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Role of QuantiFERON-TB Test in Detection of Children Infected with *Mycobacterium tuberculosis*

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ABSTRACT

Background: *Latent TB infection can persist for many years with about 10% lifetime risk of reactivation to active disease. However, in children with latent TB infection, disease develops within 2 years of infection. Recently, a new diagnostic test (QuantiFERON-TB Gold) which measures the production of interferon (IFN) gamma in whole blood upon stimulation with Mycobacterium tuberculosis has been introduced. The aim of this study is to compare the performance of the IFN-gamma assay with tuberculin skin test (TST) for the identification of latent TB infection in children in contact with active TB in the pediatric pulmonary ward.*

Materials and Methods: *This cross-sectional study was conducted on 100 children, aged 2months – 15 years admitted to the Pediatric Ward of Masih Daneshvari Hospital during 2007-2008. Whole blood was collected for measuring Interferon-gamma using QuantiFERON-TB Gold kit (QFT-Cellestis Comp). In this procedure, Mycobacterium tuberculosis specific antigens (ESAT-6 and CFP-10) are used. In the present research, 100 children were studied and divided into 3 groups of case (TB), contact and control. PPD test was performed by injecting 0.1 ml of the 5 unit solution (Pasteur Institute of Iran) for all cases.*

Results: *Twenty-eight percent of the contacts, 60% of the cases and 10% of the controls were Afghans; the remaining were Iranians. Smear of the gastric washing (3×) was prepared in contact and case (TB) groups; 30% of the cases (TB) were AFB positive, while all of the contacts had negative smears. History of BCG vaccination during neonatal period and BCG scar were present in all cases. Positive PPD test (PPD≥ 10 mm) was observed in 90% of the cases and 24% of the contacts. PPD test was negative in the control group. Out of 50 contacts, 18 (36%) showed positive QFT test; and of 20 TB patients, 18 (90%) had positive tests. Regarding age, children with positive QFT test belonged to the older age group.*

Conclusion: *To our knowledge, this is the first study to investigate the performance of the whole blood IFN-γ assay in diagnosing latent TB infection in children in Iran. This study found a fair correlation between the TST and the whole blood IFN-γ assay in children at high risk of latent TB infection. Our study also highlighted fair and moderate agreement in contact and TB groups respectively between the TST and QFT –TB test in children at high risk for latent TB infection. More studies are required to clarify this relationship.* ***(Tanaffos2010; 9(3): 22-27)***

Key words: *Tuberculosis, Infancy, Childhood, Tuberculin skin test , QuantiFERON-TB test*

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INTRODUCTION

According to the World Health Organization (WHO), one third of the world's population is believed to harbor latent TB Infection (LTI), and almost 9 million new cases occur annually. About 10% of these cases occur in children younger than 15 years of age (1-3).

Latent TB infection (LTI) can persist for many years with a lifetime risk of reactivation to active disease of approximately 10% (4,5). However, in children with latent TB infection, disease develops within 2 years of infection (6).

Accurate identification of those with latent infection is difficult as they remain asymptomatic. Also, treatment of latent TB is a major pillar in TB control (7,8). These facts show that more accurate methods are required for detection and treatment of LTI.

Tuberculin skin test (TST) is used as a screening method for diagnosing latent TB infection in adults and children. However, it has several disadvantages including false positive results due to cross reaction between environmental Mycobacteria and BCG vaccination and false negative results due to immunosuppression. Recently a new diagnostic test (whole blood assay Quantiferon-TB Gold) which measures the production of interferon (IFN) gamma in whole blood upon stimulation with specific ESAT6 and CFP-10 antigens of *Mycobacterium tuberculosis* has been introduced (9). IFN-gamma produced by T cells plays a critical role in protective immunological response to primary infection (10,11).

Potential advantages of this test over TST include: being unaffected by previous BCG vaccinations, higher specificity of this test, requiring fewer patient visits, and the exclusion of problems associated with intradermal injection technique as well as elimination of errors in reading and interpretation (12).

A study performed in Australia on whole blood interferon-gamma assay for detecting latent TB in children demonstrated a poor correlation between the whole blood interferon-gamma assay and TST for the diagnosis of TB and failed when used as a screening assay in routine practice (12).

The aim of this study was to compare the performance of the whole blood IFN-gamma assay with TST for the identification of latent TB infection in children in contact with active TB in the Pediatric Pulmonary Ward of National Research Institute of Tuberculosis and Lung Diseases.

MATERIALS AND METHODS

The present cross-sectional study was conducted on 100 children, aged 2-months – 15 years admitted to the Pediatric Ward of Masih Daneshvari Hospital during 2007-2008. The patients were divided into 3 groups:

Group 1: Included 50 children with history of contact with an active TB patient (contacts)

Group 2: Included 20 children with confirmed diagnosis of TB (cases)

Diagnostic criteria for TB were as follows:

- Presence of any clinical symptom in the child
- History of contact with a TB patient
- TB compatible radiological features
- Positive pathologic/bacteriologic findings
- Positive PPD

It is notable that presence of at least three positive criteria is essential for diagnosis of TB and initiation of anti-TB therapy.

Group 3: This group comprised 30 patients that were considered as "controls".

The exclusion criteria were presence of any underlying condition and /or immune deficiency disorders as well as use of corticosteroids and/or immunosuppressive drugs.

PPD test was performed by injecting 0.1ml of the 5unit solution (Pasteur Institute of Iran) on the

forearm. The test was read after 72 hours; a PPD induration≥ 10mm was regarded as "positive".

Three ml of venous blood was collected and sent to the referral laboratory of Masih Daneshvari Hospital for measuring Interferon-gamma using Quantiferon-TB Gold kit (QFT-Cellestis Comp). In this procedure, *Mycobacterium tuberculosis* specific antigens i.e. ESAT-6 and CFP-10, which are key elements for the diagnosis of TB and are not present in other forms of Mycobacteria (e.g. *M. avium*), are used. As a result of antigenic stimulation (by the aforementioned antigens) *M. tuberculosis* produces and releases Interferon-gamma which is detected easily by ELISA.

In this test, positive and negative cut-off points were considered as: IFN-gamma ≥0.35 IU/ml and IFN-gamma ≤0.35 IU/ml, respectively. Information was collected in a special questionnaire. Data were analyzed using SPSS-16 software and Kappa factor.

RESULTS

In this study, 100 children were evaluated and divided into 3 groups of case (TB), contact and control.

Twenty-eight percent of the contacts, 60% of the cases and 10% of the controls were Afghans; the remaining were Iranians.

There were equal number of boys (50%) and girls (50%) in the under-study population.

Growth chart was above the 50th percentile in 73% of the cases (TB) and 36.7% of the controls.

Smear of the gastric washing (3×) was prepared in controls and cases (TB); 30% of the cases (TB) were AFB positive, while all of the controls had negative smears.

History of BCG vaccination during neonatal period and BCG scar were present in all cases.

CXR and Pulmonary CT-scan were normal in 17% of the cases (TB), while 85% of them had abnormal manifestations. Radiological evaluation of contacts was normal.

Positive PPD test (PPD≥ 10 mm) was observed in 90% of the cases and 24% of the contacts. PPD test was negative in the control group.

Table 1 demonstrates and compares the results of PPD test and QFT in three groups.

Table 1. PPD test and QFT results in the understudy population.

Study Groups	No.	PPD≥10mm	QFT≥35IU/ML
Contacts	50	12(24%)	18(36%)
Cases (TB)	20	18 (90%)	18(90%)
Controls	30	0	4 (13%)
Total	100		

Evaluation of Kappa factor and statistical analysis of PPD and QFT show Kappa agreement to be 0.44 and 0.25 in the cases and contacts, respectively. *[Kappa Agreement (Poor 0-0.2; Fair 0.21-0.4; Moderate 0.41-0.6 ; Good 0.61-0.8 ; Very good 0.81-1)]* (13).

Regarding the age range of patients, children with a positive whole blood IFN-γ assay were older and had a higher mitogen control (Table 2).

Table 2. Results of QFT in correlation with age in all groups.

		QFT		Total
		Positive	Negative	
Age	0-5 yrs. (No.)	13	25	38
	%within age	34.2%	65.8%	100%
	6-10 yrs. (No.)	14	19	33
	%within age	42.4%	57.6%	100%
	11-15 yrs. (No.)	13	16	29
	%within age	44.8%	55.2%	100%
Total	No.	40	60	100
	%within age	40%	60%	100%

DISCUSSION

This Study was conducted on 100 children admitted to the Pediatric TB Ward of Masih Daneshvari Hospital. Twenty TB cases, 50 contacts

and 30 controls were evaluated during a one-year period. A similar 2-year research was performed on 28 children affected by TB in Thailand (2).

Under-study children were in the age range of 2 months – 15 yrs. In regard to clinical manifestations, fever was reported in 30% of TB patients; this rate was 78% in a similar study conducted in Australia (12).

In our study, incidence of cough and sweating was reported to be 50% and 15%, respectively. The most common age group was: less than 5 yrs (38%) followed by 6-10 yrs (33%) and 11-15 yrs (29%).

In an Indian research performed in 2005, the most prevalent age groups were: 1-4 yrs (40%), 5-8 yr (31%) and 9-12 yrs (24%) (14). CXR and pulmonary CT-scan findings were abnormal in 85% of TB patients. Normal chest x-rays were observed in only 15%.

In Lolekha study, rate of abnormal manifestations was reported to be 70% (2).

To our knowledge, this is the first study to investigate the performance of the whole blood IFN-γ assay in diagnosing latent TB infection in children in Iran.

The effect of prior BCG vaccination on TST is the subject of many reviews (15).

Many studies suggest that interferon gamma assays have higher specificity than TST, and are less influenced by previous BCG vaccination (16-22). IFN-γ assays that use more than one antigen (e.g. ESAT-6 and CFP-10) appear to be at least as sensitive as the TST in the diagnosis of active TB. Other advantages include the need for fewer patient visits, avoidance of subjective reading, and the ability to perform serial testing without boosting. A major limitation of IFN-γ assay, particularly in developing countries, is the higher cost of its material and the need for laboratory support. The need for venous blood also poses problems for its use in young children and in community-based studies.

In our study, BCG vaccination status did not appear to be associated with this bias because all cases in our study had received BCG vaccination in the past and had vaccination scar. In a same study in Japan 92% had received BCG vaccination (23).

Identifying and treating infectious cases are critical for TB control in developed countries, where resources are more readily available and secondary prevention of reactivation of the disease is feasible. Considerable emphasis is also placed on identifying individuals latently infected with *M.tuberculosis* (12).

In our study, 18 (90%) children diagnosed with TB, and 18 (36%) children in the contact group had QFT assay. This suggests that the whole blood QFT has lower sensitivity than TST, or is less likely to be more specific in revealing false positive TST results. In a similar study, there was a poor correlation between the TST and the whole blood QFT assay, with 30% positive QFT among TB cases and 30% in contact cases (12).

Children with a positive whole blood IFN-γ assay were older and had a higher mitogen control response. This association may have been due to cumulative probability of TB exposure or an increased ability to produce IFN- γ. In our study, a positive correlation was well documented between decreased IFN- γ production to various stimuli in infants and potentially increased chance of false negative assays or requiring re-evaluation of lower threshold values for positive responses (24).

Of 20 children diagnosed with TB in our study, 18 (95%) had positive whole blood IFN- γ assays.

Our study highlighted a fair and moderate agreement in contact and TB groups, respectively between the TST and the whole blood IFN- γ assay in children at high risk for latent TB infection. However, more studies are required to clarify this relationship.

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