

Diagnostic value of Line Probe Assay in Childhood Tuberculosis

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Abstract: Objective: This study was conducted in children with tuberculosis (TB) to compare the diagnostic yield of Genotype LPA assay and its comparison with MGIT culture in various body fluids and also to detect drug resistance using LPA. Methods: Children < 18 years diagnosed as TB were included in the study. Body fluids obtained from these subjects were processed for LPA assay and MGIT culture and . Drug resistance was detected using LPA. Results: 80 TB cases were registered .LPA in showed a high sensitivity of 91.1 % and specificity of 75 % .High MDR TB (44 %) was detected using LPA. Conclusion: Genotype LPA can serve as a reliable tool of clinical utility for diagnosis of TB and detection of drug resistance.

Keywords: Tuberculosis , Genotype LPA, MGIT , MDR TB

1. Introduction

Tuberculosis (TB) is among top ten causes of death in children worldwide. According to WHO Global Tuberculosis Report 2013^[1] an estimated 530,000 TB cases were reported among children under 15 years of age with India alone accounting for 26% of cases. Diagnosis of Tuberculosis (TB) in children is often challenging due to vague clinical manifestations and paucibacillary nature, making conventional methods less sensitive^[2]. Serological tests are currently not recommended for clinical use^[3]. PCR is more useful with sensitivity ranging from 70% - 90% in different studies^[4].

Apart from TB diagnosis, MDR TB is an emerging threat to TB management. According to WHO (2013), 3.6% of newly diagnosed TB cases and 20% of those previously treated for TB had MDR-TB. So a quick and reliable test is required, which can diagnose and simultaneously detect drug resistance. GenoType LPA was endorsed by WHO in 2008 as a tool to identify MTB and to detect resistance to RMP and INH. Hence, this study was conducted to assess the clinical utility of GenoType LPA in detecting mycobacterium TB in comparison to MGIT culture and detect INH and RMP resistance in children.

2. Material and Method

This prospective study was conducted in the department of pediatrics, S.N. medical college, Agra and National JALMA Institute for Leprosy and Other Mycobacterial Diseases (I.C.M.R.), Agra from May 2011 to October 2013. Subjects <18 years diagnosed as TB using national guidelines on pediatric TB, 2012 were included in the study^[5]. A written informed consent was taken from guardians. The study was approved by the ethical committee of the institute.

Clinical details were recorded for all the study subjects. Complete blood count, Chest X Ray and tuberculin test were done in all subjects. CT scan head, USG abdomen were done where required. At least 5ml of various body fluids (CSF, gastric aspirate, sputum, pleural fluid, ascitic fluid and lymph node aspirate) were subjected to microscopy, cytological and biochemical examination .MGIT culture and line probe assay was performed in all the specimens.

GenoType Line Probe Assay was done according to the protocol using DNA extraction, multiplex PCR amplification and reverse hybridization^[6].

Statistical Analysis

Sensitivity and specificity of the tests were calculated .Chi square test was applied to compare the results of various tests.

3. Results

A total of 80 specimens consisting of sputum (n = 13), gastric aspirate (n = 8), CSF (n = 44), ascitic fluid (n=7), pleural fluid (n=6) and LN aspirate (n = 2) from study subjects were processed. The clinical profile of the subjects is given table 1. Age of the subjects ranged from 1 month to 18 years (mean \pm 4.74 years) with male: female ratio of 1.6:1. History of contact was present in 43.75% . 27.5% of cases had received BCG vaccination. Most of study subjects were undernourished (63.15% < 5 years had < -2 SD and 59.52% > 5 year had BMI < -2 SD). Tuberculin test was positive in 33.75% of cases. Comparison of sensitivity and specificity of Genotype LPA, MGIT culture in various body fluids are shown in table 2. Overall sensitivity of LPA was 67.5%, followed by MGIT culture (51.25%) in various body fluids. The LPA assay showed sensitivity of 91.11 % and specificity of 75% in culture positive cases. The positive and negative predictive values were 75.9% and 90.6%, respectively.

LPA showed maximum positivity in gastric aspirate (87.5%) . In CSF diagnostic yield of LPA was 61.36% which was more than MGIT (43.18%). LPA was significantly superior to culture in CSF (p<0.05) but showed comparable results in other body fluids (p>0.05). Out of LPA positive cases 44.45% cases showed multidrug resistance, 37.03% cases were monodrug resistance. MDR TB was most common in pulmonary TB (35.71%) cases and in previously treated cases (18.75%).

4. Discussion

The most common form TB in our study was Tubercular meningitis followed by pulmonary tuberculosis, as the

subjects were from tertiary hospital. Most subjects (78.75 %) were <8 years of age group as also observed by Roelsgaard et al⁽⁷⁾ and majority were males (61.25%) as also reported by Seth et al⁽⁸⁾. Only 27.5 % of patients had received BCG vaccine which could be probable explanation for more number of severe cases of TB. Tuberculin test was positive in 33.75% ,which was lower than 52.3% reported by Udani et al. ⁽⁹⁾. This could be due to co-existent malnutrition and more severe forms of TB in the study subjects.

In our study diagnostic value of LPA was compared to MGIT culture. Overall sensitivity of MGIT culture was 51.25% which was more than that reported in prior studies (34.10% and 41%) respectively^[10,11] . The LPA assay yielded a sensitivity of 91.11% and a specificity of 75% in culture positive cases which was better than sensitivity 82.2% and specificity 66.7% reported by Hale et al⁽¹²⁾ .This could probably be due to use of newer Genotype LPA assay.

This study demonstrated a remarkably high drug resistance .Multidrug resistance was more common as also seen in another study^[13] .Resistance was more common in previously treated cases ,similar has been reported by Alrajhi et al. ^[14].The results of assay could not be compared with standard Drug resistance testing which is one of the limitations of the study.

The assay is simple, convenient, and highly reliable method with sensitivity of 93% and specificity of 75% for identification of Mycobacterium tuberculosis within 24 to 48 hour. The results show a very high multidrug resistance in children with TB needing further studies.

Table 1: Clinical profile of study subjects

Parameter		Number	Percentage
Age	1 mo – < 4 yr	25	31.25%
	4 - <8 yr	38	47.5%
	8 - <12 yr	12	15%
	>12 yr	5	6.25%
Sex	Male	49	61.25%
	Female	31	38.75%
Positive contact history	Positive	35	43.75%
	Negative	45	56.25%
History of previous ATT intake	Present	15	18.75%
	Absent	65	81.25%
Nutritional status	< -2 SD (< 5 yr)	24	63.15%
	> 2 SD (< 5 yr)	14	36.85%
	BMI < - 2 SD (>5 yr)	25	59.52%
	BMI > 2 SD (>5 yr)	17	40.48 %
BCG	Vaccinated	22	27.5%
	Unvaccinated	58	72.5%
Mantoux	Positive	27	33.75%
	Negative	53	66.25%
Duration of illness	<15 days	12	15%
	> 15 days	68	85%
Type of tuberculosis	Pulmonary TB	28	35%
	CNS TB	44	55%
	Abdominal TB	6	7.5%
	Tubercular lymphadenopathy	2	2.5%

Table 2: Comparison of MGIT Culture and LPA in various body fluids

Sample Type	MGIT Culture Positive N(%)		LPA Positive N(%)		P Value
CSF (N=44)	19	43.18%	27	61.36%	0.003
G.ASPIRATE (N=8)	4	50%	7	87.5%	> 0.05
SPUTUM (N=13)	9	69.23%	10	76.92%	>0.05
PLEURAL FLUID(N=7)	3	42.85%	4	57.14%	>0.05
ASCITIC FLUID (N=6)	4	66.66 %	5	83.33%	>0.05
LN-ASPIRATE (N=2)	2	100%	1	50%	>0.05
TOTAL (N= 80)	41	51.25%	54	67.5%	>0.05

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