Original Article

Accuracy of an Interferon-gamma Release Assay to Detect Active Tuberculosis in Children: A Pilot Study

Young Jin Lee, M.D., Peter Chun, M.D., Ju Hee We, M.D., and Su Eun Park, M.D.

Department of Pediatrics, School of Medicine, Pusan National University, Yangsan, Korea

Purpose : Early diagnosis of active tuberculosis (TB) in children is difficult. The widely used tuberculin skin test has low sensitivity and cross reactivity with non-tuberculous mycobacteria or Bacille Calmette-Guérin vaccination. Interferon gamma release assays have been shown good diagnostic accuracy for active in adults. But studies in children were limited. The purpose of this study was to examine the performance of enzyme-linked immunospot assay (ELISpot) as an initial test in the diagnosis of active tuberculosis in children.

Methods : In a hospital-based study, we prospectively examined the performance of ELISPot in 33 children suspected of active TB. TB was confirmed bacteriologically or histologically.

Results : Among 33 patients, 9 had active tuberculosis. When tested, they all had a positive test result from the ELISpot. The sensitivity and specificity of the assay were 100% (95% CI, 66.4-100%) and 95.8% (95% CI, 78.9-99.9%) respectively.

Conclusion : ELISpot might be an useful and improved clinical diagnostic method for the detection of active TB in children.

(Korean J Pediatr Infect Dis 2011;18:48-53)

Key Words : Active tuberculosis, Interferon gamma, Children

Introduction

Tuberculosis (TB) is still one of the major public health problems in the world[1]. Early diagnosis and treatment of active tuberculosis are the most important factors in reducing the morbidity, mortality, and incidence of TB[2, 3]. Because of its non-specific clinical presentation, tuberculosis is one of the most difficult diseases to diagnose early. Moreover, current diagnostic tools take a long time to come out, have poor sensitivity and cannot reliably exclude the diagnosis[4, 5].

A diagnosis of active tuberculosis can only be confirmed by positive acid-fast stains or positive cultures for *Mycobacterium tuberculosis* (*M. tuberculosis*) from any suspected site of the disease. However, suitable specimens are difficult to obtain from children and culture results are frequently negative and usually too late to manage effectively [4, 5].

The tuberculin skin test (TST), which has been widely used to lend support to clinical and radiological findings in the assessment of suspected tuberculosis, has until recently been the only test available for the indirect detection of *M. tuberculosis* infection. However, the sensitivity of TST is low and is limited by cross reactivity caused by prior vaccination with Bacille Calmette-Guérin (BCG) or by infections with non-tuberculous mycobacteria (NTM). In Korea, the accurate detection of *M. tu-*

*이 연구는 부산대학교 자유과제 연구비(2년)에 의하여 연구되었음
Received : 15 September 2010, Revised : 20 October 2010
Accepted : 20 October 2010
Correspondence : Su Eun Park, M.D.
Department of Pediatrics, School of Medicine, Pusan National University, Yangsan, Korea
Tel : 055-360-2180, Fax : 055-360-2181
E-mail : psepse@naver.com

berculosis infection by TST is particularly limited by BCG vaccination in childhood. Therefore, improved diagnostic tests are needed.

Interferon gamma release assays (IGRAs) incorporating *M. tuberculosis* specific antigens have emerged as potential replacements for TST[5]. The 2 types of IGRA are whole blood enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunospot assay (ELISpot). Tests that detect T cells producing IFN-γ in response to early secreted antigenic target 6 (ESAT-6) and culture filtrate protein (CFP-10) are now available on the market including the QuantiFERON-TB Gold (Cellestis LTD., Carnegie, Victoria, Australia) and T-SPOT. TB (Oxford Immunotec, Oxford, United Kingdom) assays.

IGRAs have been shown to have high sensitivity and specificity in TB infections in adults[7]. But there are few studies that have assessed IGRAs performance for the clinical diagnosis of pediatric active tuberculosis[8-10].

Thus, purpose of this study was to examine the performance of ELISpot as an initial test in the diagnosis of active TB in children.

Materials and Methods

From April 2007 to August 2008, 33 patients admitted to the Pusan National University Hospital with a suspected diagnosis of active TB under 18 years of age were examined and included in the study population. Patients with a prior history of TB or without symptoms or in an immunosuppressed state were excluded.

Demographic and clinical details were obtained from each patient by a detailed medical record including: sex, age, date of birth, TB exposure history, BCG vaccination history and symptoms suggestive of TB. Informed consent for inclusion in the study and a blood sampling for ELISpot was obtained from the accompanying parent. We reviewed clinical record retrospectively. All patients had a full clinical evaluation which included a TST and blood test including ELISpot, full blood count and chest x-ray. If required, CT or ultrasonography of the infected site was examined. Biopsy, acid fast stain and culture of specimens from suspected site were also done.

ELISpot (T-SPOT. TB, Oxford Immunotec, Oxford, UK) was carried out according to the manufacturer's guideline and defined as positive or negative based on the manufacturer's recommended criteria. ELISpot was performed on 3 mL of heparinized blood. A positive test result was defined as >5 spots. The laboratory scientists carrying out the ELISpot did not know the clinical status of the patients.

Diagnosis of TB was confirmed by 1) positive *M. tuberculosis* cultures or acid-fast stains, or 2) clinical manifestations or laboratory or pathological findings consistent with active TB and response to TB medication. We monitored all patients prospectively to reach a final diagnosis.

The accuracy of the ELISpot was evaluated by sensitivity, specificity with confidence intervals, using data from children with active TB vs. children without TB. Fisher's exact two sided binomial test was used to calculate sensitivity and specificity with confidence intervals.

Results

Among the 33 patients, 9 patients were confirmed

Table 1. Clinical Characteristics and Laboratory Details of Children with Active Tuberculosis

Patient	Age (yrs)	Sex	Clinical features	Diagnosis	Biopsy, site	AFB stain	AFB culture	*M. tuberculosis* PCR
1	1 m	M	Fever, otorrhea	Tb otomastoiditis	Not done	(+)	(+)	(+)
2	11	F	Fever, cough	Pulmonary Tb	Not done	(+)	(+)	(+)
3	11	M	Fever, cough	Tb pleurisy	pleural tissue	(−)	(−)	(−)
4	12	F	Hemoptysis	Pulmonary Tb	Not done	(+)	(+)	(+)
5	14	F	Supramediastinal lymph node enlargement	Tb lymphadenitis	supramediastinal lymph node	Not done	Not done	Not done
6	15	M	Bone pain	Bone Tb with pulmonary Tb	Not done	(−)	(−)	(+)
7	16	F	Palpable bilateral neck mass	Tb lymphadenitis	Not done	(−)	(−)	Not done
8	16	M	Palpable neck mass	Tb lympadenitis	cervical lymph node	(−)	(−)	Not done
9	17	M	Cough, blood tinged sputum	Pulmonary Tb	Not done	(+)	(+)	(+)

Abbreviations : m, month; Tb, tuberculosis

Table 2. Comparison of the ELISpot and Clinical Diagnosis of Active Tuberculosis

	ELISpot results		
	Positive n	Negative n	All n
Tuberculosis	9	0	9
Other diagnosis	1	23	24
All	10	23	33

to have active tuberculosis. The median age of children with active TB was 14 yrs (range: 1 month to 17 years). 3 had pulmonary TB, 3 had TB lymphadenitis, 1 had pleural TB, 1 had bone TB with pulmonary TB and 1 had TB otomastoiditis (Table 1).

Among the 33 patients included in the study, 10 had positive and 23 had negative results when using ELISpot. All of patients with active TB showed a positive result from the ELISpot assay (Table 2). The sensitivity of the assay compared with the clinical diagnosis was 100% (95% CI, 66.4−100%) in all active TB patients. ELISpot results were negative for 23 patients whose clinical diagnosis of TB was also negative. Their diagnoses were pneumonia, crohn's disease, Kikuchi disease, suppurative lymphadenitis, meningoencephalitis, meningitis, ankylosing spondylitis and toxoplasmosis (Table 3). The specificity of the assay compared with the clinical diagnosis was 95.8% (95% CI, 78.9−99.9%).

Table 3. Diagnoses of Patients with Negative ELISpot

Diagnosis	No. of patients
Pneumonia	8
Crohn disease	8
Kikuchi disease	2
Suppurative lymphadenitis	1
Meningoencephalitis	1
Meningitis	1
Ankylosing spondylitis	1
Toxoplasmosis	1
Total	23

Discussion

We assessed the clinical usefulness of ELISpot for detection of active TB in children with suspected

tuberculosis. In this study, the sensitivity and specificity of ELISpot were 100% (95% CI, 66.4-100 %) and 95.8% (95% CI, 78.9-99.9%) respectively. Recent U.S. and United Kingdom national guidelines recommend T-cell-based interferon-γ release assays for diagnosis of latent tuberculosis, but their clinical utility in evaluation of pediatric patients with suspected active tuberculosis is poorly defined. Our study was performed to evaluate its performance in the detection of active TB in pediatric patients admitted to a Korean general hospital.

In adult patients, 10 previous published studies of QFT-G among patients with active tuberculosis reported that the sensitivity of QFT-G varied from 55% to 88% with a weighted, pooled mean of 75% [11]. Recently, Nishimura et al.[12] reported that sensitivity and specificity of QFT-G test are 76.6% and 91.2% in all active TB patients. And Davinder et al. [13] reported that sensitivity for active tuberculosis was 89% with ELISpot PLUS and 85% with standard ELISpot.

In children with tuberculosis, 3 studies of IGRA have been published. One study which had been conducted in country with high burdens of TB and had included HIV patients reported that sensitivity of ELISpot was 83% for active TB[14]. Detjen et al. compared the TST, ELISpot, and QFT-IT in children with active TB, showing better correlation of IGRAs, compared with TST. In their study, the sensitivity of both QFT-IT and ELISpot was 93%. The specificity of QFT-IT and ELISpot were 100% and 98%[9]. More recently, Kampmann et al. reported that the sensitivity of QFG-IT and ELISpot were 80%, 58% in children with active TB. The sensitivity of IGRA varied from 58% to 93%[10].

It is known that ELISpot may be more sensitive than QFT to detect recent TB infection, when compared with previous reports, our estimated sensitivity for active tuberculosis is slightly higher. We believe that small study populations may induce high sensitivity. In previous studies, active TB patients were mostly pulmonary TB. In our study, 6 patients were diagnosed as extra-pulmonary TB. The sensitivity was high in patients with extra-pulmonary TB.

Although, Dewan et al.[9] did suggest that the QFT test should not be used alone because of its low sensitivity in the detection of active disease among people with suspected TB. According to our study, ELISpot test provide useful clues for the rapid diagnosis of active tuberculosis in children.

ELISpot needs only overnight (16 to 24 h) incubation times. It has no cross reactivity with prior BCG vaccination or NTM infection which means that it has higher specificity than TST. Other potential advantages of ELISpot include logistical convenience, avoidance of subjective measurement (such as skin induration), need for fewer patient visits, and the ability to perform serial testing without inducing the boosting phenomenon[11].

However, since ELISpot is a test for *M. tuberculosis* infection, not for the active disease, positive ELISpot should be interpreted in the context of the overall clinical picture to decide whether the child has active tuberculosis or an alternative diagnosis with incidental *M. tuberculosis* infection. ELISpot must be used to screen active TB in the evaluation of patients with suspected tuberculosis. It can support the decision to prescribe anti TB medication early one for highly probable tuberculosis patients before results of AFB culture or stains come out. This may be helpful for the progression and effective treatment for tuberculosis disease.

In our study, there are a few limitations. It has small study populations with only 33 patients. And we excluded the results from TST. So we couldn't compare sensitivity of TST with that of ELISpot and failed to show its statistical significance. In our study population, most of them were transferred to our hospital after having a TST at a prior hospital. Because results of TST were measured and indicated subjectively, we excluded them. An additional much larger study including the comparison of ELISpot and TST results is needed to better define its performance for active TB.

In conclusion, we found that ELISpot is sensitive and is a clinically useful initial method to detect active tuberculosis in children. Combined use of TST with ELISpot can enable rapid management of active tuberculosis.

요 약

소아 결핵 진단에서의 인터페론감마 분비 검사의 유용성

부산대학교 의학전문대학원 소아과학교실

이영진 · 천베드로 · 위주희 · 박수은

목 적: 활동성 결핵의 진단으로 투베르쿨린 피부반응검사(TST)가 널리 사용되어 왔으나 민감도가 낮고 BCG 예방접종을 한 경우 교차반응이 일어날 수 있는 한계가 있어 이를 극복하기 위해 인터페론감마검사(IGRA)에 대한 연구가 보고되고 있다. 이에 본 연구는 소아 활동성 결핵을 진단하는 초기검사로서 IGRA의 유용성에 대해 알아보았다.

방 법: 2007년 4월부터 2008년 8월까지 부산대학교 병원 소아청소년과에서 활동성결핵이 의심되어 입원한 18세 이하의 환자 33명을 대상으로 성별, 나이, BCG 접종력, 임상양상과 IGRA 검사결과를 분석하였다

결 과: 총 33명의 환아 중 9명이 활동성 결핵으로 진단되었고 ELISpot에 양성은 10명, 음성은 23명이었다. 활동성 결핵이었던 환아는 모두 양성이었다. 검사의 민감도는 활동성 결핵 환자에서 100%이었다. 23명의 환아가 ELISpot에 음성 반응을 보였는데 이들은 모두 임상적으로 결핵이 아닌 것으로 확진되었고 검사의 특이도는 95.8%였다.

결 론: 소아의 활동성 결핵 진단에 있어 ELISpot은 유용한 것으로 생각된다.

References

1) World Health Organization. 2007. Tuberculosis, fact sheet no. 104. World Health Organization, Geneva, Switzerland. http://www.who.int/mediacentre/factsheets/fs104/en/
2) Chan ED, Heifets L, Iseman MD. Immunologic diagnosis of tuberculosis: a review. Tuber Lung Dis 2000;80:131-40.
3) Keeler E, Perkins MD, Small P, Hanson C, Reed S, Cunningham J et al. Reducing the global burden of tuberculosis: the contribution of improved diagnostics. Nature 2006;444:49-57.
4) Hesseling AC, Schaaf HS, Gie RP, Starke JR, Beyers N. A critical review of diagnostic approaches used in the diagnosis of childhood tuberculosis. Int J Tuberc Lung Dis 2002;6:1038-45.
5) Shingadia D, Novelli V. Diagnosis and treatment of tuberculosis in children. Lancet Infect Dis 2003;3:624-32.
6) Pai M, Kalantri S, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis: Part I. Latent tuberculosis. Expert Rev Mol Diagn 2006;6:413-22.
7) Richeldi L. An update on the diagnosis of tuberculosis infection. Am J Respir Crit Care Med 2006;174:736-42.
8) Pai M, Dheda K, Cunningham J, Scano F, O'Brien R. T-cell assays for the diagnosis of latent tuberculosis infection: moving the research agenda forward. Lancet Infect Dis 2007;7:428-38.
9) Detjen AK, Keil T, Roll S, Hauer B, Mauch H, Wahn U et al. Interferon-γ release assays improve the diagnosis of tuberculosis and nontuberculous mycobacterial disease in children in a country with a low incidence of tuber-

culosis. Clin Infec Dis 2007;45:322-8.
10) Kampmann B, Whittaker E, Williams A, Walters S, Gordon A, Martinez-Alier N et al. Interferon-gamma release assays do not identify more children with active tuberculosis than tuberculin skin test. Eur Respir J 2009; 33:1374-82.
11) Pai M, Menzies D. Interferon-gamma release assays: what is their role in the diagnosis of active tuberculosis? Clin Infect Dis 2007;44:74-7.
12) Nishimura T, Hasegawa N. Mori M, Takebayashi T, Harada N, Higuchi K et al. Accuracy of an interferon-gamma release assay to detect active pulmonary and extra-pulmonary tuberculosis. Int J Tuberc Lung Dis 2008;12:269-74.
13) Dosanjh DP, Hinks TS, Innes JA, Deeks JJ, Pasvol G, Hackforth S et al. Improved diagnostic evaluation of suspected tuberculosis. Ann Intern Med 2008;148:325-36.
14) Liebeschuetz S, Bamber S, Ewer K, Deeks J, Pathan AA, Lalvani A. Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. Lancet 2004; 364:2196-203.