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Diagnostic Value of Real Time PCR for Neurotuberculosis

The absolute diagnosis of tuberculosis in children is often difficult and challenging because combination of a typical clinical picture with demonstration of *Mycobacterium tuberculosis* from the secretions and tissues is not possible in majority of children, who have a paucibacillary disease. Real-time PCR methods, based on hybridization of amplified nucleic acids with fluorescent-labelled probes, as evaluated in adults, has shown sensitivity of 71 to 98% and specificity close to 100%(1-3). We conducted this study to compare the diagnostic value of real time technique with conventional PCR technique (IS6110), culture (Bac T/Alert), and biochemical and cytological studies in CSF, for diagnosis of neurotuberculosis in children.

We enrolled 47 children (0-18 years) with neurotuberculosis, between March 2008 to October 2009. Another 8 patients with non tuberculous involvement of the same system were taken as matching disease controls. Inclusion and exclusion criteria were as per IAP guidelines(4). A written informed consent was taken from patients' attendants before the commencement of the study.

After a detailed clinical history and a thorough clinical examination, a complete blood count, tuberculin test, chest radiograph and CSF examination were done in all patients. CT scan of head/USG cranium was done where feasible. After processing , the CSF sample was divided in four parts and they were subjected to cytological and biochemical analysis, culture on Bac T/ALERT 3D system, PCR targeting IS6110 in thermal cyclers (Applied Biosystems) and real time PCR targeting 16SrRNA using light cycler RNA amplification syber green 1 kit (Roche applied biosciences, Germany).

Majority of our cases and controls were less than 4 years of age. The mean age in study and control groups were 4.4 ± 3.5 years and 5.0 ± 4 years, respectively. Mantoux test was positive in 30% (n=14) of cases; all the controls were mantoux negative. 53.1% (n=25) of cases had positive history of contact. 32.7% (n=15) of cases and 75% (n=6) of controls had received BCG vaccination. The mean duration of illness was 37.6 ± 27.4 days in cases and 26.2 ± 20.8 days among controls.

***Table* I** shows the sensitivity and specificity of the four methods. Statistically, real time PCR showed significantly better results than the other tests, including PCR targeting IS6110 (P<0.05).

TABLE I SENSITIVITY AND SPECIFICITY OF VARIOUS DIAGNOSTIC METHODS

S. No	Tests	Total cases	Positive cases	Total controls	Positive controls	Sensitivity (%)	Specificity (%)
1	Cytology and biochemistry	47	18	8	0	38.3	100
2	BacT/Alert	47	23	8	0	48.9	100
3	PCR IS6110	47	39	8	0	82.9	100
4	Real time PCR 16SrRNA	47	46	8	0	97.8	100

The present study, thus shows a good promise for using Real Time PCR targeting 16SrRNA to diagnose neurotuberculosis in the pediatric population. It is of particularly greater value in developing countries where the burden of the disease is high and early diagnosis is crucial to prevent mortality and morbidity. We conclude that real time PCR technique is highly sensitive and specific in diagnosing neurotuberculosis in children.

Contributors: RD and PS participated in acquisition of data and manuscript preparation, PS, VMK and DSC participated in concept, design and critical review, RD, PS and NKY participated in analysis, interpretation of data and manuscript preparation, RD and VMK participated in final approval of the version to be published.

Funding: None.

Competing interests: None stated.

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