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Diagnostic Value of Elisa Serological Tests in Childhood Tuberculosis

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Summary

Two separate studies (study I and study II) were conducted to evaluate the efficacy of ELISA serological test for the detection of IgG antibodies against specific glycolipid antigen (PGLTb1) and ESAT 6 antigen of *Mycobacterium tuberculosis*, respectively. These results were compared with bacteriological tests [Ziehl Neelson (ZN) staining for acid-fast bacilli and culture on Lowenstein Jensen (LJ) medium] and polymerase chain reaction (PCR) targeting IS6110 sequence. Both studies were carried out on children with pulmonary, central nervous system, lymph node, and gastrointestinal tuberculosis along with matching controls (65 cases and 27 controls for study I and 83 cases and 22 controls for study II). Informed consents of their parents or guardians were taken. They were subjected to clinical examination, relevant laboratory investigations, tuberculin test and chest radiograph. Relevant body fluids were subjected to bacteriological tests and PCR. Sera samples were analyzed for antibodies against PGLTbl and ESAT 6 antigen in study I and study II, respectively. ELISA tests showed a significantly higher sensitivity (49% study I; 53%, study II) as compared with LJ medium culture method (15.4%, study I; 28.9% study II) and ZN staining (27.7%, study I; 20.5%, study II) in all patients ($p < 0.05$). The results were comparable with PCR (40%, study I; 42.2% study II). Specificity of ELISA test was 100% in all the patients except in those with pulmonary disease (92.8%, study I; 84.8%, study II). In view of the convenience, low cost and comparable sensitivity with PCR, these ELISA tests have a promising future in the diagnosis of childhood tuberculosis.

Keywords: ELISA, Tuberculosis, PCR (Polymerase Chain Reaction)

Introduction

About 30% of the world's population of tuberculosis patients resides in India; [1] of these about 500 000 people die every year [2].

Diagnosis of TB in children is often difficult because the typical clinical picture and demonstration of *Mycobacterium tuberculosis* from the body fluids is not found in majority of the patients. The Paucibacillary nature of the disease in children makes Ziehl Neelsen (ZN) staining a less useful diagnostic test [3]. The growth of *M. tuberculosis* on conventional Lowenstein Jensen (LJ) culture medium takes 6–8 weeks and requires a high-bacterial load.

Among the newer diagnostic tests, polymerase chain reaction (PCR) is a highly sensitive and specific technique, and the starting material could be a single molecule of rRNA or DNA. The DNA sequence IS6110 has been commonly used for development of PCR assays with encouraging results [4–6].

ELISA was introduced by Nassau in 1976 [7]. However, due to lack of cross reactants and adequate standardization so far, the diagnostic ability is not certain. A large number of purified antigens from *M. tuberculosis* have been evaluated, but they have not stood the test of time. Even today, diagnosis is usually based on clinical signs and symptoms, chest radiography, tuberculin testing and history of contact with an open case of TB [8]. A quick, economical, sensitive and specific diagnostic modality is desirable for childhood TB. PCR, although sensitive and specific, is not in routine use in developing countries because of the high cost.

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With this background, we conducted two separate studies to evaluate the efficacy of ELISA serological tests (used for detection of IgG antibodies in serum), against two mycobacterium TB antigens, which are specific for *M. tuberculosis*, namely:

(i) Specific glycolipid antigen (PGLTb1) present in the cell wall (study I).
(ii) Secretory antigen ESAT-6 present in cytoplasm (study II).

We compared the results with ZN staining, culture on LJ medium and PCR targeting IS6110 in different body fluid specimens.

Subjects and Methods

Both studies included children of either sex, under 18 years of age, attending the Out Patient Department and admitted in the wards of Department of Pediatrics, S.N. Medical College, Agra. They were selected on the basis of criteria laid down by Indian Academy of Paediatrics [9]. Informed consent of their parents or guardians was taken.

Study I comprised 65 patients with a provisional diagnosis of TB [pulmonary=32, central nervous system (CNS) =16, lymphnode = 13, gastrointestinal (GI) = 4] and 27 matching disease controls (Table 1).

Study II comprised 83 patients with a provisional diagnosis of tuberculosis (pulmonary = 37, CNS = 16, lymphnode = 19, GI = 11) and 22 matching disease controls (Table 2).

Gastric aspirate or sputum, whichever was obtainable from pulmonary cases, cerebrospinal fluid (CSF) from CNS cases, lymphnode aspirate from lymphadenitis cases and ascitic fluid from abdominal cases were subjected to Ziehl Neelsen (ZN) staining for acid-fast bacilli (AFB), PCR targeting IS6110 [5] and culture on LJ medium.

The sera samples from all the cases and controls were subjected to ELISA test using PGLTb1 antigen in study I and ESAT-6 in study II.

The sensitivity and specificity of the various methods were calculated. Z-test was applied to the results of the diagnostic methods to compare the significance.

Results

Study I

History of contact was present in 70.7% of the cases. Controls with a negative history of contact were included.

Bacillus Calmette Guerin (BCG) vaccination had been received by 20% of the cases and 70.3% of the controls. This observation was statistically highly significant ($p < 0.01$) (Table 3).

All the patients were malnourished as compared with 63% of the controls who were malnourished.

A total of 47.7% the cases in the study group, mainly with pulmonary pathology, tested positive with Mantoux test using 5TU PPDS (TU, tuberculin units; PPDS, purified protein derivative-standard), which is equivalent to 1TU of PPD RT23. Lowest positivity was observed in cases with CNS TB. Controls who were Mantoux negative were included in the study (Table 1).

Smears for AFB were positive in 37.5, 6.3, 30.7 and 25% of the specimens from pulmonary, CNS, lymphadenitis and GI cases, respectively. A total of 27.7% cases in the study group tested positive

TABLE 1
Results of various tests in study I

System (Cases $n = 65$)	ZN Staining Positive cases (%)	LJ Medium Positive cases (%)	PCR Positive cases (%)	ELISA (PGL Tb1) Positive cases (%)	Mantoux Test Positive cases (%)
Pulmonary	12	3	16	17	18
($n = 32$)	(37.5%)	(9.3%)	(50%)	(53.1%)	(56.2%)
Sensitivity/Specificity	37.5/100	9.4/100	50/100	53/92.8	56.2/100
CNS	1	2	5	7	6
($n = 16$)	(6.3%)	(12.5%)	(31.2%)	(43.7%)	(37.5%)
Sensitivity/Specificity	6.3/100	12.5/100	31.2/100	43.7/100	37.5/100
Lymphatic	4	5	4	5	5
($n = 13$)	(30.7%)	(38.4%)	(30.7%)	(38.4%)	(38.4%)
Sensitivity/Specificity	30.7/100	38.4/100	30.7/100	38.4/100	38.4/100
Gastro-intestinal	1	0	1	3	2
($n = 4$)	(25%)	(0%)	(25%)	(75%)	(50%)
Sensitivity/Specificity	25/100	13.8/100	25/100	75/100	50/100
Total	18	10	26	32	31
($n = 65$)	(27.7%)	(15.4%)	(40%)	(49.2%)	(47.7%)
Sensitivity/Specificity	27.7/100	15.4/100	40/100	49.2/96.3	47.7/100

Comparison: ELISA vs. ZN staining: $z = 2.59$; $p < 0.05$, ELISA vs. Culture: $z = 4.42$; $p < 0.01$, ELISA vs. PCR targeting IS 6110: $z = 0.6657$; $p > 0.05$.

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with AFB smear. In the control group, all patients were smear-negative (Table 1).

Growth of *M. tuberculosis* on LJ medium culture was seen in 9.3, 12.5, 38.4 and 0% of the pulmonary, CNS, lymphadenitis and GI cases, respectively. Overall, 15.4% of the specimens in the study group tested positive with growth. No growth was observed among controls (Table 1).

PCR targeting IS6110 sequence of *M. tuberculosis* was positive in 50, 31.2, 30.7 and 25% of pulmonary, CNS, lympadenitis and GI cases, respectively. All the controls tested negative. Overall sensitivity and specificity were 40 and 100%, respectively (Table 1).

ELISA with PGLTb1 antigen was positive in 53, 43.7, 38.4 and 75% of pulmonary, CNS, lymphadenitis and GI cases, respectively. One control with pulmonary pathology (bronchiolitis) turned out to be positive. Overall sensitivity and specificity were 49.2 and 96.3%, respectively (Table 1). Statistically significant results were obtained when these results were compared with ZN staining ($p < 0.05$) and LJ medium ($p < 0.01$) (Table 1). However, an insignificant difference was found when the results of serology were compared with results of PCR, indicating the good performance and value of this test.

Study II

History of contact was present in 70.3% of the cases. Controls had no history of contact.

BCG vaccination had been received by 36.2% of cases and 72.8% of controls. This observation was statistically highly significant $p < 0.01$) (Table 3).

TABLE 2
Results of various tests in study II

System (Cases = 83)	ZN Staining Positive cases (%)	LJ Medium Positive cases (%)	PCR Positive cases (%)	ELISA (ESAT-6) Positive cases (%)	Mantoux Test Positive cases (%)
Pulmonary	13	20	20	22	21
($n = 37$)	(35.1%)	(54.1%)	(54.1%)	(59.5%)	(56.8%)
Sensitivity/Specificity	35.1/100	54.1/100	54.1/100	59.5/84.8	56.8/100
CNS	1	1	5	9	7
($n = 16$)	(6.3%)	(6.3%)	(31.25%)	(56.25%)	(43.8%)
Sensitivity/Specificity	6.3/100	6.3/100	31.25/100	56.25/100	43.8/100
Lymphatic	2	2	6	9	7
($n = 19$)	(10.5%)	(10.5%)	(31.5%)	(47.37%)	(36.8%)
Sensitivity/Specificity	10.5/100	10.5/100	31.5/100	47.37/100	36.8/100
Gastro-intestinal	1	1	4	4	6
($n = 11$)	(9.1%)	(9.1%)	(36.4%)	(36.4%)	(54.5%)
Sensitivity/Specificity	9.1/100	9.1/100	36.4/100	36.4/100	54.5/100
Total	17	24	35	44	41
($n = 83$)	(20.5%)	(28.9%)	(42.2%)	(53%)	(49.4%)
Sensitivity/Specificity	20.5/100	28.9/100	42.2/100	53/91	49.4/100

Comparison: ELISA vs. ZN staining: $z = 2.69$; $p < 0.01$, ELISA vs. Culture: $z = 2.02$; $p < 0.05$, ELISA vs. PCR targeting IS 6110: $z = 0.98$; $p < 0.05$.

TABLE 3
BCG Vaccination status

System	Study I				Study II			
	Total number of cases	BCG-vaccinated cases number (%)	Total number of controls	BCG-vaccinated controls number (%)	Total number of cases	BCG-vaccinated cases number (%)	Total number of controls	BCG-vaccinated controls number (%)
Pulmonary	32	4 (12.5)	14	10 (71.4)	37	16 (43.5)	13	9 (69.2)
CNS	16	5 (31.2)	6	5 (83.3)	16	4 (25.0)	6	5 (83.3)
Lymphatic	13	3 (23.1)	4	3 (75.0)	19	7 (36.8)	1	1 (100)
GIT	4	1 (25.0)	3	1 (33.3)	11	3 (25.0)	2	1 (50.0)
Total	65	13 (20.0)	27	19 (70.3)	83	30 (36.2)	22	16 (72.8)

Comparison of vaccinated cases with unvaccinated cases: study I: $z = 4.98$, $p < 0.01$, study II: $z = 3.27$, $p < 0.001$.

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All the patients were malnourished, as compared with 54.5% of the controls who were malnourished.

A total of 49.4% of the cases in the study group, mainly with pulmonary pathology, tested positive with Mantoux test using. Controls who were Mantoux-negative were included in the study (Table 2).

Smears for AFB were positive in 35.1, 6.25, 10.5 and 9% of the specimens from pulmonary, CNS, lymphadenitis and GI cases, respectively. A total of 20.5% cases in the study group tested positive with AFB smear. In the control group all the patients were smear negative (Table 2).

Growth of *M. tuberculosis* on LJ medium culture was seen in 54.05, 6.3, 10.5 and 9% of the pulmonary, CNS, lymphadenitis and GI cases, respectively. Overall 28.9% specimens in the study group tested positive with growth. No growth was observed among controls (Table 2).

PCR targeting IS6110 sequence of *M. tuberculosis* was positive in 54.05, 31.2, 31.5 and 36.4% of pulmonary, CNS, lympadenitis and GI, respectively. All the controls tested negative. Overall sensitivity and specificity were 42.5 and 100%, respectively (Table 2).

ELISA with ESAT-6 antigen was positive in 59.5, 56.2, 47.4 and 36.4% of pulmonary, CNS, lymphadenitis and GI cases, respectively. One control with pulmonary pathology (bronchiolitis) turned out to be positive. Overall sensitivity and specificity were 53.01 and 91%, respectively (Table 2). Statistically significant results were obtained when these results were compared with ZN staining ($p < 0.01$) and LJ medium ($p < 0.05$) (Table 2). However, insignificant difference was found when the results of serology were compared with results of PCR, indicating the good performance and value of this test.

Discussion

Positive history of contact was obtained in 70.7% of cases in study I and 70.3% in study II. According to Park [2], 25% of adult patients with TB are infectious, transmitting aerosol-borne infection among healthy individuals. This observation supports the intra familial source of contact in majority of cases.

In this study, 20% of cases in study I, 36.2% in study II, 70.3% of controls in study I and 72.8% of controls in study II had received BCG vaccine.

Also, 47.7% cases in study I and 49.4% in study II tested positive (>10 mm induration) with Mantoux test using 5TU PPDS. In a review by Udani *et al.* [10], tuberculin testing was positive in 52.3% of children with tuberculosis. A lower positivity in our studies may be because all the children were malnourished [11].

A total of 27.7% cases in study I and 20.5% in study II tested positive with AFB smear. Lowest positivity was seen in cases with tubercular meningitis. Similar results have been reported by Molani and LeFrock [12].

Poor yield from pulmonary specimens ($3/32 = 9.3\%$) in study I on the LJ medium in spite of higher positivity in the smear for AFB can be explained on the basis of the predominance of the gastric lavage specimens ($n = 19$), which generally yielded poor growth because of the lower number of viable bacilli in these specimens owing to the acidic pH of the gastric lavage. This condition was not seen in study II because of the predominance of sputum specimens. In study II, $20/37 = 54.1\%$ of the cases were found to be positive.

Overall sensitivity and specificity of 49.2 and 96.3% was observed using ELISA for detecting IgG antibodies against PGLTbl antigen (Table 1), while this was 53 and 91% when ESAT-6 antigen was used (Table 2). These results were comparable with results of Simonney *et al.* [13], who have reported a sensitivity and specificity of 61.2 and 98.1%, respectively, with ELISA using PGLTbl glycolipid antigen in adults. The relatively lower sensitivity in our study may be due to inadequate immune response in pediatric population as compared with the adults.

On statistical analysis, ELISA showed a sensitivity (49.0% in study I and 53.0% in study II) that was significantly higher than that of LJ medium (15.4% in study I and 28.9% in study II; $p < 0.01$ in study I and $p < 0.05$ in study II) and ZN staining (27.7% in study I and 20.5% in study II; $p < 0.05$ in study I and $p < 0.01$ in study II). However, it was comparable with that of PCR (40% in study I and 53% in study II; $p > 0.05$ in both the studies).

Specificity <100% (96.3%, study I; 91% study II) of ELISA tests can be explained by cross reactivity of antigen to other antigenic material present in the body because of which controls with bronchiolitis turned out to be positive.

Overall sensitivity of PCR targeting IS6110 was 40% in study I and 53% in study II while specificity was 100% in both the studies (Table 2). Eisenach *et al.* [5] have reported higher sensitivity of 72% and specificity of 72–100%. However, their study was done on the sputum samples in which yield was relatively higher. Half of the specimens in our study were from extrapulmonary cases. Furthermore, IS6110 could be absent or in low copy numbers in a section of the *M. tuberculosis* isolates in India.

To conclude, an overview of the results of various diagnostic methods highlights the fact that ELISA gave the best results with good sensitivity and specificity. The cost of performing an ELISA test is much lower than PCR. Thus, the ELISA test has a promising future. The diagnostic yield would be further enhanced if the ELISA test is used in combination with the Mantoux test. In a developing country, like ours, where resources are limited,

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it would be advisable to carry out larger surveys to further evaluate the efficacy of ELISA in the diagnosis of childhood TB.

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