ORIGINAL ARTICLE

Evaluation of polymerase chain reaction and adenosine deaminase assay for the diagnosis of tuberculous effusions in children

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Aim: To evaluate and compare the utility of polymerase chain reaction (PCR) for the diagnosis of tuberculous effusions in children.

Methods: PCR, adenosine deaminase (ADA) activity and absolute lymphocyte count (ALC) were evaluated in the fluid of 31 tuberculous (20 pleural, 8 ascites and 3 pericardial) and 24 non-tuberculous (10 transudative ascites, 8 empyema thoracis, 3 malignant pleural and 3 pyopericardium) effusions.

Results: Fluid PCR for *Mycobacterium tuberculosis* was positive in 74% of tuberculous effusions, whereas it was falsely positive in 13% of the non-tuberculous group. The mean fluid ADA and ALC values were significantly higher in tuberculous effusions than in non-tuberculous effusions ($p < 0.001$). The sensitivity and specificity of PCR, ADA (≥ 38 IU/l) and ALC ($\geq 275/\text{mm}^3$) were 74% and 88%, 81% and 75%, and 90% and 83%, respectively, in diagnosing tuberculous effusions. The sensitivity of PCR, ADA and ALC was 100%, 100% and 88%, respectively, for confirmed tuberculous effusions. When the two tests were combined (either/or positive), the sensitivity increased (90–100%) at the expense of specificity. When both the tests were positive, then the specificity markedly increased (92–96%), but sensitivity of the tests decreased.

Conclusion: Fluid PCR alone should not be relied on as a single test; rather, combined analysis with either ADA or ALC could be more useful in the diagnosis of tuberculous effusions in children.

Tuberculosis is a major health problem in developing countries, including India. Children represent 5–15% of all reported tubercular cases.¹ Although pulmonary tuberculosis is a major manifestation of the disease in childhood, the proportion of extrapulmonary tuberculosis has increased to 25–30% in children over the past two decades.² Pleural effusion is the second most common form of extrapulmonary tuberculosis, with an incidence of 4.9%.³

The diagnosis of tuberculosis in children is based on compatible history, clinical features, suggestive lesions in chest x ray, positive tuberculin or Bacillus-Calmette Guérin (BCG) test and, in effusions, characteristic fluid biochemistry, demonstration of acid-fast bacilli (AFBs) in smear or culture for *Mycobacterium tuberculosis*, and response to treatment. The conventional tests, such as tuberculin or BCG, may be falsely negative and even the yield from gastric aspirate or fluid specimens for AFB may be low. The AFB positivity in the fluid is <20% and fluid culture grows AFB in 18–38% of samples, which takes 6–12 weeks.⁴

In the past, several biochemical and serological tests, such as antigen or antibody detection by ELISA⁵ and detection of increased levels of γ -interferon,⁶ lysozyme⁷ and ADA,⁸ have been used in tuberculous effusions. But these tests have variable results and limitations of non-availability at most centres. DNA amplification by polymerase chain reaction (PCR) has changed the entire approach in the identification of pathogens. This has enabled early detection of mycobacterial DNA directly from clinical specimens, especially in effusions, which are paucibacillary in nature. Insertion sequence IS6110 has the potential to overcome the limitation of conventional methods, as it is specific for *M. tuberculosis*.^{4–9} Various studies have been conducted in adults with tuberculosis,^{4–10} but there are few reports in children, especially with tubercular effusions. Therefore, this study was undertaken to evaluate the usefulness of PCR for the

diagnosis of tuberculous effusions in children and to compare the results with fluid ADA activity and other diagnostic tests.

PATIENTS AND METHODS

Patients and controls

Our study included 55 consecutive patients aged 8 months to 14 years, who presented with pleural effusions, ascites and pericardial effusions to the Department of Pediatrics, Chest and Tuberculosis and Cardiothoracic Surgery, Institute of Medical Sciences, Varanasi, India, between January 2003 and March 2005. A detailed history was taken, and physical and systemic examinations and investigations carried out. The clinical specimens were sent in a blinded manner to the laboratory. Adequate precautions were taken to avoid any cross-contamination of samples during processing. Informed consent was obtained from the parents of each patient. The institute's postgraduate board, Institute of Medical Sciences, Banaras Hindu University, India, approved the protocol of the study.

The cases were categorised into tuberculous (20 pleural, 8 ascites and 3 pericardial) and non-tuberculous (10 transudative ascites, 8 empyema thoracis, 3 malignant pleural and 3 pyopericardium) effusions on the basis of aetiology. Table 1 shows the modalities used for classifying tuberculous aetiology. The non-tuberculous group included patients with effusions due to other causes—that is, transudative (5 cirrhosis of liver and 5 nephrotic syndrome), empyema thoracis (n = 8), pyopericardium (n = 3) and malignant pleural effusions (1 Hodgkin's and 2 non-Hodgkin's lymphoma).

Abbreviations: ADA, adenosine deaminase; AFB, acid-fast bacillus; ALC, absolute lymphocyte count; BCG, Bacillus-Calmette Guérin; PCR, polymerase chain reaction

Table 1 Modalities used for the diagnosis of tuberculosis

Method	No of Cases (n)	Status of effusions (n)
Lymph node biopsy showing tuberculous granuloma	3	Confirmed pleural (2) and confirmed ascites (1)
Biopsy of pleura	2	Confirmed pleural
Fluid culture positive for AFB	2	Confirmed pleural (1) and ascites (1)
Gastric aspirate culture positive for AFB	1	Confirmed pleural
Compatible clinical features, positive tuberculin or BCG tests, exudative fluid biochemistry, suggestive radiographs and response to antituberculous treatment	23	Probable (pleural, ascites and pericardial)

AFB, acid-fast bacillus; BCG, Bacillus-Calmette Guerin.

Clinical specimens

Fluid specimens from each patient were aspirated from pleural, pericardial and peritoneal cavities using a sterile needle, and collected in clean, sterile and dry vials kept in ice. A minimum of 20 ml of the sample was taken and divided into two. One part was centrifuged at 3000 g for 20 min, and the supernatant was used for ADA estimation and the pellet for DNA extraction. The other part was used for AFB smear preparation by Ziehl-Neelsen staining and culture on Lowenstein-Jensen medium.

Samples of 2–5 ml of gastric aspirate were collected on three consecutive days without lavage, decontaminated with 4% sodium hydroxide (equal volume), incubated at 37°C for 45 min and neutralised with 15% sulphuric acid. Thereafter, samples were centrifuged at 3000 g for 20 min and pellets were sent for AFB staining, culture and PCR processing.

Fluid ADA assay was carried out according to the method described by Giusti.¹¹ Fluid absolute lymphocyte count (ALC) was calculated from fluid total cell count and percentage of lymphocytes present in the fluid, and expressed as per mm³.

DNA extraction and PCR

DNA isolation was carried out by the classic phenol-chloroform method to obtain pure DNA mixture, and single tube-nested PCR was carried out to detect a sequence of IS6110 as described by Chan *et al.*¹²

Statistical analysis

Student's t test was used to analyse the data for statistical significance. The sensitivity, specificity, predictive values and accuracy of the tests were calculated using SPSS version 10.0.

RESULTS

The age of patients ranged from 2 to 14 years and included 12 (39%) female and 19 (61%) male patients. The participants in the non-tuberculous group were 8 months to 13.5 years of age, and included 8 (33%) girls and 16 (67%) boys.

Fluid and gastric aspirate culture were positive in 2 (6%) and 1 (3%) patients with tuberculous effusions. Pleural biopsy was performed in two patients with empyema thoracis not responding to adequate treatment with antibiotics and chest-tube drainage, who responded to antitubercular treatment and hence were included in the tuberculous group. Lymph node biopsy from cervical lymph nodes was suggestive of tubercular lymphadenitis in three patients with tuberculous effusions. Thus, 8 (26%) of 31 cases could be diagnosed as confirmed tuberculous effusion by these methods.

The mean (standard deviation (SD)) fluid ADA level in tuberculous effusions (56.1 (21.3)) was significantly higher ($p < 0.001$) than in the non-tuberculous group (29.4 (14.7)). Of the 31 patients, 25 (81%) patients with tuberculous effusions had levels ≥ 38 IU/l, whereas 6 (25%) of the 24 in the non-tuberculous group had values above this level, with sensitivity and specificity at 81% and 75%, respectively (fig 1). The mean (SD) value of fluid ALC was also significantly higher ($p < 0.001$) in tuberculous effusions (536 (277)) than in non-tuberculous effusions (235.2 (211.2)). By taking a cut-off value of $\geq 275/\text{mm}^3$, the test had a sensitivity and specificity of 90% and 83%, respectively (fig 2).

PCR was positive in 23 (74%) patients with tuberculous effusions, whereas it was falsely positive in 3 (13%) patients

Table 2 Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of individual diagnostic methods for tuberculous effusions

Groups	PPD	BCG	Fluid culture	Fluid ALC	Fluid ADA	Fluid PCR
Confirmed (n = 8)						
Sensitivity	63	75	25	88	100	100
Specificity	92	92	100	83	75	88
PPV	71	75	100	64	57	73
NPV	88	92	80	95	100	100
Accuracy	83	88	81	84	81	91
Probable (n = 23)						
Sensitivity	39	74	0	91	74	65
Specificity	92	92	0	83	75	88
PPV	82	90	0	84	74	83
NPV	61	79	0	91	75	72
Accuracy	66	83	0	87	75	77
Total (n = 31)						
Sensitivity	45	74	7	90	81	74
Specificity	92	92	100	83	75	88
PPV	88	92	100	88	81	89
NPV	56	73	45	87	75	72
Accuracy	76	82	47	87	78	80

ADA, adenosine deaminase assay; ALC, absolute lymphocyte count; BCG, Bacillus-Calmette Guerin; NPV, negative predictive value; PCR, polymerase chain reaction; PPD, purified protein derivative; PPV, positive predictive value.
Values are percentages.

Table 3 Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of combined (either/or) tests for tuberculous effusions

Groups	ADA+ or PCR+	ADA+ or ALC+	PCR+ or ALC+	PPD+ or ADA+	PPD+ or PCR+	PPD+ or ALC+
Confirmed (n=8)						
Sensitivity	100	100	100	100	100	88
Specificity	67	67	75	71	79	79
PPV	50	50	57	53	62	58
NPV	100	100	100	100	100	95
Accuracy	75	75	81	78	84	81
Probable (n=23)						
Sensitivity	87	100	96	87	87	100
Specificity	67	67	75	71	79	79
PPV	71	74	79	74	80	82
NPV	84	100	95	85	86	100
Accuracy	77	83	85	79	83	89
Total (n=31)						
Sensitivity	90	100	97	90	90	97
Specificity	67	67	75	71	79	79
PPV	78	80	83	80	85	86
NPV	84	100	95	85	86	95
Accuracy	80	86	87	82	85	89

ADA, adenosine deaminase assay; ALC, absolute lymphocyte count; BCG, Bacillus-Calmette Guérin; NPV, negative predictive value; PCR, polymerase chain reaction; PPD, purified protein derivative; PPV, positive predictive value.
Values are percentages.

of the non-tuberculous group. Therefore, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the test were 74%, 88%, 89%, 72% and 80%, respectively. Gastric aspirate PCR was positive in two patients with tuberculosis (sensitivity 7%); one sample was also positive for AFB culture.

Table 2 shows the comparative utility of different tests applied for the diagnosis of tuberculous effusions. The sensitivity of fluid PCR and ADA was 100% in the confirmed group, with an overall sensitivity of 74% and 81%, respectively. The fluid ALC had a sensitivity of 88% in confirmed cases, with an overall sensitivity of 90%. The sensitivity of the combined tests, ADA and PCR positivity, was 100% in the confirmed group, with an overall sensitivity of 90%. Overall,

Figure 1 Individual fluid adenine deaminase assay (ADA) levels in different study groups. Short horizontal lines indicate mean values; horizontal dotted line indicates cut-off level. Asc., ascites; Emp., empyema thoracis; Malig., malignant pleural; Per., pericardial; Pl., pleural; Pyo., pyopericardium; Trans., transudative ascites; Tub., tuberculous.

Figure 2 Individual fluid absolute lymphocyte count (ALC) values in different study groups. Short horizontal lines indicate mean values; horizontal dotted line indicates cut-off level. Asc., ascites; Emp., empyema thoracis; Malig., malignant pleural; Per., pericardial; Pl., pleural; Pyo., pyopericardium; Trans., transudative ascites; Tub., tuberculous.

the purified protein derivative test, in combination with ALC showed the best diagnostic utility, with a sensitivity of 97% and a specificity of 79% (table 3).

Table 4 shows the combination of the two methods, requiring both to be positive for the diagnosis of tuberculous effusions. The overall specificity of all the tests increased at the expense of sensitivity. The sensitivity of PCR and ADA positivity was 100% in the confirmed group. The positive predictive value showed marked improvement (92–96%), whereas accuracy was almost similar to that of individual tests (78–80%).

Table 4 Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of combined (both) tests for tuberculous effusions

Groups	ADA+ and PCR+	ADA+ and ALC+	PCR+ and ALC+	PPD+ and ADA+	PPD+ and PCR+	PPD+ and ALC+
Confirmed (n=8)						
Sensitivity	100	88	88	63	63	63
Specificity	96	92	96	96	100	96
PPV	89	78	88	83	100	83
NPV	100	96	96	89	89	89
Accuracy	97	91	94	88	91	88
Probable (n=23)						
Sensitivity	52	65	61	26	17	30
Specificity	96	92	96	96	100	96
PPV	92	88	93	86	100	88
NPV	68	73	72	58	56	59
Accuracy	74	79	79	62	60	64
Total (n=31)						
Sensitivity	65	71	68	36	29	39
Specificity	96	92	96	96	100	96
PPV	95	92	96	92	100	92
NPV	68	71	70	54	52	55
Accuracy	78	80	80	62	60	64

ADA, adenosine deaminase assay; ALC, absolute lymphocyte count; BCG, Bacillus-Calmette Guérin; NPV, negative predictive value; PCR, polymerase chain reaction; PPD, purified protein derivative; PPV, positive predictive value.
Values are percentages.

DISCUSSION

The diagnosis of tuberculosis in children is difficult and often delayed. Bacterial evidence of infection, especially positive smears, is rarely obtained. Amplification of *M tuberculosis*-specific IS6110 in clinical specimens is the most sensitive method. AFB staining lacks sensitivity and this was also reflected in our study. Fluid cultures showed the presence of *M tuberculosis* in 6% of cases. This was in accordance with the findings of a previous study with a culture positivity of <20%.⁴

The fluid mean ALC was significantly higher in patients with tuberculous effusion than in controls ($p<0.001$). However, the difference was not significant when compared with malignant pleural effusions. By taking a cut-off value of $\geq 275/\text{mm}^3$, the test was found to be useful, with high sensitivity (90%) and specificity (83%) for the diagnosis of tuberculous effusions, as emphasised by Burgess *et al.*¹³ Hence, this test can be an useful adjunct to the diagnosis, as it is simple and inexpensive.

Previous studies have shown significantly higher levels of ADA in effusions of tuberculous aetiology, which had a sensitivity of 91–100% and a specificity of 81–100%.^{8, 13, 14} ADA is predominantly a T lymphocyte enzyme, and its activity increases in diseases where cell-mediated immunity is stimulated.¹³ In tuberculous effusions, both absolute number as well as T lymphocytes are significantly higher in fluids than in peripheral blood, and this could explain the increased fluid ADA activity as well as ALC.¹⁵

In this study, fluid PCR was positive in 74% of the tuberculous cases and 13% of the non-tuberculous cases. Nagesh *et al.*⁴ also reported almost similar sensitivity (70%) but higher specificity (100%) in their study. However, some workers had reported both higher sensitivity (94–100%) and specificity (88–100%) for fluid PCR in the diagnosis of tubercular effusions.^{10, 16} In contrast, Reechaipichitkul *et al.*¹⁷ observed lower sensitivity (50%) and specificity (61%) of PCR in the pleural fluid of tuberculous aetiology. The low sensitivity found in our study may be because of the paucibacillary nature of tuberculous effusions in children, or strains of *M tuberculosis* causing disease in Asia, having a single copy or lacking the gene sequence IS6110 completely.¹⁸ The false positive results with PCR have been reported earlier,

What is already known on this topic

- Fluid polymerase chain reaction has variable sensitivity and specificity in the diagnosis of adult tuberculous effusions.
- Fluid adenosine deaminase assay is increased in tuberculous effusions.

What this study adds

- Fluid polymerase chain reaction (PCR) alone has limited utility in diagnosing tuberculous effusions in children.
- Combined evaluation of PCR with adenosine deaminase assay or absolute lymphocyte count is preferable.

and it could be because of contamination of extraneous DNA or detection of non-viable organism present in patients already treated for tuberculosis.^{19, 20} However, our false positive cases did not have any history of tuberculosis in the past, and adequate precautions were taken to avoid any cross-contamination during laboratory processing. The fluid PCR was less sensitive but more specific than fluid ADA level in diagnosing tuberculous effusions in our study, which was similar to the observations reported by Lima *et al.*²¹

The observations of different tests in combination for their diagnostic utility showed that the combination ADA and ALC was most useful, followed by the combination PCR and ALC. The conventional tests, such as fluid staining and culture, purified protein derivative or BCG test, showed a lower sensitivity pattern (0–74%) and were less useful. Thus, it seems that neither PCR nor ADA activity is useful as a single test in diagnosing tuberculous effusions. Hence, it would be a rational approach to use these tests in combination as an adjunct to clinical presentation for rapid and precise diagnosis in children.

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