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Original Research Article

GeneXpert: a game changer in the detection and diagnosis of childhood tuberculosis

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ABSTRACT

Background: Battling against tuberculosis (TB) is still a major challenge in India, despite measures undertaken by the government and medical fraternity. Delay in diagnosing tuberculosis is a challenge, causing hurdle in the prevention of spread of the disease.
Methods: This retrospective study analysed the samples by geneXpert assay. Samples (n=403, from 359 children) included pulmonary (sputum and gastric aspirate, 359), extrapulmonary (lymph node aspirate (LNA), 41), cerebrospinal fluid (CSF, 03), pus from the lesion at the elbow joint (01). Only sputum was analysed for 315 children, both sputum and LNA for 41.
Results: Mean age of patients was 9.08±2.85 years, range 3-15 years. There were 221 (61.56%) males and 138 (38.44%) females. Fever (71, 19.78%), fever with cough (87, 24.23%), fever with weight loss (41,11.42%) were the main symptoms. There were three patients with high fever, headache and seizures with neck rigidity, clinically diagnosed as Tuberculous meningitis. There was history of contact with Tuberculosis in 15 (4.18%) patients. Mean ESR was 112.09mm/1st Hr±56.05 (range 54 -750 mm/1st Hr). Mantoux test was positive in 270 (75.42%). Chest X-ray was normal in 33 (9.19%); consolidation in 189 (52.65%), mild pleural effusion in 94 (26.18%) mild pleural effusion associated with consolidation in 43 (11.98%) were reported. Positive GeneXpert assay (106 samples, 27.39%; sputum (87, 24.23% %), pus (01), CSF (03), LNA (15, 57.69%) was reported in 87 patients. Results were obtained ≤36 hours, mean 2 hours± 2.34 (range 6- 36 hours).
Conclusions: GeneXpert is an effective tool for rapid detection of tuberculosis. Present study supports its inclusion in the battery of routine investigations. It can revolutionise the scenario in prevention and management of tuberculosis.

Keywords: GeneXpert assay, Lymph node aspirate, Rapid Detection, Sputum, Tuberculosis

INTRODUCTION

Battling against tuberculosis (TB) is still a major challenge in India, despite measures undertaken by the government and medical fraternity. It is estimated that India accounts for 1/4th of global burden of TB with an incidence of 28 lakh (104 lakh globally) and mortality of 4.8 lakh (14 lakh globally). Tuberculosis in pediatric population accounted for 6% of the total burden in India in 2016.[1] World Health Organization (WHO) estimated that in 2016, one million children are affected with tuberculosis with a mortality of 210,000 per year.[2]

Actual cases may be higher as tuberculosis in children is difficult to diagnose and the symptoms mimic many other childhood diseases.[3] Non-specific symptoms, delayed diagnosis often make the exact estimation of the disease burden difficult in this population.

Statistics show that 67 million children have latent TB, and about 850,000 develop active tuberculosis each year.[4] It is estimated that globally upto 500 children die from TB; nearly 1/4th of a million children fall prey to TB. Child aged <5 years, with history of household contact with TB, severe malnutrition and HIV infection are more prone to develop tuberculosis. Age is an important factor as disseminated TB is more common among young children <3 years. Pulmonary tuberulosis is the most common compared to extra pulmonary (20%-30%) TB in children. Poor accessibility to health care, inadequate screening for TB, emergence of multidrug resistant strains adds to the existing problem.

Rapid diagnosis is still a challenge; Culture is the gold standard, but time consuming (2-8 weeks) and the yield may not be satisfactory. Smear for acid fast bacilli though rapid, inexpensive is often considered less sensitive and of poor positive predictive value.

Moreover, smear cannot detect drug resistance. Nucleic acid amplification methods are being considered for rapid detection of acid fast bacilli (AFB).[5] WHO recommends genexpert assay as the initial test to detect pulmonary and extrapulmonary tuberculosis.

With the introduction of geneXpert assay, there has been a huge change in the detection time and more accuracy. It is proved to be highly effective not only in the detection of tuberculosis but also in identifying rifampicin resistant strains.[6]

METHODS

This retrospective, observational study was conducted by the Departments of Pediatrics and Pulmonology, in a teaching hospital, Hyderabad, India. Data was collected from January 2015 to March 2017 and the role of GeneXpert assay in the detection of tuberculosis particularly when other tests for diagnosing tuberculosis are negative was evaluated.

Data of children aged 5-15 years whose samples were sent for geneXpert analyseis, based on the clinical symptoms and suspicion of tuberculosis was collected.

Sputum from the patients with lung consolidation, lymph node aspirate (LNA)from those who had lymphnode involvement and cerebrospinal fluid (CSF) from those who had symptoms of meningitis were analysed. In children <5 years, gastric aspirates were analysed.

Data of patients with a probable diagnosis of tuberculosis were collected from the hospital records. Demographic data, clinical features, history of (h/o) household contact with tuberculosis, relevant laboratory investigations i.e., Erythrocyte sedimentation rate, Mantoux test, Chest X-ray, Cerebrospinal fluid (CSF) analysis and GeneXpert assay were recorded.

Results of basic investigations (Mantoux test, chest X-ray, sputum for AFB) were compared with that of GeneXpert assay.

Statistical analysis

Data was captured on Microsoft Excel worksheets (2007) and analysed. Results were expressed as mean, standard deviation (SD), median and inter quartile Range for continuous variables and as %, frequency distribution for categorical variables. Chi square and Fischers extract test were applied.

Analysis was carried out using statistical package for social sciences (SPSS 20th version). A p value of <0.05 with two sided was considered significant.

RESULTS

A total of 403 samples (359 paatients, (sputum–359, lymph node aspirate – 41 and CSF- 03) were sent for analysis. Only sputum was analysed for 315 patients, both sputum and LNA for 41 and CSF for three patients. Mean age of patients was 9.08±2.85 years, range of 3-15 years.

There were 221 (61.56%) males with a mean age of 8.96±2.83 years (range 5-15 years) and 138 (38.44%) females with a mean age of 9.25±2.89 years (range 5-15years). Fever (71, 19.78%), fever with cough (87, 24.23%), fever with weight loss (41, 11.42%) were the presenting symptoms (Table 1).

Table 1: Clinical presentation.

Clinical features	Frequency (percent)	Cumulative frequency (cumulative percent)
Cough	1 (0.28%)	1 (0.28%)
Fever	71 (19.78%)	72 (20.06%)
Fever, weight loss	41 (11.42%)	113 (31.48%)
Fever, cough	87 (24.23%)	200 (55.71%)
Fever, cough, weight loss	59 (16.43%)	259 (72.14%)
Fever, cough, lymphadenopathy	90 (25.07%)	349 (97.21%)
Fever, lymphadenopathy	10 (2.79%)	359 (100.00%)

There were three patients with high fever, headache, neck rigidity and seizures, clinically diagnosed as Tuberculous meningitis. There was history of contact with Tuberculosis only in 15 (4.18%) patients.

Laboratory investigations

Data of 358 samples were available. Mean±SD ESR was 112.09mm/1st Hr±56.05 with a range of 54 -750 mm/1st

Hr (Table 2). There was no statistical significance between the ESR of male and female.

Mantoux test was performed for all patients but data of one patient was unavailable. Reading of >10mm was seen in 270 (75.42%) and <10mm was seen in 88 (24.58%) patients.

Table 2: Erythrocyte sedimentation rate among both genders.

ESR	Female	Male
Mean	112.95	109.05
Std deviation	81.08	31.40
Minimum	79	54.00
Maximum	750	450.00

Chest X-ray was normal in 33 (9.19%); consolidation was seen in 189 (52.65%), mild pleural effusion in 94 (26.18%) and mild pleural effusion associated with consolidation in 43 (11.98%).

Positive GeneXpert assay (106, 27.39%) was reported in 87 patients (sputum (87, 24.23% %), pus (01), CSF (03), LNA (15, 57.69%)).

GeneXpert assay was negative for 271 patients. There was no statistically significant gender difference in GeneXpert results (Figure 1).

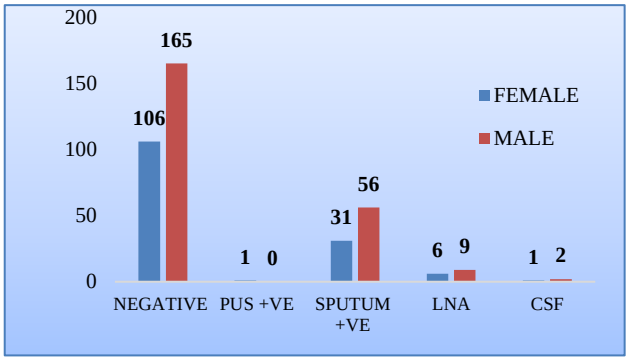

Figure 1: Results of GeneXpert assay among males and female.

Table 3: Comparison of results of geneXpert assay with ESR.

Genexpert	n	Variable	n	Mean	Std Dev	Minimum	Maximum
Negative	271	Age	271	9.20	2.97	33 years	15
		ESR	270	108.19	29.98	54.00	450.00
Pus +ve	01	Age	1	6.00	-	6	6
		ESR	1	148.00		148.00	148.00
Sputum +ve	87	Age	87	8.85	2.82	5.00	15.00
		ESR	87	123.78	100.15	82.00	450.00
LNA +ve	15	Age	15	8.33	2.65	5	13
		ESR	15	112.6	31.73	79	147
CSF	03	Age	3	9.66	2.63	8	11
		ESR	3	106	2.5	104	108

Table 4: Comparison of GeneXpert and Mantoux test.

Mantoux test	GeneXpert assay					
	Negative	Pus +ve	Sputum +ve	LNA	CSF	Total
<10mm	2	0	86	3	0	91
>10mm	268	1	1	12	3	285
Total	270	1	87	15	3	376
Frequency Missing = 1						

There was no statistically significant gender difference among the participants in GeneXpert assay.

Results of the assay was obtained within 36 hours, mean of 12 hours±2.34, and a range of 6-36 hours. It took a longer time in few cases due to technical issues.

In those without any h/o contact with tuberculosis (n=344); GeneXpert was positive for 79 (23.0%) sputum samples, one pus sample, and LNA (14, 4%). 264 samples were negative. Of those who had h/o contact with tuberculosis (n=15), geneXpert assay was positive for eight (53.3%) sputum samples, and one (6.6%) LNA.

Of those with tuberculous meningitis and positive CSF for geneXpert assay, none had h/o contact with tuberculosis. There was statistically no significant relationship between geneXpert and ESR (1st Hr)

(p>0.005). Table 3 compares the results of geneXpert assay with ESR.

There was no statistically significant (p>0.005) relationship between Mantoux test, Chest X-ray findings and geneXpert results. Table 4 compares Mantoux test, Table 5 findings of chest X-ray and Table 6 the clinical features with geneXpert assay. There was no statistically significant association between presenting clinical features and GeneXpert assay.

Table 5: Comparison of chest X-ray findings with results of geneXpert assay.

Chest X-ray	GeneXpert					
	negative	Pus +VE	Sputum +VE	LNA +VE	CSF	Total
Consolidation	141	1 0,	47	8	0	189
Normal	26	0	7	4	1	33
Pleural Effusion with consolidation	34	0	9	2	1	46
Pleural effusion	70	0	24	1	1	94
Total	271	1	87	15	3	359

Table 6: Comparison of clinical features and results of GeneXpert assay.

Clinical features	Genexpert					
	Negative	Pus +ve	Sputum +ve	LNA +ve	CSF	Total
Cough	1	0	0			1
Fever	68	0	3	2	1	71
Fever, Weight loss	41	0	0	4		41
Fever, Cough	85	0	2	8	2	87
Fever, Cough, Weight loss	58	0	1			59
Fever, Cough, Lymphadenopathy	18	1	71	1		90
Fever, Lymphadenopathy	0	0	10			10
Total	271	1	87	15		359

DISCUSSION

Delayed diagnosis of tuberculosis, which is a common observation in clinical practice has its impact on the disease course, treatment outcome and spread of the disease in the community. Early diagnosis is a major step towards effective disease control and preventing dissemination. Introduction of GeneXpert in the initial testing of tuberculosis is a game changer in the detection of tuberculosis and in its management since it can delineate rifampicin resistant strains. This technique has well established specificity and sensitivity.[7] This simple test has shown to be of comparable sensitivity as that of culture in smear positive cases; moreover, it can be performed by the staff with minimal training. Turnaround time for assay is ≈ 3-24 hours which is shorter compared to culture.[8] Specificity of detecting tuberculosis by geneXpert technique is reported to be around 99%. Reechaipichitkul W et al report a good specificity (92.1%) and sensitivity (83.9%) along with positive predictive value (81.3%) and negative predictive value (93.3%). Its role in diagnosis of smear negative tuberculosis was satisfactory.[9] Similar observations were reported by Pandey P et al for the diagnosis of multi-drug resistant tuberculsosis.[10] Narute S et al too support these obsevations.[11] Sachdeva KS et al assessed the impact of this assay on the disease burden and conclude that utilization of this technique can reduce the number of cases over a decade. This can help in reducing India's epidemic tuberculosis, multi drug resistant tuberculosis, in particular.[12] There are similar reports globally. GeneXpert assay is more sensitive than direct smear but as accurate as culture with less turanaround time.[13-19] The focus of these studies were on pulmonary tuberculosis while there is limited data on the diagnostic utility of extrapulmonary tuberculosis.[20-27]

Studies of diagnostic utility of geneXpert study in the pediatric population is limited, more so from Indian population. Available data is on adult Indian population.[28]

Present study population included children aged 3-15 years. There was only one child <5 years (aged 3 years) in whom gastric aspirate was analysed. In one patient with lesion in the elbow, we analysed the pus sample from this region. In 2015, of 89 (13 LNA) samples tested, 21 samples of sputum were found positive for geneXpert while results for sputum for AFB was nil; three samples of LNA was positive. In 2016, of 239 (16 LNA) samples tested, 49 samples sputum were positive for geneXpert,

while four tested positive for AFB, 09 samples of LNA was positive. In 2017, of 75 cases (12 LNA), 14 samples of sputum and three samples of LNA were positive for geneXpert analysis. In our study, 106 samples tested positive for geneXpert assay. In those with tuberculous meningitis (n=03), CSF samples were analysed, and all were positive for geneXpert assay.

There were more females in our study, but there is no statistical significance in gender difference. Those with h/o Tuberculosis were only 4.18%. Clinical Presentation was typical of fever, fever with cough, fever with weight loss and combination of symptoms. Mantoux test was positive in significant proportion (n=270, 67.0%). Consolidation, pleural effusion and combination of these two features were frequent findings on chest X-ray.

Contact with tuberculosis is an important factor to be considered in the diagnosis and preventing the further spread. Use of GeneXpert assay helped in enhancing the detection and diagnosis of tuberculosis.[29]

Present analysis did not show any significant correlation with clinical features, other diagnostic tests i.e., ESR, Mantoux test, and Chest X-ray findings. We did not correlate the results of smear and culture with geneXpert assay. Three patients who were clinically diagnosed as tuberculous meningtis were positive for geneXpert suggesting greater possibility of accurate diagnosis using CSF sample; however, the sample size is very less to derive concrete results. We did not consider identification of multidrug resistant strains and did not analyse special cases such as HIV positive patients in our study population.

Current study supports the fact that geneXpert is an effective tool for rapid diagnosis of tuberculosis (mean time of 12 hours), a blessing for countries like India, which are rampant in tuberculosis. Similar reports are available from our neighbouring country.[30] With early detection of the disease it is possible for efficient management and reduction in the spread.

Marouane C et al opine that though it is a useful tool, it can be used as an adjuvant to the existing methods of diagnosis, not as a replacement.[31] Bunsow E et al support the accuracy and rapidity of the test, but suggest other confirmatory tests for drug resistant mycobacteria.[32] Centre for disease control too recommends combination of tests, in particular for smear negative cases.[33]

This technique is not free from drawbacks. Although it is helpful in detecting new cases; it is possible to have dead bacilli in positive samples. Another possible scenario is bacilli may be resistant to only one drug – either rifampicin or Isoniazid, which requires isolation using cultures and test drug sensitivity. Moreover, at this stage, its role is restricted to diagnosis, and is difficult to assess the therapeutic response based on geneXpert assay. Raizada N et al analysed the implementation of this technique in 18 centres in India and assessed the reasons for failure as processing errors and equipment malfunction, electricity related issues, issues due to non maintanence of accurate temperature, cartridge related issues which required attention.[34]

Hence, one should not forget the ageold tested methods, though yield is delayed and less, till more roles of this assay is established. There is no doubt that this assay has revolutionised the early and rapid detection of tuberculosis particularly in countries burdened with the disease and struggling to fight it.

Let's work together to make the Slogan for world TB day 2017 'Unite to End TB' and goal of eliminating TB by 2025 come true.

CONCLUSION

GeneXpert is an effective tool for rapid detection of tuberculosis, and our study supports the inclusion of this test in the battery of routine investigations. It can revolutionise the scenario in the disease management and prevention. However, in durg resistant cases, other diagnostic tests may be required.

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