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# **Original Research Article**

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# Incidence of rifampicin resistance, HIV status and efficacy of fluid analysis among tuberculosis suspect pediatric cases

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#### **ABSTRACT**

Background: Body fluids are commonly used for diagnosis as sputum is not reliable in children. Hence it is essential to study efficacy of body fluids in comparison to GeneXpert which is a new diagnostic modality. Objectives of this study was to incidence of rifampicin resistance, HIV status and efficacy of fluid analysis among tuberculosis suspect pediatric cases.

Methods: Present study was hospital based cross sectional study carried out over a period of two years at Department of Pediatrics, ACPM Medical College and Hospital from February 2016 to January 2018 among children with suspected tuberculosis. Rifampicin resistance was determined by using GeneXpert. Body fluids like CSF, pleural fluid, gastric aspirate etc were analyzed and compared with GeneXpert results.

Results: Rifampicin resistance was present in 5 cases i.e. 3.3%. 15.15% Patients were HIV positive and in them 12 were GeneXpert positive with 1 Rif resistance. Different Fluid analysis showed maximum positivity with pleural fluid >TB Lymphadenitis >TBM. GeneXpert done on different body fluids showed extra case detection in different fluid analysis negative patients i.e. 28.6% extra case detection in CSF (2 cases), 87.9% in gastric aspirate (25 cases), 85.4% in induced sputum (35 cases), 14.3% in Lymph node aspirate (1 case), 50% in pleural fluid (2 case).

Conclusions: : Rifampicin resistance found in present study is alarming. Among body fluids, FNAC, CSF and pleural fluids can be used reliably for diagnosis of tuberculosis where GeneXpert is not available.

**Keywords:** Body fluids, Diagnosis, FNAC, HIV status, Resistance, Tuberculosis

# **INTRODUCTION**

Prevalence of tuberculosis infection among pediatric age group is for 0 to 4 years is 1.0%, 5 to 9 years is 6.4%, and 10 to 14 years is 15.4%. About 5 per cent of those infected are likely to develop disease in the first year after infection and the remaining 5 per cent during their lifetime. Nearly 8-20% of the total deaths caused by TB, occur in children.1

Disease progression after infection occurs within 12 months in majority of children who develop disease. Therefore, most childhood TB reflects ongoing transmission, and the pattern of drug resistance observed in children, provides an accurate estimate of primary drug resistant TB within communities. Children can therefore be considered a sentinel population, with childhood TB incidence as an indicator to measure the effectiveness of TB control programs.<sup>2</sup>

TB can occur in any site in children, but mostly affect lungs (pulmonary TB). Following inhalation, MTB bacilli may disseminate to other organs via hematogenous route, causing extra-pulmonary TB (EPTB). Cervical lymphadenitis is the most common type of extra-pulmonary TB in children, followed by tuberculous meningitis. Most severe form of EPTB is Miliary TB and tuberculous meningitis. Other extra-pulmonary sites are less commonly described including pleura, genitourinary, skin, larynx, eyes, middle ear, and intestinal tract etc.<sup>3</sup>

Children may also acquire infection with M. bovis through the drinking of unpasteurized milk and "BCG-osis" that may occur in children with HIV following BCG vaccination. Due to this reason BCG vaccination is not recommended in HIV positive children.<sup>4</sup>

In children with HIV infection, the clinical evaluation for TB is even more challenging because it is difficult to differentiate the TB-related symptoms from those caused by other HIV associated conditions and opportunistic infections.<sup>5</sup>

Most important factor for diagnosis is appropriate specimen. Induced sputum (IS) and gastric aspirate are most commonly used. It is collected using hypertonic saline to irritate the airways to induce cough and obtain material from lung airways or by taking gastric aspirate.<sup>6</sup>

Present study attempts to study the incidence of rifampicin resistance among children and HIV status. At the same time, we attempted to study the value of fluid analysis for TB diagnosis in children in comparison to GeneXpert test.

#### **METHODS**

Present study was hospital based cross sectional study carried out over a period of two years at Department of Pediatrics, ACPM Medical College and Hospital among 150 study subjects from February 2016 to January 2018 among children with suspected tuberculosis. Rifampicin resistance was determined by using GeneXpert. Body fluids like CSF, pleural fluid, gastric aspirate etc. were analyzed and compared with GeneXpert results.

## Inclusion criteria

- Suspected cases of tuberculosis meningitis
- With or without de-cerebrate posturing
- Willing to participate in the study

#### Exclusion criteria

- Cases with severe morbidity which made them bed ridden
- Not willing to participate in the study

CSF analysis of all suspected TBM cases giving history of fever with convulsions, headache, apathy, having neck

rigidity may or may not present with or without decerebrate posturing, with or without H/O contact was done. CSF was collected under all aseptic precautions through lumber puncture needle (as per the age) at the level of L3-L4 or L4-L5 space. Sample was sent for cytology and biochemistry and one sample in falcon was sent for GeneXpert. Very high Protein level (as per the age), with high cell counts having lymphocytic predominance (>50% of total counts) and low sugar level (< 60% of parallel serum sugar) in CSF gives suspicion of TBM.

Pleural fluid analysis was done in cases of suspected TB effusion giving H/O fever, cough and distress. On auscultation air entry was much reduced with dull node on percussion and X-ray S/O effusion. Diagnostic pleural fluid tapping has been done by using 20 gauze needles, 5-10 cc fluid was collected and sent for proteins, sugar TLC and DLC, ADA and culture. One sample was also sent for GeneXpert. Total protein >3.5 with lymphocytic predominant cells suggest Exudative fluid, most commonly TB as a suspicion.

FNAC (Fine needle aspiration cytology) procedure was performed in all suspected TB lymphadenitis (having >2 cm, non-tender lymph nodes, matted or non-matted) by using 23 gauze needles, under all aseptic precautions and cytological analysis was done. One sample was also sent for GeneXpert. Cytological evaluation showing granulomatous appearance gives most commonly a diagnosis of TB lymphadenitis.

In all TB cases (diagnosed by GeneXpert), Rif Resistance has been checked and treatment has been started with AKT regimen for 6 months to 12 months (on the basis of category and site involved) along with supportive treatment. All the patients who has Rif resistance and all negative GeneXpert cases with highly suspicious of TB on clinical basis have been sent to higher centre in Pune or Nagpur by RNTCP to STDC Centre i.e. State TB Training and Demonstration center where 2 cultures i.e. LPA and LJ Culture is sent for the patient and diagnosed as TB and Resistance pattern was being checked. Treatment was started on the basis of Resistance pattern (like mono-resistant, multidrug resistance or extensively drug resistant TB). Data was entered in the Microsoft Excel worksheet and analyzed using proportions, chi square test.

## **RESULTS**

Table 1 shows rifampicin resistance status in GeneXpert positive suspected TB patients. It was found from the above table in the present study that rifampicin resistance was present in 3.3% of suspected patients who were suspected to be having tuberculosis meningitis.

It was found from the above table in the present study that rifampicin resistance was present in 96.7% of

suspected patients who were suspected to be having tuberculosis meningitis.

Table 2 shows HIV status in suspected tb patients. It was found from the above table in the present study that 10% were HIV positive suspected patients who were suspected to be having tuberculosis meningitis. It was found from the above table in the present study that 90% were HIV negative suspected patients who were suspected to be having tuberculosis meningitis.

Table 3 shows relation of history of Koch's contact to rifampicin resistance in GeneXpert positive suspected tb patients. it was found from the above table in the present study that rifampicin resistance was present in 4.3% of suspected tb patients without history of Koch's contact while it was found from the above table in the present study that resistance was not noted in those with history of Koch's contact, there was statistically no significant (p

>0.05) difference of rifampicin resistance with Koch's contact history in suspected tb patients.

Table 1: Rifampicin resistance status in GeneXpert positive suspected tb patients.

Rifampicin resistance	Number	Percentage
Yes	5	3.3
No	145	96.7
Total	150	100

Table 2: HIV status in suspected TB patients.

HIV status	Number	Percentage
Positive	15	10
Negative	135	90
Total	150	100

Table 3: Relation of history of Koch's contact to rifampicin resistance in genexpert positive suspected TB patients.

Rifampicin resistance					— Total	■ Total		
Koch's contact	Yes		No		Total		Fisher's —— exact test	P value
Contact	Number	%	Number	%	Number	%	exact test	
Yes	0	0	34	100	34	100		
No	5	4.3	111	95.7	116	100	1.516	0.218
Total	5	3.3	145	96.7	150	100		

Table 4: Relation of Sputum and ZN AFB positive to Rifampicin resistance in GeneXpert positive suspected TB patients.

7N	Rifampicin	resistance			Total		Fisher's	
ZN staining	Yes		No		Total	Total		P value
stanning	Number	%	Number	%	Number	<b>%</b>	exact test	
Positive	3	30	7	70	10	100		
Negative	2	1.4	138	98.6	140	100	23.645	< 0.001
Total	5	3.3	145	96.7	150	100		

Table 5: Relation of HIV status to Rifampicin resistance in GeneXpert positive suspected TB patients.

THE	Rifampicin	resistance			— Total	Fisher's		
HIV status	Yes		No		Total	Total		P value
status	Number	%	Number	%	Number	%	exact test	
Positive	1	6.7	14	93.3	15	100		
Negative	4	3	131	97	135	100	0.575	0.414
Total	5	3.3	145	96.7	150	100		

Table 4 shows relation of Sputum and ZN AFB positive to Rifampicin resistance in GeneXpert positive suspected TB patients. It was found from the above table in the present study that 30% patients were ZN positive and It was found from the above table in the present study that 1.4% with ZN negative for AFB had rifampicin resistance. There was statistically very highly significant (p<0.001) difference of Rifampicin Resistance within

the ZN staining status so that it was more with positive ZN staining.

Table 5 shows relation of HIV status to Rifampicin resistance in GeneXpert positive suspected TB patients. It was found from the above table in the present study that 6.7% TB suspected HIV positive patients and It was found from the above table in the present study that 3%

of HIV negative were Rifampicin Resistant. There was statistically no significant (p>0.05) difference of Rifampicin Resistant according HIV status of suspected TB patients staining.

Table 6 shows body Samples evaluated in suspected TB patients. In the present study CSF sample was evaluated in 22.7% of the cases. It was found out from the above table that the Gastric aspirate was evaluated in 22% of the cases. In the present study 14% of the studied cases had lymph-node aspirate done.

It was found from the above table that 11.3% of the cases had pleural fluid analysis. In the present study induced sputum was done in 30% of suspected TB patients to confirm the diagnosis. Table 7 shows comparison of TB positivity by fluid Analysis and by GeneXpert. When CSF sample positivity was compared with GeneXpert it was found that both the tests were able to detect equal

number of TB positive cases. Similar findings were seen in comparison of pleura fluid analysis with GeneXpert. But GeneXpert gave 84.4% and 87.9% more yield in case of induced sputum and gastric aspirate respectively. Only FNAC of lymph nodes positivity was slightly more compared to GeneXpert.

Table 6: Body Samples evaluated in suspected TB patients.

Sample type	Number	Percentage
CSF	34	22.7
Gastric aspirate	33	22
Lymph node aspirate	21	14
Pleural fluid	17	11.3
Induced sputum	45	30
Total	150	100

Table 7: Comparison of TB positivity by fluid Analysis and by GeneXpert.

Sample type	Total number	TB positive as per sample test	TB positive as per GeneXpert	% extra diagnosed by GeneXpert
CSF	34	27 (79.4%)	27 (79.4%)	0
Pleural fluid	17	13 (76.5%)	13 (76.5%)	0
FNAC of lymph nodes	21	14 (66.7%)	12 (57.1%)	-9.6%
Induced sputum	45	4 (8.9%)	38 (84.4%)	75.5%
Gastric aspirate	33	0	29 (87.9%)	87.9%

#### **DISCUSSION**

In this study total 5 patients out of 119 GeneXpert positive were found to have Rif Resistance which is a very significant finding. 3 out of 5 Rif Resistant cases were ZN AFB positive, 1 patient had HIV Co-infection and 1 patient had H/O Koch's contact positive.

In a cross-sectional observational study conducted by Shah I and Chilkar S in Mumbai in children, of 500 children analyzed, 34 (6.8%) had drug resistant TB. Mean age of presentation was 6.8±3.2 years. (M:F ratio 13:21). Eighteen (52.9%) children had been treated for tuberculosis in the past (1 defaulted), 7 patient had been contact with an adult suffering from drug resistant TB and 3 patients (10.3%) were HIV co-infected.<sup>7</sup>

In a study by Seddon JA et al. in Cape Town, South Africa, a total of 294 children with a median age of 26 months (range 3 days-13 years) were diagnosed with culture-confirmed TB. DST results were available for 292 (99.3%); 41 (14%) were INH-resistant, including 26 (8.9%) with MDR-TB. Four children (1.4%) had RMP mono-resistance.<sup>8</sup>

In a study by Lapphra K et al, in Thailand, among 230 children diagnosed with TB, the median age was 6.5 years, of the 195 (85%) specimen submitted, 57 (25%) were positive using culture or polymerase chain reaction. Of the 53 positive specimens available for drug susceptibility testing (DST), 18 (34%) had any resistance, 13 (24.5%) were mono-resistant, 2 (3.8%) poly-resistant and 3 (5.7%) were multidrug resistant.<sup>9</sup>

This shows that emergence of drug resistance especially in pediatric age group is a critical scenario coming nowadays. To find cases and to treat them appropriately is a difficult task. Culture is the gold standard for it, but it is a cumbersome process not available in small settings and also gives result very late. So, GeneXpert is a novel diagnostic modality, which does not require much setting or expertise, and gives result within 2 hours in OPD itself with Rif resistance pattern. So, we can diagnose them, find them, categorize them (to send for culture) to know the resistance pattern and treat them appropriately and cut the vicious cycle.

In the present study, different body fluid sample have been used on the basis of site involved, suspecting type of tuberculosis. Like for suspected Pulmonary TB cases, we have taken gastric aspirate (in smaller children <5 year age) and Induced sputum (in elder children >5 year age) and ZN staining had been done. In suspected TBM, CSF cytology and biochemistry was done. In suspected TB lymphadenitis, FNAC was done. So, CSF sample was evaluated in 22.7%, Gastric aspirate in 22%, 14% had lymph-node aspirate done, 11.3% had pleural fluid analysis while induced sputum was done in 30% of suspected TB patients to confirm the diagnosis. Results were suggesting that by clinical diagnosis maximum positivity was present in Pleural fluid examination i.e. 76.5% followed by FNAC examination suggesting 66.7% positivity. CSF analysis shows 21.3% positivity and Induced sputum ZN staining showed only 8.9% positive ratio. There was not any single case present positive with Gastric aspirate ZN staining. Friedrich et al, Study done on Pleural fluid diagnosis suggested good sensitivity of Pleural fluid examination. 10 Lightelm et al, study done on FNAC sample also showed 84.8% sensitivity of it.<sup>11</sup>

Other studies showed good sensitivity and specificity of Induced sputum and Gastric aspirate AFB sensitivity. 12-13 In this study sensitivity is very low because of errors in taking samples; problems with its storage may be there.

In comparison of the GX positivity with the type of sample evaluated it was found that, GeneXpert was positive in 87.9% in gastric aspirate analysis, 84.4% induced sputum analysis, 57.1% within lymph node aspirate, 76.5% in pleural fluid analysis and 79.4% in CSF analysis. So as compare to other fluid analysis the case detection rate has increased. Like in CSF two patients were fluid analysis negative found positive with GeneXpert, same as with others, 29 cases which were missed in GA analysis were GX positive. 35 patients were positive in IS with GX as compare to fluid analysis. One more case detected with GX along with FNAC and 2 more cases were detected in pleural fluid GX. Case detection has been drastically increased upon using GeneXpert.

#### **CONCLUSION**

Rifampicin resistance found in present study is alarming. Among body fluids, FNAC, CSF and pleural fluids can be used reliably for diagnosis of tuberculosis where GeneXpert is not available. Gastric aspirate and induced sputum cannot be relied upon for diagnosis of tuberculosis among children.

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Institutional Ethics Committee

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