Journal of Epidemiology and Global Health 7 (2017) 141–145

Contents lists available at ScienceDirect

Journal of Epidemiology and Global Health

journal homepage: www.elsevier.com/locate/jegh

Drug resistance detection and mutation patterns of multidrug resistant tuberculosis strains from children in Delhi

Jyoti Arora [a], Ritu Singhal [a], Manpreet Bhalla [a], Ajoy Verma [a], Niti Singh [a], Digamber Behera [b], Rohit Sarin [c], Vithal Prasad Myneedu [a,*]

[a] Department of Microbiology, National Institute of Tuberculosis and Respiratory Diseases, Sri Aurobindo Marg, New Delhi, India
[b] Department of Pulmonary Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India
[c] Department of TB and Chest, National Institute of Tuberculosis and Respiratory Diseases, Sri Aurobindo Marg, New Delhi, India

ARTICLE INFO

Article history:
Received 1 June 2016
Received in revised form 26 November 2016
Accepted 15 December 2016
Available online 7 February 2017

Keywords:
Drug resistance
Line probe assay
Retreatment cases
Smear positive
Tuberculosis

ABSTRACT

A total of 312 sputum samples from pediatric patients presumptive of multidrug resistant tuberculosis were tested for the detection of drug resistance using the GenoTypeMTBDRplus assay. A total of 193 (61.8%) patients were smear positive and 119 (38.1%) were smear negative by Ziehl–Neelsen staining. Line probe assay (LPA) was performed for 208 samples/cultures (193 smear positive samples and 15 cultures from smear negative samples). Valid results were obtained from 198 tests. Of these, 125/198 (63.1%) were sensitive to both rifampicin (RIF) and isoniazid (INH). 73/198 (36.9%) were resistant to at least INH/RIF, out of which 49 (24.7%) were resistant to both INH and RIF (multidrug resistant). Children with tuberculosis are often infected by someone close to them, so strengthening of contact tracing in the program may help in early diagnosis to identify additional cases within the household. There is a need to evaluate newer diagnostic assays which have a high sensitivity in the case of smear negative samples, additional samples other than sputum among young children not able to expectorate, and also to fill the gap between estimated and reported cases under the program.

© 2017 Ministry of Health, Saudi Arabia. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Childhood tuberculosis (TB) is one of the major causes of childhood morbidity and mortality. An estimated 74,000 children die from TB each year and account for around half a million new cases annually worldwide. It is estimated that childhood TB constitutes 10–20% of all TB in high-burden countries [1].

In 2013, 63,919 pediatric TB cases were notified in India, under the Revised National Tuberculosis Control Program (RNTCP) [2]. Although child TB in India is estimated to be approximately 10% of the total adult incidence, only 6% of the total cases reported to the program are children. A large number of children with TB remain undiagnosed each year which makes it difficult to assess the actual magnitude of the childhood TB epidemic. Dodd and colleagues [3,4] modeled TB infection and estimated a prevalence of 50 million infected children. India is predicted to account for 27% of the total burden of pediatric TB in 22 countries with high disease burden. They interpreted that far more drug-resistant TB occurs in children than is diagnosed. According to their estimates, 850,000 children developed TB in 2014; 58,000 with isoniazid (INH) monoresistant TB, 25,000 with multidrug resistant (MDR) TB, and 1200 with extensively drug resistant (XDR) TB.

The challenges in diagnosing TB in children include the paucibacillary nature of the disease in many, difficulty to obtain a sample from young children, and low sensitivity of the commonly used smear microscopy technique. A molecular diagnostic test, GenotypeMTBDR plus Line probe assay (LPA; Hain Life Sciences, Nehran, Germany) was introduced in the RNTCP in 2011. The method is based on nucleic acid amplification directly from smear positive pulmonary specimens, permitting rapid detection of mutations in genes coding for resistance to rifampicin (RIF) and INH (Hain test). With LPA, turnaround time to diagnose MDR TB among smear positive pulmonary samples has decreased markedly [5,6].

Although the role of pediatric TB in the transmission of disease may be lower than that of adult patients, pediatric TB can be a reservoir which constitutes a significant number of future adult cases. Therefore, the epidemiology of the disease in children reflects the efficiency of the control programs and also enables bet-

Peer review under responsibility of Ministry of Health, Saudi Arabia.

* Corresponding author at: Department of Microbiology, National Institute of Tuberculosis and Respiratory Diseases, Sri Aurobindo Marg, New Delhi 110030, India.

E-mail address: tbmicro@gmail.com (V.P. Myneedu).

http://dx.doi.org/10.1016/j.jegh.2016.12.003
2210-6006/© 2017 Ministry of Health, Saudi Arabia. Published by Elsevier Ltd.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

ter predictions of the resources required for management of children with TB/DR TB.

Since there is limited data on TB among children and its genetic determinants, the present study was conducted at the National Institute of Tuberculosis and Respiratory Diseases, National Reference Laboratory (NRL), to determine the proportion of MDR TB among South Delhi children presumptive of MDR and to detect associated mutations in *rpoB*, *katG*, and *inhA* genes using the GenoType MTBDRplus assay.

2. Materials and methods

2.1. Study setting

Delhi has an area of 1483 sq km, with a total population of 17 million and population density of 11,000/sq km. For the management of RNTCP, the state has been divided into 24 chest clinics. Under each chest clinic, there is one TB unit for half a million population having a designated microscopy center for every 0.1 million population. The Department of Microbiology, National Institute of Tuberculosis and Respiratory Diseases receives samples of presumptive MDRs from six South Delhi districts (population – 25 lakhs) for performing LPA under the program. A total of 312 samples from children ⩽15 years were received from six districts of South Delhi and outpatient departments during October 2011 to December 2013.

2.2. Patient demographic details and inclusion criterion

Sociodemographic characteristics of the study population were sourced from the referral for culture drug susceptibility testing (DST) forms and laboratory register. The data included age, sex, type of TB, and the presumptive MDR criterion of the patient.

The criteria for presumptive MDR-TB under the national program were: treatment failures among new TB cases, smear positive cases that remained smear positive after the 4th month of treatment with retreatment regimen, and pulmonary TB cases who were contacts of known MDR-TB cases (Criterion A). Criterion B included any smear positive follow up or smear positive retreatment case at diagnosis in addition to criterion A. Criterion C included smear negative retreatment cases and all HIV/TB co-infected cases at diagnosis in addition to Criteria A and B. Children who fulfilled the criteria for presumptive MDR-TB were screened in the peripheral DOT (Directly Observed Treatment) centers by medical officers and lab technicians, and referred to the lab for diagnosis.

2.3. Sample collection processing

Sputum samples (spot and morning) were collected from each patient in 50 mL wide-mouthed sterile falcon tubes. All specimens were screened for the presence of acid fast bacilli by Ziehl–Neelsen staining. The samples were processed by the N-acetyl-L cysteine–sodium hydroxide method [7]. Smear positive samples were subjected to LPA directly from processed samples. All smear negative samples were inoculated in MGIT 960 liquid culture tubes. Tubes which flashed positive were subjected to smear microscopy and to immune-chromatographic assay for detection of the mpt64 antigen to confirm the presence of the *Mycobacterium tuberculosis* complex. These cultures were further subjected to LPA.

2.4. LPA

All smear positive samples and smear negative culture positive isolates were subjected to the Genotype MTBDR V 2.0 plus assay as per the manufacturer's instructions. Each LPA strip had five control zones (conjugate, amplification, and a locus control each for *rpoB*, *katG*, and *inhA* genes). The test was considered as invalid in the case of a missing amplification band in a negative test result due to the presence of inhibitors or mistakes during amplification set up.

For RIF susceptibility determination, there were eight *rpoB* wild-type (WT1–WT8) and four mutant probes (MUT1 D516V, MUT2A H526Y, MUT2B H526D, and MUT3 S531L). For INH susceptibility determination, *katG* WT with two mutant probes (MUT1 S315T1 and MUT2 S315T2), and two *inhA* WT with four mutant probes (MUT1 C15T, MUT2 A16G, MUT3A T8C, MUT3B T8A) were present. Either missing of the WT band or the presence of a mutant band was taken as an indication of a resistant strain. The presence of all WT probes with no signal from the mutant probe was considered as sensitive. The presence of all wild type probes along with the presence of one or more mutant bands was considered as hetero resistant.

3. Results

3.1. Acid fast bacilli microscopy and culture

A total of 312 children who fulfilled the criteria of presumptive MDR and whose sample was sent for DST were analyzed for the study. The distribution patterns regarding age groups (years) and male to female sex-ratios were: 0–5 $n = 2$ (1/1); 6–10 $n = 43$ (11/32 or 0.34); 11–15 $n = 267$ (58/209). The mean age of patients was 13 years with 85.5% (267/312) of children in the age group of 11–15 years. Overall, there were more females than males with a male to female sex-ratio of 70/242 or 0.28 (Table 1). The proportion of cases between 0–15 years was 4–6% (312/5663) of the total presumptive MDR cases received for culture and DST from different districts (Table 1). All patients were retreatment cases: Category (Cat) I failure ($n = 13$); Cat II failure ($n = 4$); retreatment cases before starting Cat II treatment ($n = 91$); any follow up positive during Cat I or Cat II treatment ($n = 89$), smear negative retreatment cases ($n = 101$); contact of MDR patients ($n = 1$); and no information regarding criterion ($n = 13$).

Of the total patients, 193 (61.8%) were smear positive and 119 (38.1%) were smear negative. Smear positivity was higher among the 11–15 year age group (179/267; 67%) followed by those aged 6–10 years (24/43; 55.8%). Of 119 smear negative samples, 15 (12.6%; 5 boys and 10 girls) were culture positive for *M. tuberculosis* complex. Of these there was one patient each of 9 and 12 years of age, two patients each of 10, 11 and 13 years, three patients of 15 years and four patients of 14 years of age.

3.2. Drug susceptibility and mutation patterns

Samples/cultures of 208 children were subjected to LPA (193 directly from samples and 15 from culture). Valid results were obtained from 198 tests. Ten invalid samples were either scanty ($n = 4$) or 1+($n = 6$). Of the 198, 125 (63.1%) were sensitive to both RIF and INH; 49 (24.7%) were found to be resistant to RIF and INH, 6 (3.0%) as mono-RIF resistant, and 18 (9.1%) as mono-INH resistant. In all, 27.8% (55/198) children were resistant to RIF (Table 1).

The mutation pattern for RIF and INH resistance using GenoTypeMTBDRplus is presented in Table 2. Among 55 RIF resistant strains, the commonest mutation was at codon S531L of the *rpo*B gene (41/55; 74.5%) followed by H526Y (3/55; 5.5%) and D516V (2/55; 3.6%). In 10 (18.2%) RIF resistant strains, resistance was determined by absence of one or more wild type probes with no gain in mutant probe. Hetero-resistance to RIF was found in seven samples (12.7%), with S531L being the most common mutation.

Download English Version:

https://daneshyari.com/en/article/5662965

Download Persian Version:

https://daneshyari.com/article/5662965

Daneshyari.com