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RIFAMPICIN RESISTANT PAEDIATRIC TUBERCULOSIS: PREVALENCE AND RISK FACTORS

Microbiology

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ABSTRACT

Rifampicin resistance in children reflects the intensity of ongoing transmission of DRTB in community. This study was undertaken to determine the prevalence of rifampicin resistant tuberculosis and the common risk factors in paediatric patients which is important from epidemiological point of view.

210 children with clinically suspected tuberculosis were enrolled in the study. Pulmonary and/or extrapulmonary specimens submitted to the lab were processed for fluorescent microscopy, solid culture and Xpert MTB/RIF assay and risk factors were noted.

12 specimens showed unsatisfactory results in Xpert assay or culture hence excluded from analysis. Of the 198, 22(11.11%) children were microbiologically confirmed for tuberculosis. Positivity in male and female children was 10.81% and 11.49% respectively. 154 were pulmonary and 44 were extrapulmonary specimens with positivity of 3.89% and 27.27% respectively. As a standalone test, microscopy, culture and Xpert assay detected MTB in 8 (4.04%), 19 (9.59%) and 20 (10.10%) of the cases respectively. Rifampicin resistant was observed in seven (31.81%) cases.

Among confirmed cases, 11 had history of contact with sputum positive case, 6 had malnutrition, four were living in overcrowded and inadequately ventilated area. Univariate analysis showed contact with sputum positive cases, malnutrition, overcrowding and inadequate ventilation significant. Multivariate analysis showed contact with sputum positive cases and malnutrition as significant. Contact with sputum positive case, overcrowding and inadequate ventilation were significant risk factors in seven children diagnosed with RRTB.

Increasing prevalence of rifampicin resistant tuberculosis detected in paediatric cases is alarming. Greater emphasis on modifying risk factors such as close contact and malnutrition will help the TB program to prevent transmission in community.

KEYWORDS

Paediatric tuberculosis, prevalence, rifampicin resistance, risk factors.

INTRODUCTION:

Tuberculosis [TB] caused by *Mycobacterium tuberculosis*, is a major health concern worldwide. According to Annual TB report of India published in 2020, around 3,42,000 incident cases are estimated to occur every year which accounts for 31% of the global burden and 13% of the overall TB burden in the country. In 2019, around 1,51,286 (only 44% of estimated) cases of paediatric TB were notified in India. In 2018, globally around 11 lakh children became infected with TB and 2,50,000 children died of TB. It has been one of the top 10 causes of childhood mortality [1]. Multi drug resistant TB [MDRTB] is resistance to most common anti-tuberculosis drugs, isoniazid and rifampicin. Rifampicin resistance is considered as a good and reliable proxy for MDR-TB in high burden setting. Because of diagnostic difficulties and exclusion of children in most of the drug resistant survey, there are no exact estimates of overall burden even though it is documented in paediatric age group [2].

Various factors play role in predisposing a child to easily acquire TB. Poor cell mediated immunity in younger children allows proliferation of bacilli with progressive parenchymal lung damage and dissemination [3]. Malnutrition, close contact with sputum smear positive cases, overcrowding in the house, poor housing & inadequate ventilation, Vitamin D deficiency in children, infection with HIV, steroid therapy, cancer chemotherapy, hematologic malignancies may contribute as a risk factor. Infections such as measles, varicella, pertussis may activate quiescent TB [4,5].

Rifampicin resistance tuberculosis [RRTB] develops mainly in adult patients with high bacillary load who receive inadequate antituberculosis treatment or are poorly compliant with treatment. Children usually acquire drug resistant TB from close contacts, hence primary drug resistant TB is common in paediatric cases. Also, history of antituberculosis treatment is easy to exclude in children. Rifampicin resistance in children reflects the intensity of ongoing transmission of DRTB in community. If drug resistance in such cases is detected earlier, appropriate treatment can be started and mortality can be reduced. Understanding the common risk factors resulting in increasing prevalence of MDR TB is important both from management and programmatic point of view. Hence this study will be conducted to determine the prevalence of RRTB in paediatric patients and to know the common risk factors in children leading to RRTB.

MATERIALS AND METHODS:

Prospective study was carried out in department of Microbiology at a tertiary care hospital after obtaining institutional ethics committee permission (IEC-II reference No EC/236/2016).

Consecutive 210 clinically &/or radiologically suspected paediatric TB cases (up to 14 years of age) submitting two sputum or gastric lavage specimen and extrapulmonary specimen at least 2 ml quantity were included in the study. Patients already on anti-tuberculosis treatment were excluded from the study. All processing was carried out in a class IIA biosafety cabinet with level 2 biosafety practices. All specimens were processed for fluorescent microscopy, Xpert MTB/RIF assay and culture on Lowenstein Jensen medium followed by drug susceptibility test for rifampicin using proportion method. Clinical details and findings of various investigations were recorded from patient's file.

Risk factors such as close contact with sputum positive cases within last 2-3 years, malnutrition (arm circumference &body weight), HIV status of the patient, ongoing chemotherapy with immunosuppressants or steroids, environmental conditions were asked for and noted in the case record form.

Microscopy:

All specimens were subjected to microscopy of both direct and concentrated smears. Sterile body fluids were concentrated by centrifuging at 3000 rpm for 15 minutes and the deposit was used for microscopy. Sputum specimen and highly contaminated specimens such as pus were decontaminated by NALC-NaOH method and subsequently concentrated [6]. Both direct and concentrated smears were stained by fluorescent stain and screened for the acid-fast bacilli. Specimens were considered positive if either of the direct or concentrated smears showed fluorescent acid-fast bacilli [7].

Culture [6]:

Two loopful of the deposit obtained by NALC-NaOH method were

inoculated on each of two slants of Lowenstein Jensen (LJ) medium which were incubated aerobically at 37^{0}C. Cultures were observed daily for one week for contamination and up to 8 weeks for any visible growth suggestive of mycobacteria. The isolate was characterized as MTBC or NTM based on phenotypic characteristics and presence or absence of MPT64 antigen using "SD BIOLINE TB Ag MPT 64 Rapid®" kit [8]. A slow growing, buff coloured, acid fast isolate, positive for MPT64 was identified as MTBC.

Rifampicin susceptibility testing by Proportion method:
All MTBC isolates obtained on LJ medium were tested for rifampicin (40µg/ml) susceptibility by proportion method according to the protocol laid down by Canetti *etal* [9].

Readymade LJ medium containing rifampicin (Himedia) was used. Drug containing as well as drug free LJ media were incubated at 37^{0}C. The culture bottles were examined for contamination for one week. On the 28th day, both drug containing media as well as drug free media were checked for any growth. If no growth was observed in drug containing medium, it was incubated further and the second reading was taken on the 42nd day as the final reading.

Interpretation of the test:
Susceptible strain: Colonies of a mycobacterial strain seen on drug free LJ media but not on drug containing media.

Resistant strain: A strain showing number of colonies on drug containing media which are more than 1% (the critical proportion) of number of colonies on drug free media.

Xpert MTB/RIF assay:
Xpert assay was performed as per manufacturer's instructions [10]. Cartridges and sample reagent were first brought to room temperature. Sample reagent was added carefully in a ratio of 3:1 (Sample Reagent: pellet) to the original specimen container. The lid was tightened and mixed properly by shaking vigorously followed by incubation at room temperature for 15-20 minutes till the specimen was completely liquefied. Using the pipette, minimum 2 ml pellet – reagent mixture was added to the sample chamber of the cartridge, taking care to avoid bubble formation. The lid was closed properly. The cartridge was loaded in the machine. The results generated were recorded. Negative results are generated as "MTB NOT DETECTED". Positive results are generated as" MTB DETECTED" as well as" RIF RESISTANCE DETECTED / NOT DETECTED/ INDETERMINATE.

Statistical analysis was performed using Microsoft Excel. Fisher's exact test and logistic regression analysis were used for statistical analysis. A p value of less than 0.05 was considered significant.

RESULTS:
Consecutive 210 specimens from same number of paediatric patients of clinically and / or radiologically suspected PTB or EPTB, submitting required quantity of specimen and satisfying the inclusion criteria were enrolled in the study. 12 specimens were excluded from analysis as they either showed contamination in culture or error in Xpert assay that could not be retested. 198 patients showing valid results of all tests were considered for analysis.

Out of 198 children, 111 (56.06%) were male children and 87 (43.93%) were female children. 85 (42.92%) children were less than 5 years and 113 (57.07%) children were between 6-14 years. TB positivity in male and female children was 10.81% and 11.49% respectively. Positivity was higher in age group 6-14 years (14.15%) as compared to total positivity in age group 0-5 years (7.05%).

All specimens were categorized according to nature of specimen and analyzed for positivity by different tests performed (table 1). 154 were respiratory specimens and 44 were extrapulmonary specimens. Specimen was considered as positive for TB if any of the above three microbiological tests is positive. 22 (11.1%) specimens tested positive by one or more of the tests. TB was detected by fluorescent microscopy in eight (4.04%) specimens, culture in 19 (9.59%) specimens and Xpert assay in 20 (10.10%) specimens. All specimens positive by microscopy were also positive by culture and Xpert assay. In comparison to culture, microscopy had a sensitivity & specificity of 42% and 100% respectively.

Table 1- Specimen wise detection of MTB

Specimen	Total Number (%)	Total positives (%)	Culture positives (%)	Xpert assay positives (%)	Microscopy positives (%)
Gastric lavage	66 (33.33)	2 (3.03)	1 (1.51)	2 (3.03)	1 (1.51)
Sputum	88 (44.44)	8 (9.09)	8 (9.09)	8 (9.09)	4 (4.54)
Sterile body fluid	36 (18.18)	8 (10.10)	6 (16.66)	6 (16.66)	1 (2.77)
Pus	7 (3.53)	3 (42.85)	3 (42.85)	3 (42.85)	2 (28.57)
Tissue	1 (0.50)	1 (100)	1 (100)	1 (100)	0
Total	**198**	**22(11.11)**	**19(9.59)**	**20(10.10)**	**8(4.04)**

Of the 198 TB suspects included in the study, 19 were culture positive (9.59%) by solid culture. Twenty (10.10%) specimens were positive by Xpert assay. Discordance between culture and Xpert assay result was observed in five i.e., three (two cerebro-spinal fluids and one gastric lavage) were culture negative Xpert assay positive and two (pleural fluids) were culture positive and Xpert assay negative. The sensitivity of Xpert assay when compared to culture as gold standard was 89.47% and specificity was 98.32%. Positivity in extrapulmonary samples such as sterile body fluids (10.10%), pus (42.85%) and tissue (100%) were more than pulmonary specimens such as sputum (9.09%) and gastric lavage (3.03%).

Overall prevalence of paediatric tuberculosis was 11.1% and of rifampicin resistant tuberculosis was 3.5%. Seven specimens showed rifampicin resistant result by Xpert assay. Concordant results were obtained by proportion method of DST in all seven specimens except in one where proportion DST was not performed as no growth was observed on culture.

Various risk factors were analyzed for their association with paediatric tuberculosis. Univariate analysis showed contact with sputum positive cases, malnutrition, overcrowding and inadequate ventilation were associated with acquisition of TB. Multivariate analysis showed only contact with sputum positive cases and malnutrition to be associated risk factors (p value <0.05). (Table 2)

Table 2: Association of various risk factors in paediatric tuberculosis

Risk factors	Total	MTB detected (n=22)	MTB not detected (n=176)	Univariate analysis	Multivariate analysis
Close contact with sputum positive cases	Present- 23 (11.61%)	11 (47.82%)	12 (52.17%)	0.001 (<0.05)	0.001
	Absent- 175 (88.38%)	11 (6.28%)	164 (93.71%)		
BCG vaccination	Present- 197 (99.49%)	22 (11.16%)	175(88.38 %)	1 (>0.05)	-
	Absent- 1 (0.50%)	0	1 (100%)		
Malnutrition	Present- 8 (4.04%)	6(75%)	2(25%)	0.001(<0.05)	0.007
	Absent- 190 (95.95%)	16 (8.42%)	174 (91.57%)		
Indoor air pollution	Present-4 (2.02%)	1(25%)	3(75%)	0.378 (>0.05)	-
	Absent- 194 (97.97%)	21 (10.82%)	173 (87.37%)		
Overcrowding in house	Present- 11(5.55%)	5 (45.45%)	6(54.54%)	0.003 (<0.05)	0.458
	Absent- 187 (94.44%)	17 (9.09%)	170 (90.90%)		
Inadequate ventilation	Present- 6(3.03%)	4 (66.66%)	2(33.33%)	0.002(<0.05)	0.526
	Absent- 192 (96.96%)	18 (9.37%)	174 (90.62%)		
Others	Present- 2(1.01%)	1(50%)	1(50%)	(>0.05)	

	Absent-196 (98.99%)	21 (10.71%)	175 (89.28%)		

Among seven children of RRTB, risk factors such as close contact with sputum positive case, overcrowding, inadequate ventilation and malnutrition were observed and found significant (p value < 0.05) except malnutrition (p value 0.25).

DISCUSSION

MDRTB is a major problem in elimination of tuberculosis. Primary as well as secondary DRTB is common in adult. Children usually encounter primary MDR TB by coming in close contact with infectious family member. Its further progression to clinical disease and development of complications depends on the immunity of the child. Associated risk factors may facilitate transmission and delay recovery of the child. As treatment of MDRTB is of longer duration, it is a task to sustain the compliance of the child. Children in the house or in the neighbourhood may share the common risk factors resulting in easy spread of MDRTB. Hence, knowing the prevalence of RRTB and studying various risk factors is necessary to formulate guidelines for paediatric tuberculosis.

In the present study, MTB was detected in 22(11.11%) patients either by microscopy or culture or Xpert assay, of which seven (3.5%) patients had strain resistant to rifampicin. As a standalone test, microscopy, culture and Xpert assay detected MTB in 4.04%, 9.59% and 10.10% of the cases respectively. Other studies done on paediatric TB cases shows positivity rate ranging from 5.44% to 14% by microscopy, 16.2% to 30.66% by culture and 10.36% to 33.33% by Xpert assay [11-14, 15-18].

All smear positive cases were positive by culture and Xpert assay in both pulmonary and extrapulmonary specimens. Smear grading correlated very well with the bacterial load in Xpert assay. Five specimens showed discordance in culture and Xpert assay. Two specimens (pleural fluids) were culture positive and Xpert assay negative which may be due to the better level of detection by culture as it detects 10–100 organisms per ml of specimen, whereas Xpert assay has a limit of detection (as defined by 95% sensitivity) of 131 bacilli per ml of sputum [10].Three specimens (cerebrospinal fluids and gastric lavage) were culture negative and Xpert assay positive which may be due to over decontamination while processing specimens for culture or uneven distribution of mycobacteria in the specimen resulting in absence of growth on culture medium. It has also been reported that the sample reagent provided with the Xpert assay is a better liquifying agent as compared to NALC-NaOH. [19] In the present study, H37Rv strain was used as quality control for culture. A suspension of the same also underwent digestion and decontamination for each batch of specimens as decided for other specimens for verifying the decontamination protocols. Another reason for culture negativity could be use of solid LJ medium instead of the more sensitive liquid culture (MGIT 960) system.

Concordant results for rifampicin resistance were observed between Xpert assay and proportion method of DST in all cases except in one where proportion DST was not performed as no mycobacterial growth was observed on culture.

Univariate analysis showed close contact with sputum smear positive cases, malnutrition, overcrowding in house and inadequate ventilation as significant risk factor associated with tuberculosis. Also, same risk factors were observed and found significant in children with RRTB except malnutrition. Multivariate logistic regression considering TB as a dependent factor and individual risk factor as independent variable was constructed, which showed close contact and malnutrition as a significant risk factor.

Close contact is defined as "living in the same household or in frequent contact with a source case (e.g., care giver) with sputum smear positive TB. Source cases who are sputum smear negative but culture-positive are also infectious, but to a much lesser degree." [20] In the present study, 23 (11.61%) children had close contact with sputum positive patients in the same household. Out of those, eleven children (47.8%) had tuberculosis which was found significant (p value < 0.001) as an individual factor. These children had close contact with index case for duration ranging from 2 to 3 years.

Exogenous factors influencing transmission of TB from close contact includes the quantity of bacilli expelled by the index patient, environment into which the organisms are dispersed, and duration of exposure to the infectious patient. Transmission occurs most frequently to contacts who live in a close environment and have spent prolonged periods with index case. The children sleeping in the same room as the index patient are more likely to get infection [21].

Malnutrition is defined as "a pathological state resulting from a relative or absolute deficiency or excess of one or more nutrients. It comprises undernutrition which results when insufficient food is eaten over an extended period of time." [22]. To evaluate malnutrition in children, arm circumference was measured and body weight was recorded from case paper to decide level of nutrition. In the present study, 8(4.04%) patients were having malnutrition out of which 6 (75%) developed tuberculosis.

Having active TB leads to loss of weight, and being underweight is considered as a risk factor for developing TB, either through reactivation of latent TB or developing progressive primary disease upon infection, which shows bidirectional relationship between nutrition and TB [23]. Decreasing appetite and changes in metabolic processes in TB disease can itself lead to malnourishment which results in further spread and progression of disease [24].

Routine TB risk assessment among acutely malnourished children, would help increase TB case finding and improve outcomes for children with TB and undernutrition by improving linkages with TB services [25].

Overcrowding refers to the situation where more people are living in a single dwelling than there is a space for, so that movement is restricted, privacy secluded, hygiene impossible, rest and sleep difficult.' [22] Overcrowded housing conditions have the potential to increase exposure of susceptible people to those with infectious respiratory disease, and may increase the probability of transmission. It is possible that communities with overcrowded housing also experienced a higher prevalence of latent tuberculosis infection [21].

In present study, overcrowding was observed in 11 (5.55%) children, out of which 5(45.45%) children developed TB which was statistically found as a significant risk factor (p value 0.003). Studies done by Clark (2002) and Lienhardt et al (2003) suggested that TB incidence was higher in communities with a higher average housing density [26,27]. Study done by Taifur Rahman et al showed that children who were living in an over-crowded room (64%) (>4 persons in a room) were 2.64 times more likely to be in the TB case group as compared to the children who were living in a less crowded room (44%) (<=4 persons in a room) [28].

Poorly ventilated living conditions favors TB transmission. [29, 30] Standards of ventilation are defined as, in living rooms there should be 2 or 3 air changes per hour whereas in work rooms or assemblies it should be 4-6 air changes per hour. Doors and windows facing each other provide cross ventilation [22]. Ventilation provides outdoor air into a room, dilute the pollutants originating in the room, and provide an airflow rate to change this air at a given rate [31].

M. tuberculosis is carried in airborne particles of 1– 5 microns in diameter called as droplet nuclei which are generated when persons who have pulmonary or laryngeal TB disease cough, sneeze or shout. These tiny particles can remain suspended in the air for several hours in closed environment facilitating transmission [32].

Few limitations of the study are use of solid culture instead of both solid and liquid culture. HIV status of all children was not known. In few children, quality of sputum was salivary or inappropriate.

Diagnosis of paediatric tuberculosis is challenging because of nonspecific clinical features and difficulty in specimen collection for laboratory diagnosis. As this study shows discordance between Xpert assay and culture in few samples, use of more than one laboratory test may be helpful for more accurate and rapid diagnosis in paediatric tuberculosis as TB in children reflects the ongoing transmission in the community. The risk factors which have been studied in the present study are modifiable and if more awareness is created among people, can prevent major part of population from acquiring TB and reduce the overall incidence of TB in community.

CONCLUSION-

Compared to the national average, the prevalence of TB in paediatric suspects in the present study is low. However, the higher proportion of rifampicin resistant in paediatric TB cases is a cause for concern. Close contact and malnutrition were observed as most frequently associated risk factors. NTEP should emphasize more on early screening of close contacts of diagnosed TB cases and improve nutrition in paediatric age group to prevent transmission and acquisition of TB.

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