Efficacy of gene Xpert over other diagnostic modalities of tuberculosis among children

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ABSTRACT

Background: Diagnosis of tuberculosis is a challenge especially among children. GeneXpert has been recommended as a diagnostic test in children. Objectives of this study was to efficacy of GeneXpert over other diagnostic modalities of Tuberculosis like Sputum smear microscopy, Mantoux testing, X-ray chest among children.

Methods: A cross sectional hospital-based study was conducted over a period of 24 months among 150 children. All the patients who were having suspicion of Tuberculosis on the basis of History & Examination (fulfilling inclusion criteria) had been enrolled in the study. After doing all preliminary investigations clinical diagnosis has been made and Gene X’pert was carried out for all the samples collected. Pearson chi square test and Fishers exact test was applied wherever appropriate.

Results: There was statistically no significant (p >0.05) difference of GeneXpert positivity within different age groups of Suspected TB patients. GeneXpert was positive in 80% with symptom of Cough lasting more than 2 weeks, in 78.8% with fever more than 2 weeks, in 88.9% with FTT, in 76.5% with H/O Koch’s contact, in 77.8% with H/O convulsion, in 69% with significant lymphadenopathy. GeneXpert was positive in all suspected TB patients having ZN staining positive for AFB. In clinically TB diagnosed patients, 86.5% were positive for GeneXpert.

Conclusions: GeneXpert is a novel diagnostic modality of choice in all suspected Pulmonary & Extra-pulmonary TB cases among children. It can be used as a primary tool in Pulmonary TB with smear negative samples in pediatric age group.

Keywords: Children, Diagnostic modalities, Efficacy, Genexpert, Tuberculosis

INTRODUCTION

Tuberculosis (TB) is a pandemic that causes serious morbidity and mortality. According to the Global Tuberculosis report 2014 of W.H.O., Tuberculosis (TB) remains one of the deadliest communicable diseases in the world.¹

India is the country with highest burden of TB. WHO estimates in 2013 revealed that, India alone shares the incidence of 2.1 million (24%) cases per year (one fourth of global incidence), out of the estimated global annual incidence of 9 million TB cases. WHO estimates in 2013, revealed that children accounts over a half a million new cases annually and up to 80000 children die every year from TB.³

Since most children acquire the infection from adults living in the same environment, the epidemiology of
childhood TB follows that in adults. Because of the difficulty in diagnosing and confirming the diagnosis, the global burden of childhood tuberculosis in the world is unclear. Another important reason is that children do not make a significant contribution to the spread of tuberculosis. Available data which links the incidence of TB to case load suggest that children may constitute nearly 40 percent of the case load in certain high incidence communities.6

Tuberculosis is currently said to be the leading cause of death among the curable infectious diseases.3

Diagnosis of TB is a challenging health care issue due to its nonspecific symptoms and radiological signs. Hence diagnosing TB as early as possible is the milestone for its eradication.6

Although Culture is the gold standard for diagnosing TB, it is time-consuming, can take 2-8 weeks and a cumbersome process. Acid-fast bacilli (AFB) detection by smear microscopy is a fast and cheap, but it has low sensitivity and positive predictive value (PPV).7

There has been lot of attempts to come up with a method which is cheap, rapid, affordable, and accurate in the diagnosis of TB. GeneXpert is a Nucleic Acid Amplification test; it has proven to satisfy all the required factors and has been successfully used in TB diagnosis, due to the affordability, rapidity and accuracy. GeneXpert MTB/RIF were approved by the WHO in 2011 and recommended for diagnosis of TB and MDR-TB in developing and high prevalence countries. GeneXpert MTB/RIF is a real time PCR (RT-PCR)-based molecular assay that amplifies a specific sequence of rpoB gene in Mycobacterium tuberculosis (MTB) and detects RIF resistance mutations as a marker for MDR-TB.8

The reaction takes place in a single-use cartridge easy to operate, without cross contamination, and giving a result in as early as 2 hours and thus decreasing default due to delayed diagnosis.9

GeneXpert could detect MTB in all smear-positive clinical samples and approximately 75% of smear-negative samples. Although there are many GX evaluation studies, the information is still limited in size and geographic representation.10

Hence present study was carried out to study the efficacy of GeneXpert over other diagnostic modalities of Tuberculosis like Sputum smear microscopy, Mantoux testing, X-ray chest.

METHODS

The study was approved by ethical committee MUHS in 2015. This study was conducted in the department of pediatric and child health unit in Tertiary health care centre in Dhule. A cross sectional hospital based study has been conducted over a period of 24 months from November 2015 to October 2017. Total sample size was 150 patients.

Inclusion criteria

1. All suspected cases of tuberculosis in Paediatric age group (2 months-17 yrs.)
2. Giving written informed consent from parents
   - Any Two of the following
   - History of contact
   - Unexplained weight loss/failure to thrive: 5% reduction in weight compared with the previous weight of 3 months
   - Persistent (> 2 weeks) of unexplained fever
   - Persistent (> 2 weeks) of cough and rhinorhea
   - X-ray suggestive of TB
   - Significant Lymphadenopathy
   - Clinical suspicion of TBM

Exclusion criteria

1. People who are not giving consent for GeneXpert
2. People who are already taking AKT for > 2 months

Procedure

All the patients who were having suspicion of Tuberculosis on the basis of History and Examination (fulfilling inclusion criteria) had been enrolled in the study. Procedure had been explained to the parents and written informed consent has been taken from them.

Detailed History of the patient regarding present illness, significant past illness (having multiple hospitalization for the similar type complaint), immunization history (mostly BCG) with a history of Koch’s contact was noted. Thorough examination of the patients including vitals, general examination including thorough lymph nodes examination and whole systemic examination was carried out. Complete Anthropometry has been noted and plotted under growth chart (WHO or IAP). BCG scar was being noted. Preliminary tests like Complete Hemogram was done using five-part differential cell counter, ESR using Westergeen’s method, HIV testing has been done in all patients and if age is < 6 months, mothers testing has been done. Chest X-ray (PA view) of all the patients was done.

DLC (leucocytosis), TLC (lymphocytic predominance), raised ESR as per the age and method criteria and X-ray Chest showing infiltrates, cavitations, Para-hilar lymphadenopathy, Primary complex (any of the above) has been suggested as suspicion of Tuberculosis. In all suspected Pulmonary TB patients, after doing initial investigations as described above, other Investigations performed like induced sputum/gastric lavage ZN AFB test by Cold Method in all the patients (smaller the child, gastric lavage has been taken and older the child induced
sputum has been taken). To collect the sample for induced sputum (early morning sample), Hypertonic saline nebulization was given to the baby and allow baby to cough and the sample was collected in sterile container and sent for ZN staining for two consecutive days and the same sample in Falcon tube was being sent for GeneXpert. To collect sample for Gastric aspirate, at least 6 hr. fasting at night was needed and at early morning one feeding tube was inserted in stomach, checked its presence by 5 cc syringe. Then gastric aspirate was collected in sterile container and Falcon tube (at least 1 ml for each) and sent for ZN staining and for GeneXpert.

Mantoux testing of all patients has been done with 2 TU (RT-23) PPD injected intra-dermal in left forearm volar aspect at junction of upper 1/3rd and lower 2/3rd and results were noted after 48 hour and 72 hours in form of induration at the site, > 10 mm induration for immune-competent and >5 mm for HIV co-infected was considered as positive Mantoux test.

After doing all preliminary investigations clinical diagnosis has been made. After clinical diagnosis, we also did Gene X’pert for all the samples collected. It tells about MTB detected or MTB not detected. So, confirmed diagnosis was made as TB and Non-TB by using Gene X’pert. Gene X’pert (CBNAAT) is a newer diagnostic modality. We Used version 4, model 2012, Cepheid Company for our study. It is a molecular assay, a real time PCR (RT-PCR) that amplifies a specific sequence of rpoB gene in Mycobacterium tuberculosis (MTB) and detects RIF resistance as well within 2 hours. Technique is very simple and required less experience. Other important profit is that we can make diagnosis by using number of samples of body fluids or tissue like CSF, sputum, gastric lavage, pleural fluids, biopsy specimens etc. to diagnose TB but having different sensitivity.

The manual operation has only 3 steps: 1) mix sputum sample with sample reagent; 2) transfer the mixed solution to a test cartridge; 3) put the cartridge into GeneXpert test platform and get results in 2 hrs. Thus, this assay can be introduced to the areas where highly trained lab technicians are scarce. X’pert Cartridge is a closed-system vessel. It is a disposable unit with necessary reagents for real time PCR. Inside the cartridge, the specimen is isolated. PCR inhibitors are removed, and specimens are ultrasonically lysed. After the sample is processed, it is mixed with reagents and moved into the integrated reaction tube. The reaction tube is automatically inserted into the I-CORE module when the cartridge is loaded into the instrument.

The data was analyzed using proportions and appropriate statistical tests

RESULTS

Almost 150 patients were included in my study having suspicious of TB as per the criteria, from November 2015 to October 2017 of total 24 months duration. Data has been collected and all necessary tests were performed with permission and after taking consent. Diagnosis is made and the treatment has been started.

Table 1 shows relation of age group to GeneXpert. In suspected TB patients, GeneXpert positivity was in 72.7% of up to 1 year patients, 80% of those in 1 to 5 years, 77.3% within 5 to 10 years, 79.5% in 10 to 15 years and 100% more than 15 years of age. There was statistically no significant (p > 0.05) difference of GeneXpert positivity within different age groups of Suspected TB patients.

Table 2 shows relation of Clinical findings to GeneXpert. GeneXpert was positive in 80% with symptom of Cough lasting more than 2 weeks as well as 77.5% in those without the cough more than 2 week duration. In patients suspected of TB due to fever lasting more than 2 weeks 78.8% were GeneXpert positive and 21.2% were negative. In patients suspected of TB due to FTT, 88.9% were GeneXpert positive and 11.1% were negative. In patients suspected of TB due to H/O Koch’s contact, 76.5% were GeneXpert positive and 23.5% were negative. In patients suspected of TB due to H/O convulsion, 77.8% were GeneXpert positive and 22.2% were negative. In patients suspected of TB due to significant lymphadenopathy 69% were GeneXpert positive and 31% were negative. All these findings were statistically not significant.

Table 1: Relation of age group to genexpert.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>GeneXpert Negative</th>
<th></th>
<th>GeneXpert Positive</th>
<th></th>
<th>Total</th>
<th></th>
<th>Chi square</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1</td>
<td>3</td>
<td>27.3</td>
<td>8</td>
<td>72.7</td>
<td>11</td>
<td>100</td>
<td>1.984</td>
<td>0.739</td>
</tr>
<tr>
<td>1-5</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>80</td>
<td>50</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-10</td>
<td>10</td>
<td>22.7</td>
<td>34</td>
<td>77.3</td>
<td>44</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-15</td>
<td>8</td>
<td>20.5</td>
<td>31</td>
<td>79.5</td>
<td>39</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-17</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>100</td>
<td>6</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>20.7</td>
<td>119</td>
<td>79.3</td>
<td>150</td>
<td>100</td>
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Table 2: Relation of clinical findings to genexpert.

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>GenXpert</th>
<th>Total</th>
<th>Chi square</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number %</td>
<td>Number %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough &gt; 2 weeks</td>
<td>Yes</td>
<td>88 80  22 20 110</td>
<td>0.112</td>
<td>0.738</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>31 77.5 9 22.5 40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever &gt; 2 weeks</td>
<td>Yes</td>
<td>67 78.8 18 21.2 85</td>
<td>0.031</td>
<td>0.140</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>52 80 13 20 65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTT/wt. loss &gt; 5%</td>
<td>Yes</td>
<td>32 88.9 4 11.1 36</td>
<td>2.638</td>
<td>0.140</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>87 76.3 27 23.7 114</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H/O Koch’s Contact</td>
<td>Yes</td>
<td>26 76.5 8 23.5 34</td>
<td>0.220</td>
<td>0.639</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>93 80.2 23 19.8 116</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H/O Convulsion</td>
<td>Yes</td>
<td>28 77.8 8 22.2 36</td>
<td>0.070</td>
<td>0.791</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>91 79.8 23 20.2 114</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant Lymphadenopathy</td>
<td>Yes</td>
<td>20 69 9 31 29</td>
<td>2.357</td>
<td>0.133</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>99 81.8 22 18.2 121</td>
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<td></td>
</tr>
</tbody>
</table>

Table 3: Relation of Sputum and ZN AFB status to genexpert.

<table>
<thead>
<tr>
<th>ZN staining</th>
<th>GeneXpert</th>
<th>Total</th>
<th>Fisher’s exact test</th>
<th>P value</th>
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<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number %</td>
<td>Number %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>10 100 0 0</td>
<td>10 100</td>
<td>2.791</td>
<td>0.123</td>
</tr>
<tr>
<td>Negative</td>
<td>109 75.7 31 22.1</td>
<td>140 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>119 31</td>
<td>150 100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Relation of Chest X-ray findings to genexpert.

<table>
<thead>
<tr>
<th>Chest X ray</th>
<th>GeneXpert</th>
<th>Total</th>
<th>Fisher’s exact test</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number %</td>
<td>Number %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>28 87.5 4 12.5</td>
<td>32 100</td>
<td>1.655</td>
<td>0.229</td>
</tr>
<tr>
<td>Negative</td>
<td>91 77.1 27 22.9</td>
<td>118 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>119 79.3 31 20.7</td>
<td>150 100</td>
<td></td>
<td></td>
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Table 5: Relation of clinical diagnosis compared to genexpert diagnosis for TB.

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>GeneXpert</th>
<th>Total</th>
<th>Fisher’s exact test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number %</td>
<td>Number %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>115 86.5 18 13.5</td>
<td>133 100</td>
<td>36.416</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Negative</td>
<td>4 23.5 13 76.5</td>
<td>17 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>119 79.3 31 20.7</td>
<td>150 100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3 shows relation of Sputum and ZN AFB status to GenXpert. GenXpert was positive in all suspected TB patients having ZN staining positive for AFB while GenXpert was positive in 75.7% in those with ZN staining negative.

Table 4 shows relation of Chest X-ray findings to GenXpert. Out of 32 chest X-ray positive patients, 87.5% were GenXpert Positive and 12.5% were GenXpert Negative while GeneXpert was positive in 77.1% with Chest X-ray Normal. There was statistically no significant (p > 0.05) difference of GeneXpert status within the Chest X-ray finding in Suspected TB patients.

Table 5 shows relation of clinical diagnosis compared to GenXpert diagnosis for TB. In 133 Clinically TB diagnosed patients, 86.5% were true positive as GenXpert Positive while 13.5% were GenXpert
Negative. While in 17 clinically non-tuberculosis patients 23.5% were GeneXpert Positive while 76.5% were negative. There was statistically very highly significant (p<0.001) difference of positive relation of Clinical diagnosis with the GeneXpert Positivity in suspected TB patients.

DISCUSSION

Mantoux test was positive in 7.4% suspected TB patients in our study. Results were nearly similar with other studies like Chadha et al, in 2007 and Rao et al, in 2008. Mantoux test is a hypersensitivity reaction; its positivity shows tubercular infection or disease. The reason for such a low positivity may be explained by fact that in malnourished and HIV co-infected patients it may come negative. It is not 100% specific as its positivity doesn’t differentiate between Tubercular Mycobacterium to NTM and may get positive as a reactivation of previous BCG Vaccination. Other reason for negative result may be deactivation of PPD by excessive heat and freezing so proper storage is necessary and also proper dose and proper method to implementation is needed for its success rate.

AFB sensitivity was found to be very low with 100% specificity. On ZN staining AFB positive was noted in 6.7% i.e. 10 patients of total 150 suspected TB patients, while 93.3% were AFB negative. It showed that ZN AFB has not a good diagnostic value in our study in children, while GeneXpert detects 109 cases of TB in ZN negative patients and all positive in ZN positive cases, so having high diagnostic value over ZN staining while specificity was very good with ZNAFB also.

Study done by Bunsow et al, showed AFB sensitivity of 78.5% and specificity of 98.3%. Other studies reported low sensitivity and specificity for AFB. GeneXpert was positive in all patients having ZN staining positive for AFB but at the same time it was positive in 75.7% of the patients with ZN AFB negative. There was statistically no significant (p < 0.05) difference of the GeneXpert status with the ZN staining finding in TB suspected patients.

Based on an updated Cochrane systematic review when GeneXpert was used as an initial test replacing smear microscopy for the diagnosis of PTB, X’pert MTB/RIF has an overall sensitivity of 88% and a pooled specificity of 98%, as compared to culture. The pooled sensitivity is 98% for smear-positive, culture-positive cases and 68% for smear-negative cases; the pooled sensitivity is 80%.

The reason behind such a low sensitivity in our study might be same as of Sampling error and method error. For induced sputum and Gastric aspirate 6 hr. fasting is required, which is sometimes unacceptable by parents. So, the ability of X’pert to rapidly confirm TB in smear-negative cases offers the possibility of improving early TB case detection. On Chest X-ray, Positive signs of TB were noted in 21.3% patients i.e. 32 patients in 150. It was found that 2.7% patients had infiltrate without cavitations, 11.3% had pleural effusion, primary complex was seen in 5.3% and 2% had miliary pattern on X-ray chest.

Out of 32 chest X-ray positive patients, 87.5% were GeneXpert Positive and 12.5% were GeneXpert Negative while in Chest X-ray normal patients, GeneXpert was positive in 77.1%. All the patients showed miliary pattern had 100% Gene X’pert positivity followed by all the patients with primary complex had 100% Gene X’pert positivity. So Positive X-ray findings add on to the diagnosis of TB but Negative X-ray doesn’t mean that patient is not suffering from TB as seen in the present study which showed GeneXpert positivity in 77.1% of negative X-ray cases. So Gene X’pert was able to diagnose good chunk of X-ray negative patients, which is significant.

The introduction of GeneXpert MTB/RIF as initial diagnostic test for TB has significantly increased the case-notification rates of all bacteriologically confirmed TB by 39% and rifampicin- resistant TB case by fivefold with an overall sensitivity of 97.6% and specificity of 99.2%.

In our study, out of 150 suspected TB patients 79.3% had positive GeneXpert and 20.7% had negative GeneXpert finding.

In 133 Clinically TB diagnosed patients, 86.5% were true positive as GeneXpert Positive while 13.5% were GeneXpert Negative. While in 17 clinically non-tuberculosis patients 23.5% were GeneXpert Positive while 76.5% were negative.

Finally GeneXpert confirmed TB in 79.3% patients while 20.7% did not have TB even though they were suspected. Pulmonary TB was confirmed in 44.7%, which were only pulmonary in 42.7% while 2% having Miliary TB. Extra-pulmonary TB was in 34.6%in which TB effusion was found in 8.7%; TBM in 18%, and8% had TB lymphadenitis.

So by adding Gene X’pert the diagnostic case finding has increased and also we were able to find some false positive patients in whom the confirmation can be done by culture and these patients will be saved from AKT side effects.

The clinical presentation of pulmonary tuberculosis is often non-specific and there is increased variability in the interpretation of radiological findings. This variability becomes even more evident in the presence of HIV co-infections when other opportunistic infections present with overlapping clinical and radiological findings. In our study we found total 67 Pulmonary TB positive patients out of 78 suspected pulmonary TB patient.
Pulmonary Koch’s was more common than EPTB as the primary route of infection for EPTB is also through lungs. X-ray findings may miss the diagnosis with ZN AFB. So adding GeneXpert with other diagnostic modality increases the case finding and false positive patients.

Worldwide, extra-pulmonary tuberculosis (EPTB) accounts for 25% of all TB cases, and even higher percentages in HIV-infected individuals and children. Existing tests for the diagnosis of EPTB are limited in accuracy and takes time, and often require invasive procedures and special expertise. For pleural TB, culture of pleural fluid has low sensitivity (on average, 30–50%). For lymph node TB, culture of an aspirate has a sensitivity of 60–70%. Culture specificity is 100% if the presence of Mycobacterium tuberculosis complex is confirmed with antigen tests or nucleic acid amplification tests (NAATs). Often, biopsy with culture and histopathological examination is necessary to achieve a diagnosis. For TB meningitis, the yield of culture is even lower (on average, 30%), although repeat examination of the cerebrospinal fluid (CSF) or the use of NAATs may increase sensitivity and is associated with high specificity (98%). All the above studies showed sensitivity and specificity pattern is compared with culture. We were not able to do the culture, as it is not available in our setting.

We assumed that the specificity of Xpert is high, as every study performed to date and meta-analysis has indicated consistently high specificity of 99%. Therefore, although there is no culture confirmation for comparison, this was not an evaluation of diagnostic accuracy, which has been comprehensively reported elsewhere and it can be reasonably assumed that these cases are genuine, and the rate of false positive diagnosis is unlikely to exceed that of culture.

CONCLUSION

GX is a novel diagnostic modality of choice in all suspected Pulmonary and Extra-pulmonary TB cases. Case detection rate got increased if GX is added along with other diagnostic modalities. It can be used as a primary tool in Pulmonary TB with smear negative samples in pediatric age group (smear ZN AFB have poor sensitivity as compare to adults). GeneXpert shows better sensitivity in those patients.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES


