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Original Research Paper

Pulmonary Medicine

COMPARATIVE STUDY OF EFFICACY OF ZIEHL– NEELSEN STAIN AFB LIQUID CULTURE AND GENE XPERT ALONG WITH RADIOLOGICAL CORRELATION IN SUSPECTED PATIENTS OF PULMONARY TUBERCULOSIS

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ABSTRACT Tuberculosis occurs in every part of the world with over 95% of cases and deaths occurring in developing countries . People infected with TB bacteria have 5-15 % lifetime risk of falling ill with TB . However ,persons with compromised immune system , such as people living with HIV, malnutrition or diabetes , or people who use tobacco, have a much higher risk of falling ill [1]. Health Assembly in 2014 adopted a new End TB strategy targeting the "end of the epidemic " by 2035.defined as a global incidence of less than 10 cases per 100,000 people , similar to the situation of advanced economies of today . The strategy , besides proposing a patient- centred care approach that consist of the basics of TB control complemented by all modern technologies available and a bold health policy environment that emphasizes universal coverage and social protection , promotes rapid intensification of research efforts in a way that results in new means and their immediate application every where [2].

KEYWORDS : Neelsen stain – Acid Fast Staining (AFB stain), Gene Xpert – Molecular test .

INTRODUCTION –

Tuberculosis remains one of the most deadly infectious and has claimed millions of lives for many years. While significant progress has been made towards controlling the global burden of TB over the past decade, more efforts are still needed. It is evident from the studies that patients primarily resort to self–medication and take the help of private practitioners and traditional healers as an initial consultation for their TB care at private health facilities. An initial period of relief from the symptoms after receiving treatment from a variety of private setups creates a pseudo-impression of cure among the patients which invariably leads to delay in help seeking at appropriated public health facilities for complete treatment. The delay in seeking treatment is a major problem for the individual patient and the community as well .

Diagnosis of TB can be clinically challenging. M .Tuberculosis complex is highly infectious. Therefore its diagnosis as early as possible is of paramount importance .The highly infectious mature of tuberculosis (TB) urges the need to increase the efficiency and rapidity of lab methods .The present study was undertaken to evaluate the sensitivity of Nucleic acid amplification assay (Gene Xpert) using respiratory samples in patients with suspected pulmonary tuberculosis and compare with AFB smear microscopy (Ziehl Neelsen Stain) and Acid Fast Bacilli (AFB) liquid culture . The study helped to evaluate and compare negative predicative value of all three methods in addition to help find the correlation between chest X ray finding and results of above three methods.

MATERIALS AND METHODS –

Patients with clinical suspicion of pulmonary tuberculosis including symptoms of cough with or without expectoration for >2 weeks , weight loss, fatigue , haemoptysis and loss of appetite .

A cross sectional study was conducted in the department of pulmonary medicine of a tertiary care teaching hospital .Approval was obtained from the institutional ethics committee prior to the commencement of the study.

Study Design – Hospital based cross sectional study.

Source of Data – 100 outdoor / indoor patients in Department of Pulmonary Medicine from November 2017 to Nov 2018 presenting with clinical suspicion of pulmonary tuberculosis.
Selection of Cases .

INCLUSION CRITERIA

1) Age >12 years .
2) Patient who were ready to sigh informed consent for study participation .
3) Patients with clinical suspicion of pulmonary tuberculosis including symptoms of cough with or without expectoration for > 2 weeks , low grade fever , weight loss , failure , hemoptysis ,chest pain and loss of appetite.
4) Patient with no history of lung malignancies or fungal infections.

Sample Size – 100 calculated using $n = Zpq/L^2$

The Global TB Report 2017 released by World Health Organization (WHO) states that iin India, an estimated 27.9 lakh patients were suffering from TB in 2016 and up to 4.23 lakh patient were estimated to have died during the year .

According to the Global tuberculosis report 2016 the incidence of tuberculosis in India was 217 per 100 population .India shares on fifth of global tuberculosis load . therefore with 20 percent proportion , the sample size is estimated at 95% confidence interval and with precision of 10% using the following formula .

$n = Zpq/L^2$ where n = Sample Size
p= Estimated true proportion of disease = 0.2 q=1 1-p 0.8
L= Allowable relative error = 0.1
$N = 1.96x1.96\ (0.2)(0.8)/0.1)^2 = 61.46$

Considering the dropout cases and noted errors in staining method and for better calculation we have considered a sample size of 100. Therefore 100 patients were included in the study fulfilling inclusion and exclusion criteria.

Written informed consent was taken from patients that fulfilled the inclusion criteria A case study proforma was filled and detailed clinical history was obtained from patients including symptoms of cough ,expectoration ,chest pain , hemoptysis , loss of appetite and weight loss. Clinical examination was conducted and the information from the patient was gathered by means of direct interviews.

Either of Sputum Sample of BAL sample was collected for following investigation.

1. Sputum Z.N . Microscopy.
2. Liquid AFB culture .

3. Gene Xpert .

All the participants included in the study were send to radiology department for obtaining Chest X ray chest PA view All Data was compiled using Microsoft excel and analysed using SPSS and Medical software's.

Laboratory Methods .

Each sputum and BAL samples received in the lab from the centres as per the collection and transportation policy of the laboratory were divided into three parts one part was immediately tested using Genexpert Second part used for ZN smear microscopy and third part for MGIT BACTEC 320 liquid culture and performed on same day . Only one sample either BAL or sputum from a single patient was divided and processed on same day. For liquid culture as much as sample was taken after sending for Genexpert and ZN stain but it should be checked that volume remaining should not be less than 2ml for processing . GeneXpert testing was performed according to the manufacturer's instructions [3]. Sample reagent was added to untreated sputum and BAL at a ratio of 2:1, manually agitated and kept for 10 min at the room temperature, then shaken again and kept for 5 min; 2ml of the inactivated material was transferred to the test cartilage and inserted into the test platform . Only electronic results were used for comparison .Direct Smear microscopy was performed to investigate presence of acid fast bacilli with the second part of the specimen using conventional ZN staining method. Slides showing red coloured acid fast bacilli were taken as positive and negative slides were those without any acid fast bacilli [4]. Third part was processed using the N-acetyl –L cysteine –sodium hydroxide method (NACL- NaOH) as per the manufacturer's instruction, cultured on MGIT and incubated in MGIT BACTEC 320 liquid culture system .[5] Sodium hydroxide (NaOH) is a decontaminating agent and also acts as emulsifier and NALC acts as mucolytic agent and also reduces the concentration of NaOH required .[4] when the tubes were flagged positive by the system, ZN staining and culture on 5% sheep blood agar were performed from the tube directly to see any contamination as per the manufactures instruction. All tube were checked for positivity till 42 days. MOTT and Mycobacterium tuberculosis testing from positive culture tubes were done by rapid immunochromatography test kit using MPT 64 antigen according to the manufacturer's instruction .

Analysis :-

The data was tabulated in Microsoft excel spread sheet in a chart and studied for correlation . Statistical analysis of the data was conducted with statistical package for the social science system version SPSS17.0

Sensitivity , specificity ,PPV and NPV was calculated for the diagnosis of Pulmonary tuberculosis for AFB smear microscopy and the GeneXpert , using culture of Mycobacterium tuberculosis from sputum or BAL specimens as Gold standard .

By taking culture method as reference, samples that were positive and negative in culture were considered true positive and true negative .Culture negative and test in consideration (Gene Xpert/ZN Smear) positive samples were taken as false positive samples while test n consideration (Gene Xpert/ZN Smear) negative and culture positive samples were considered false negative respectively .

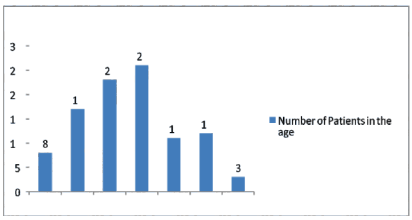
RESULT :

Figure 1- Age wise distribution of patients.

Table 1

SEX	No of Pt	Age%
Male	53	53
Female	47	47
Total	100	100

2:- Gender wise distribution of study population



In our study the total number of male patients was 53 and the number of female patients were 47.

Out of 100 patients who participated in the study, nased upon primary history. X ray findings and positive liquid culture reports, 39 patients were diagnosed as pulmonary tuberculosis cases. Rest of the cases were termed as not confirmed cases which involved similar symptoms as PTB but they could not be diagnosed based on Chest X ray finding and liquid culture method. This group comprised of patient where Non tubercular mycobacteria (NTM) bacterial culture and other patients whose liquid culture turned out negative.

Table 3 - Symptoms present in study participants

	Confirmed PTB Cases Symptoms				Non Confirmed cases including NTM			
	Present		Absent		Present		Absent	
Symptoms	NO	%	N0	%	No	%	No	%
Fever	25	64.10	14	38.89	53	86.8	8	13.1
H/O fever	36	92.31	3	8.57	45	73.7	16	26.2
Cough	35	89.74	4	10.26	50	81.9	11	18.0
Wt loss	37	94.87	2	5.13	53	86.8	8	13.11
Malaise	31	79.49	8	20.51	48	78.6	13	21.31
Night Sweats	28	71.79	11	28.21	34	55.7	27	44.26
Anorexia	37	94.87	2	12.82	39	63.9	22	36.0
Breathlessness	16	41.03	23	58.97	29	47.5	32	52.4

Table :4 Sputum AFB Smear Report .

Sputum AFB	Number of Patients	Percentage %
Positive	35	35
Negative	65	65
Total	100	100

In our study it was observed that out of the 100 patients were screened ,35% patients turned out to be sputum AFB smear while 65% turned out negative .

Table 5:- MGIT Liquid culture Report .

MGIT liquid Culture	Number of Patients	Percentage
Positive	39	39
Negative	61	61
Total	100	100

The MGIT liquid culture report were positive for 39 cases while they came out negative for 61% of the cases.

Table 6:- Gene xpert Test Report

MGIT liquid Culture	Number of Patients	Percentage
Positive	45	45
Negative	55	55
Total	100	100

Table 7:- Gene Xpert Positive Test Report as per Concentration.

GeneXpert Test	Number Samples	Percentage %
Detected Low	36	80
Detected Medium	08	17.78

Detected High	01	2.22
Total	45	100

A total of 100 respiratory specimens (20 BAL and 80 Sputum samples) were tested . Of the 100 Specimen , 29 samples were positive all three methods and whereas 50 specimens were negative by all three methods used . Among 100 samples ,45 samples were Genexpert TB positive . Altogether 39 specimens were culture positive for AFB. Total 35 samples were AFB smear positive. Among 65 AFB smear microscopy negative samples ,50 samples were negative for all three methods In rest 15 AFB smear negative samples,10 samples were culture positive , 13 samples were GeneXpert positive.

Formula Used -

PPV = No. of True Positive / Number of True Positive + No of False Positive.

NPV = No of True Negative / Number of True Negative + Number of False Negative.

Specificity = No of True Negative / Actual Number of cases not having disease.

Sensitivity = No of True Positive / Actual Number of cases having disease.

Overall Sensitivity , Specificity , PPV and NPV of GeneXpert when ZN Microscopy was taken as reference method is illustrated in

Table 8

Sensitivity	Specificity	PPV	NPV
82.05	85.25	91.43	80.00

Over all Sensitivity , Specificity , PPV and NPV of Gene Xpert when Culture was taken as reference method is

Table 9

Sensitivity	Specificity	PPV	NPV
94.87	86.89	82.22	96.36

Over all Sensitivity , Specificity , PPV and NPV of ZN Microscopy when Culture was taken as reference method is

Table 10

Sensitivity	Specificity	PPV	NPV
74.35	90.16	82.85	84.61

DISCUSSION :-

In this cross sectional study , we have evaluated the diagnostic yield of Genexpert to detect MTB in respiratory samples (BAL and Sputum) and compared it with AFB culture which was taken as gold standard.

Mycobacterial cultures for detection of Mycobacterium tuberculosis can be done either using solid (Lowenstein Jensen Media) or Liquid broth system (MGIT 320) Results by MCIT liquid culture medium come earlier as compared to LJ medium[8,7]. In our study result form MGIT 320 culture were included . GeneXpert is a simple bench to point of care diagnostic assay that can be performed with minimal training . The results are available within 2 hours, much earlier than the culture which usually takes days to come positive [8,9].

Number of studies have demonstrated the utility of GeneXpert in diagnosis of pulmonary tuberculosis [10].In our study, overall sensitivity ,specificity, PPV and NPV of GeneXpert were 94.87%,86.89% ,82.22% and 96.36% respectively that is comparable with other studies [11,12].In other studies ,GeneXpert sensitivity and Specificity for BAL sample was from 81%- 92% and 71%-100% ,it is in conjunction with our studies [11,12,13].

Although specificity in our study is 86.89% , it is because 2 culture samples were positive for MOTT and GeneXpert only dectects MTB . In other 2 samples although MTB growth is in culture but it is possible that the bacterial load may have been too low for the GeneXpert to detect the DNA from MTB – Complex .It shows that a patient with a negative GeneXpert can still have TB with MTB or MOTT [11,14,15]. The NPV value of GeneXpert is high in our study in comparison to the study done by Kanwal et al. as LJ media was used in their study whereas in our and other studies ,liquid culture method was used[16].

CONCLUSION :-

1. Both GeneXpert and AFB smear microscopy have almost same specificity but sensitivity of GeneXpert is found to be much higher than AFB smear microscopy in respiratory samples.
2. AFB culture is considered as a gold standard method but as it takes days to come positive and simultaneous detection of Rifampicin resistance is not possible with it. On other hand GeneXpert can be a useful diagnostic method in patients of suspected pulmonary tuberculosis either AFB smear negative or positive due to its rapidity and simultaneous detection of Rifampicin resistance especially beneficial in patient with MDR and HIV associated tuberculosis .
3. Samples which were culture negative and GenXpert positive the result of GeneXpert was low positive In case with history of treatment with ATT or with low bacterial load PCR test amplifies any DNA. Of live or dead bacilli . Therefore while diagnosing a person with active tuberculosis physicians should be very cautious using it as a sole method .Clear history of previous treatment with ATT is required to avoid false positive results [9,14].
4. Our study further strengthens the use of GeneXpert in smear positive pulmonary sample as endorsed by WHO[8].

In patient with incongruous results of smear microscopy and GeneXpert pulmonary samples but high clinical evidence of pulmonary tuberculosis like HIV positive or critically ill, clinicians may exercise their clinical decision to start anti tubercular treatment after sending sample of culture [14].

5. GeneXpert does not eliminate the need of conventional microscopy, culture and anti- tubercular drug sensitivity that are required to monitor the progression of treatment and to detect resistance to drug other than Rifampicin [17].

Limitation of Study :-

1) In this study sample size was limited so further studies with large sample size need to be done .

2) In this study culture and drug sensitivity testing was not done so the sensitivity and specificity of MTB /RIF assay to detect Rifampicin resistance was not evaluated .

3) Only sputum and BAL samples results were studies were studied. Result of non-respiratory samples were not checked

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