB infection. Children were between 0 and 17 years old.

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Inclusion criteria

Diagnosis of TB was made as a result of:

1. Signs and symptoms of active TB (detailed case history and status)
2. Microbiological and cultural confirmation of *M. tuberculosis* (MTB)
3. Abnormal X-ray (X-ray morphological changes typical for primary tuberculosis)
4. History of contact with active disease adults

Examination for active TB

1. In all children gastric lavage was taken and examined for tuberculosis bacteria directly and in culture.
2. TST Mantoux test was made on the left forearm strictly intra dermal with 5IU Bulgarian PPD tuberculin. Results were obtained after 72 hours. They were separated in 3 groups. Size of infiltration 0-5 mm-negative, size of infiltrate 6-14 mm and over 15 mm.
3. In all children presence or absence of BCG vaccination scar was determined on the left shoulder /in Bulgaria compulsory BCG vaccination has been done since 1960/.

Laboratory analysis

Quanti FERONH-TB GoldIn-Tube (QFT) was done according to the instructions of the manufacturer Cellestis Limited Cha dstone, Australia. (www.cellestis.com). Analysis was performed in Cibalab laboratory.

Statistical analysis

Statistical analysis was done with SPSS17. Unsuccessful results were not included in the analysis. The percentage of correlation between both tests was determined in different forms of the TB disease and different diagnostic criteria. The percentage of concordance and kappa was established.

Results and comments

All 97 children were admitted in the clinic for treatment and were sent either by GP or by regional dispensaries. All children are Bulgarian citizens aged 0 to 17 years. The study duration was 12 months.Girls and boys were respectively 49 and 48.The biggest group was children with TB of the tracheobronchial lymph nodes – 49 children; followed by Latent TB infection - 35 children; infiltrative pneumonic TB - 7 children and Pleuritis, Primary TB complex and extra pulmonary TB each having 2 children.Children were distributed in four age groups: 0-3 years – 10 children; 4-7 years – 15 children; 8-12 years - 35 children and 13-17 years – 37children.

All children were checked for presence or absence of BCG vaccination scar. In 38 /39, 9%/ of the children BCG scar was missing which is most probably due to oversight in our vaccination program. Since having a scar is the only criteria for exact BCG vaccination, we will follow these children in regard to severity of the disease.Almost half of the children 49 /50,5%/ were admitted in the clinic after having a contact with diagnosed bacilli producing adult. Knowledge of the etiopathogenesis of tuberculosis in children tells us that very small percentage of them is bacilli producing. In this study only 9,3% of the children were producing bacilli observed directly or on culture, which is much lower than the value for children, which is normally around 14%. This is most probably due to the fact that a great number of the examined children were with latent tuberculosis infection. (Mandalakas *et al.,* 2011; Dogra *et al.,* 2007)

We discovered that only nine /9,3%/ children had a negative reaction towards TST with 5 IU PPD tuberculin. 18 /18,65/ had infiltrate sizes from 6 to 14 mm at 72 hours, and 70 /72,2% / were with tuberculin test over 15 mm.

In testing with QFT 43/44,3%/ of the children had negative results and 54/55,7%/ were with positive results. The average age of the tested children was 10, 59.± 4,61. Mean values of the tuberculin testing were 14, 87 mm ± 6,2. As statistics demonstrates the mean size of the infiltrate is in the reference range, but in most children the quality difference showed a virulent character of the infiltrate – with bullous, vesicular, punctate hemorrhages, unequal infiltrate borders, residual pigmentation or crusts. This all shows us one more time how important is the interpretation of one, at first look, easy to perform testing. (Machado *et al.,* 2009; Lienhardt *et al.,* 2010)

QFT was with mean values of 1,33 ± 2,48 IU/ml. These values are significantly lower than in adults, which is due to production of effector cells in childhood. Out of all tests there were no intermediate results. We discovered that the largest share of children who reacted to both TST and QFT are those in the highest age groups. Of course the relative part of the children in younger age was too small to make cardinal conclusions. Many authors think that in primary infection, as it is the case in younger children due to the specific immune reaction, there is lower production of gamma interferon from the effector cells in the peripheral blood, whilst in reactivation of infection in adults there is much more (Zhang *et al.,* 2011; Ling *et al.,* 2011; Pavić *et al.,* 2011; Bergamini *et al.,* 2009) (Fig.1).

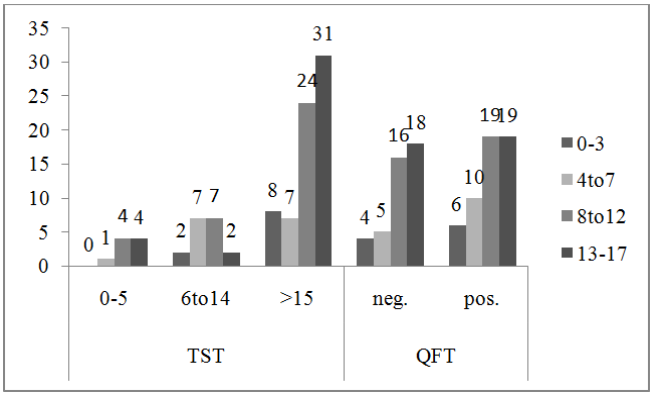

Fig.1. TST and QFT reaction to the age

We intended to follow up TST and QFT in different forms of the tuberculosis disease. In the most common form of tuberculosis disease in children – TB of the tracheobronchial

lymph nodes, 34/69,4%/ of the children had TST > 15 mm and 13/26,5%/ - TST 6-14 mm, negative were only 2/4,1%/. 32 /65,3%/ of the children reacted positive with QFT, and 17/37,4%/ were negative. This shows that there is almost equal diagnostic agreement between both TST and QFT.In Bulgaria the vaccination program includes compulsory BCG vaccination at birth with three booster shots afterwards. We examined all children for presence or absence of BCG scar and does that influence both immunological tests. In children without BCG scar 25/65,8%/ reacted with size of the infiltrate above 15 mm, whilst in these with BCG scar 45/76,3%/ reacted with size of infiltrate above 15 mm, which undoubtedly shows cross reactivity in testing with TST. With QFT 21/55,3%/ of the children without BCG scar reacted positive and 33/55,9%/ of the children with BCG scar reacted positive, which once more demonstrates absence of influence of the BCG strain on the reactivity of QFT (Haustein *et al.,* 2009; Starke, 2006).

On the next diagram it is seen that in children in contact with active diseased adult we have obvious reaction towards both TST and QFT, which shows once again that having close contacts with individuals with active TB disease reflects on immunological reaction of the organism and more often leads to disease. There is obvious correlation between the presence of contact and QFT reaction - likelihood ratio 10,147-df1-p0, 001. (Fig.2)

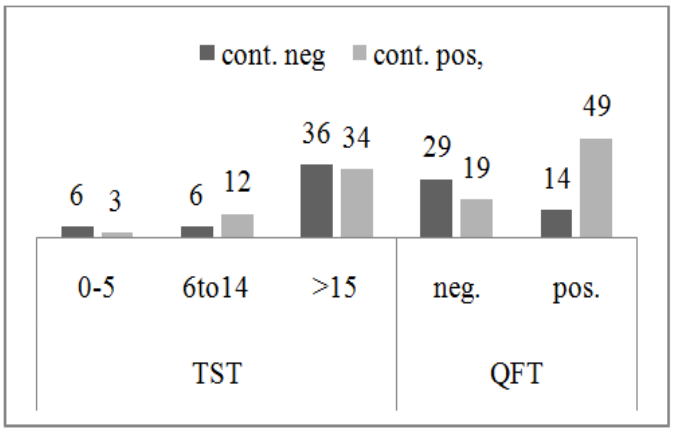

Fig.2. Reaction by contact

Tuberculosis bacteria positive children are only 9 /9,3%/ thus the interpretation of reaction of both immunological tests is statistically insignificant. For us, it was of great importance to follow up if there is any kind of correlation between both tests and what it is. For this purpose we used percentage of concordance and kappa.In the tested children we determined percentage of concordance 62,88%., kappa 0,19 with p=0,011 and likelihood ratio 6,614-df1-p0,01. This shows a relatively low correlation between the two immunological tests.

On the following diagram it is seen that in TST reaction above 15 mm, QFT is positive in 42 /60%/ of the children, which shows that using both tests we receive higher diagnostic value of the results especially in cases with absent diagnostic criteria for establishing the TB diagnosis. (Fig.3)

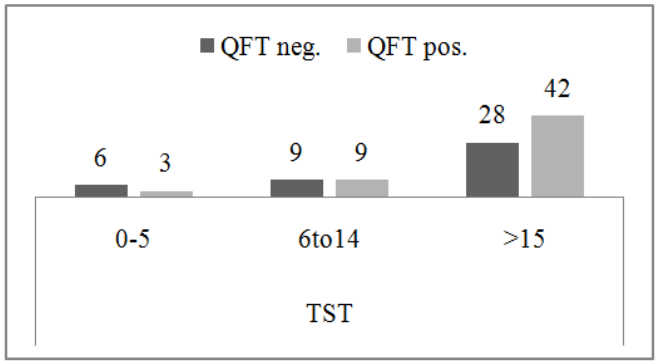

Fig.3. Relationship between TST and QFT

Sensitivity of TST was 77,7%, whilst with QFT GIT it was 88,9%.

Diagnosis of tuberculosis in childhood remains a challenge in pediatric practice since bacteriological confirmation is more of an exception than a rule. In most cases diagnosis is made over symptoms, history of exposure, X-ray changes and data from BCG vaccination. In this particular study in children with active TB we tried to investigate diagnostic potential of QFT in tube, which shows the same sensitivity as in adults. Many authors consider that in primary infection, as it is the case in young children due to the specifics of the immune reaction, there is less production of gamma interferon from effector cells in peripheral blood, whilst in reactivation of infection in adults there is significantly more specific effector cells which makes the sensitivity of QFT in tube higher. In this study we did not establish this phenomenon. Many studies determine that it is necessary to have certain amount of antigen-specific effector cell in peripheral blood in order to have adequate results with QFT in tube test. In our study there are no intermediate results despite the statements of many authors statements for presence of intermediate results especially in young childhood age. (Starke, 2006; Dyrhol-Riise *et al.,* 2010; Roumiana Markova *et al.,* 2011; Michala *et al.,* 2012; Sester *et al.,* 2011)

We have noticed that the biggest part of children who reacted towards TST and QFT is in the higher aged groups. According to the obtained results, negative QFT in tube does not exclude active tuberculosis especially in children below 3 years of age. In QFT 21/55,3%/ of the children without BCG scar reacted positively and 33/55,9%/ of the children with scar from BCG vaccination reacted positive, which supports the statement of the efficacy of QFT in tube test in cases with compulsory BCG vaccination. Nevertheless testing with QFT in tube especially in countries with compulsory BCG vaccination can contribute to the whole diagnostic process especially in combination with TST. In negative QFT in tube, but with presence of other diagnostic criteria for active TB, a tuberculostatic therapy is initiated. This is especially important for children below 5 years. Those are also the recommendations of ECDC GUIDANCE - Use of interferon-gamma release assays in support of TB diagnosis Stockholm: 2011.

This gives us reason to conclude that despite the difficulties for making the tuberculosis diagnosis in this particular age, when using both tests we have 60,7% positive results. Despite the complicated immunological relationships between micro and

macro organism in young childhood, the two immunological tests have their value. Of course, in children with negative QFT in tube with presence of the remaining diagnostic criteria for making the diagnosis at presence, treatment should be initiated. From the above we can conclude the following:

1. The diagnostic possibilities of QFT in tube and TST in the most common form of primary TB – TB of the tracheobronchial lymph nodes are almost equal 69,4% of the children were with TST > 15мм. and 65,3% were positive with QFT GIT
2. QFT GIT test can be successfully used in complex diagnosis of infection with *Mycobacterium tuberculosis* in BCG vaccinated children.
3. QFT GIT is effective in testing children in greaterage group.
4. Sensitivity of QFT GIT 88,9% is higher than the sensitivity of TST 77,7% and showed high diagnostic value in culture confirmed TB.

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