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Sero Diagnosis of Tuberculosis in Children Using Two ELISA Kits

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Abstract. The diagnosis of childhood tuberculosis is based on circumstantial evidence in the absence of a gold standard in the majority of cases. Sero-diagnosis offers scope for an early diagnosis in a variety of clinical conditions and is simple to perform. A number of mycobacterial antigens have been used for antibody detection assays and several are available as kits in the market. This study was done to evaluate the value of antibody detection kits (ELISA) against the A60 antigen and 38kDa antigen of *Mycobacterium tuberculosis* in the diagnosis of childhood tuberculosis at the outpatient department of the Institute of Social Paediatrics, Government Stanley Hospital in collaboration with Tuberculosis Research Centre, Chennai. Thirty five children with pulmonary tuberculosis, 7 with TB lymphadenitis and 22 healthy controls were studied. In addition to routine investigations including gastric lavage for AFB culture, serum antibodies against the A60 and 38kDa antigens were assayed using commercially available ELISA kits. With A60, IgM serum levels were positive in 74% of pulmonary TB cases, 57% of TB lymphadenitis cases and 50% of controls. A60 IgG was positive in 17% of pulmonary TB, 86% of TB lymphadenitis and 14% of controls. The 38 kDa IgG antibody was positive in 37% of pulmonary and 86% of TB lymphadenitis cases and 27% of controls. Among 10 culture confirmed cases, A60 IgM was positive in 8, A60 IgG in 3 and 38kDa IgG in 5 patients. The sensitivity of the tests ranged between 29% and 71% and specificity between 50% and 86%. Although the numbers are small, the results suggest that serodiagnosis using the currently available antigens of *M. tuberculosis* is unlikely to be a confirmatory test for tuberculosis in children. **(Indian J Pediatr 1999; 66 : 837-842)**

Key words *: Sero-diagnosis; Tuberculosis; Children.*

There is no uniform consensus in the diagnosis of tuberculosis in children. In the absence of a gold standard, diagnosis is usually based on a combination of clinical features, chest X-ray findings and tuberculin skin test results[1]. Gastric lavage cultures are slow and positive only in 20-30% of pulmonary tuberculosis cases. Although the BACTEC method is quicker, it is prohibitively expensive for routine use[2]. Molecular biologic techniques like PCR can detect very small amounts of bacterial DNA and could be usefu1[3,4]. However, because of the extreme sensitivity of the technique, detection of mycobacterial DNA from recently infected children may not distinguish infection from disease. In ex-

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trapulmonary TB, the diagnosis is even more difficult because of lack of tissue specimens, except in TB lymphadenitis.

Serodiagnosis is advantageous because it is easy, relatively inexpensive and results can be obtained easily. Moreover, it offers scope for an early diagnosis in a wide variety of clinical situations[5]. A number of mycobacterial antigens have been used for antibody detection assays but have given varying results and none has been sensitive or specific enough for routine use.

Despite its limitations, serodiagnostic kits for TB are being aggressively marketed and used by medical professionals. Two of these commercially available kits. (Anda-TB kit to detect IgG and IgM antibodies to A60 antigen and Pathozyme TB complex kit to detect IgG antibodies to 38 kDa antigen) are evaluated here for their value in the diagnosis of tuberculosis in children.

MATERIALS AND METHODS

This investigation was part of a larger study to develop diagnostic criteria for tuberculosis in children in which about 2400 children have been enrolled to date.

This study was carried out from July to September 1996 and all the children diagnosed with tuberculosis during that period and those considered free of tuberculosis, who were available for blood collection, were included. Children in the age group l-12 years attending the outpatient department with signs and symptoms suggestive of tuberculosis were evaluated further. Blood counts, Mantoux test, chest X-ray and gastric lavage for AFB (on two consecutive days) were done. Chest X-rays were done for all children and lymph node biopsies in those who had significantly enlarged lymph nodes.

Sixty four children were included in this study and formed three groups :

(A) Healthy controls (22) : children investigated for tuberculosis and found to be normal.

(B) Pulmonary TB (35) : children presenting with respiratory signs and symptoms, diagnosed to have tuberculosis on work up.

(C) TB lymphadenitis (7) : children presenting with a glandular swelling diagnosed to have TB lymphadenitis on biopsy.

The diagnosis of pulmonary TB was based on clinical and radiographic criteria, (persistent X-ray shadows not responding to antibiotics) with or without a history of contact and positive tuberculin test. Gastric lavage cultures (done at Tuberculosis Research Centre) were positive for ***M. tuberculosis*** in 10 of the 35 patients. The diagnosis of TB lymphadenitis was confirmed by histopathology or bacteriology or both. Children who had no clinical or laboratory evidence of TB were taken as controls. ELISA using two commercially available kits was performed on blood (serum) obtained from all study patients.

(a) Anda TB kit to detect IgG and IgM antibodies to A60 antigen.

(b) Pathozyme-TB complex kit to detect IgG antibodies to 38 KDa antigen.

The cut off level for a positive test was defined as the average optical density (OD) of the low positive control divided by 1.5 and tests results were reported as positive, negative or equivocal (manufacturer‘s instructions).

RESULTS

Of the 42 patients with a diagnosis of tu-

TABLE 1. Results of Serodiagnostic Tests in TB Patients and Controls

Group	No. of patients	IgM A60		IgG A60		IgG 38 kDa	
		No.	%	No.	%	No.	%
Control	22	11	50	3	14	6	27
Pulm. TB	35	26		6		13	
lymphadenitis	7	4		6		6	
Total TB	42	30	71	12	29	19	45

berculosis, 36 had a Mantoux reaction $\geq$ 10 mm (86%). Chest X-ray was abnormal in 35/42 cases (83%) and sputum/gastric lavage was positive for *M. tuberculosis in* 10 cases (24%). Of the 22 healthy controls, Chest X-ray was normal in all of them and Mantoux was positive in 9 (41%).

Table 1 shows the ELISA sero-positivity among the different study groups according to the kit used. Cases were defined as those with culture or biopsy confirmation as well as those with a clinical diagnosis of tuberculosis who responded to specific anti-TB treatment. This was taken as the gold standard for calculating various statistical parameters.

Table 2 shows the sensitivity, specificity, positive predictive value, false negativity and accuracy of the three tests. It can be seen that there is a reciprocal relationship between the sensitivity and specificity and none of the tests was adequate in both. The A60 IgG and 38KDa IgG had sensitivities that were too low while the specificity of the A60 IgM was only 50%. When only the 10 children with bacteriologically confirmed tuberculosis were considered, 8/ 10 had positive titres for A60 IgM, 3/10 for A60 IgG and 5/10 for 38 kDa IgG. Hence even in bacteriologically confirmed cases, these tests were not sensitive enough to pick up all the cases. In the TB lymphadenitis group, however, both the IgG assays were positive in 6 out of 7 cases. Regarding the specificity, considering only the 13 healthy children with negative Mantoux tests (as truly uninfected controls), the specificity of the three tests was 38%, 85% and 54%. The specificity of the tests, therefore, did not improve by taking into account only tuberculin negative controls.

TABLE 2. Results Using Different Antigens

Test results	A60 IgM	A60 IgG	38 kDa IgG
Sensitivity	71%	29%	45%
Specificity	50%	86%	73%
Pos. Pred. value	73%	80%	76%
False negativity	52%	61%	59%
Accuracy	64%	48%	55%

DISCUSSION

Immunodiagnosis using different antigens from *M. tuberculosis* has been attempted for the past 100 years. There are a number of crude, semipurified and highly purified antigens that have been proposed as useful antigens for diagnosis. The only *M. tuberculosis* specific antigens shown to date are the 38kDa of Ivanyi[6] and 14kDa[7]. Only a small number of monoclonal antibodies are *M.*

tuberculosis specific (TB 23, 68, 71, 78)[6]. The value of a serodiagnostic test depends on its sensitivity (number of positive tests out of total number of true cases) and specificity (number of negative tests out of the total number of control samples). The development of antibodies to a particular antigen depends on its immunogenecity, dose and duration of exposure and possibly, the genetic make up of the host. Also, with "natural exposure" to environmental mycobacteria, antibodies develop to common mycobacterial antigens that could cross react with the test antigen[8].

In the present study, an attempt was made to assess the value of 2 commonly available sero diagnostic kits namely the Anda TB kit to detect IgG and IgM antibodies to A60 antigen and the Pathozyme-TB kit to detect IgG antibodies to the 38kDa antigen. The results suggest that neither of the two kits exhibit sufficient sensitivity and specificity to be useful for routine diagnostic use. Even the bacteriologically confirmed cases were not uniformly detected by these tests. This suggests that antibodies to these two antigens are not uniformly increased in childhood tuberculosis cases.

Reported studies on serodiagnostic tests for TB in children have given conflicting results (Table 3). Turneer *et al* did not find the anti A60 IgM and IgG measurements useful in the diagnosis of primary TB or mycobacterial adenitis in children[9]. Delacourt *et al* found that using the anti-A60 IgG test and a specificity of 98%, a positive serodiagnosis was observed in 68% of children with clinically active TB[10]. The IgM test, however, had a low sensitivity of 19%. They also found that the IgG antibody values were influenced by BCG vaccination and age, increasing with both.

Rosen used mycobacterial sonicates but

TABLE 3. Summary of Serodiagnostic Studies in Children

Reference	Test	Population	Sensitivity	Specificity
Delacourt[10] (1993)	Antigen 60 (IgG)	Pulm. TB	0.67	0.98
Rosen" (1990)	Mycobacterial sonicates	Pulm. TB	0.21	0.40
Barrera[12] (1989)	PPD	Bacteriologically confirmed cases	0.51	0.98
Alde[13] (1989)	Antigen 5	Bacteriologically confirmed cases	0.85	1.00
Turneer[9] (1994)	Antigen 60 (IgG)	Pulm. TB	0.14	0.95
Seth[14] (1997)	PPD	Pulm. Primary complex	0.86	0.92
Present study *(1997)*	A 60 (IgG)	Pulm. TB	0.29	0.86
	A 60 (IgM)		0.71	0.50
	38 Kda (IgG)		0.45	0.73

sensitivity and specificity were poor, essentially due to cross reactivity with BCG[11]. Barrera *et al* had better results using purified protein derivative and showing a specificity of 98% and a sensitivity of 51% in bacteriologically confirmed cases[12]. The best results were obtained by Alde *et al* using antigen 5 which is the same as the 38kDa antigen[13]. They found a specificity of 100% and sensitivity of 85.7% in bacteriologically confirmed tuberculous children but the number of control subjects was low in that study.

In children, antibody levels to *M. tuberculosis* antigens are generally lower than in adults. Such a hyporesponsiveness could be due to the nature of exposure, extent of disease, immunocompetence and genetic background of the children[15]. The presence of cross reacting antibodies also depends on the environment the child lives in. Hence, studies done in one part of the world may not be applicable elsewhere. The issue of reliability of the commercially available serodiagnostic kits can be answered only by testing children in whom the diagnosis has been confirmed. In this study, the kits tested did not diagnose all those children in whom TB was bacteriologically confirmed. At the same time the kits gave positive results in a significant number of healthy (control) children. Hence, they cannot be recommended for routine diagnostic use.

CONCLUSION

Sero diagnosis requires further development and prospective evaluation. An optimal combination of antigenic epitopes in assay may offer the best prospect of a good diagnostic test for tuberculosis.

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