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COMPARATIVE STUDY OF SPUTUM SMEAR EXAMINATION, GENE X-PERT AND AFB CULTURE IN DIAGNOSIS OF PULMONARY TUBERCULOSIS

DR.M. M NAYAK PROFESSOR & HOD,DEPARTMENT OF PULMONARY MEDICINE,TERNA MEDICAL COLLEGE, NERUL, NAVI MUMBAI.

DR. H. S. THAKKER SENIOR RESIDENT,DEPARTMENT OF PULMONARY MEDICINE,TERNA MEDICAL COLLEGE, NERUL, NAVI MUMBAI.

DR.RUHEE GOTMARE JUNIOR RESIDENT,DEPARTMENT OF PULMONARY MEDICINE,TERNA MEDICAL COLLEGE,NERUL, NAVI MUMBAI.

ABSTRACT Tuberculosis is one of the commonest infectious disease in India with one-fourth of the world's annual incidence reported in our country.Gene X-pert is a comparatively new rapid diagnostic test endorsed by WHO.

Aims & Objectives -This study was carried out with the aim of determining sensitivity and specificity of Gene X-pert for detection of tuberculosis in pulmonary specimens and compare it with both smears and TB cultures.

Material & Methods - A retrospective study of 60 TB patients was studied to evaluate the sensitivity and specificity of Gene X-pert and compare the results with sputum smear and culture reports in pulmonary TB patients.

Results - Sensitivity of Gene X-pert in sputum samples was found to be 96.67%. In our study specificity was found to be 100% in smear positive patients & 63.6% in smear negative patients. Conclusion - Gene X-pert was found to be a highly specific and sensitive rapid diagnostic tool that should preferably be employed in all suspected TB patients to facilitate early detection of pulmonary TB.

KEYWORDS : .

INTRODUCTION -

Tuberculosis is a serious public health problem in India with prevalence reported to be 2.5 million according to WHO 2015 report. The incidence of Tuberculosis in India is 2.2 million cases per year out of an estimated 9.6 million new cases detected globally, which is approximately 1/4th of the global burden [1].

The proportion of Drug Resistant Tuberculosis cases among new cases is 2-3 % and that among previously treated cases is 12-17 % [2].

Early diagnosis of tuberculosis and prompt initiation of treatment are essential components of TB control especially in cases of drug resistant tuberculosis due to high risk of transmission and emergence of primary drug resistant tuberculosis.

Sputum smear microscopy is an inexpensive, easily available and rapid diagnostic test for detection of Acid Fast Bacilli, presently employed under RNTCP programme. However it has poor sensitivity and poor positive predictive value [3] and also requires multiple visits leading to higher default [4].

Mycobacterial culture , although considered as gold standard, is slow and usually takes 2-6 weeks time to yield a final result and requires proper infrastructure and technical expertise [5,6,7]. X-pert MTB/RIF is a commercially available cartridge based nucleic acid amplification test for diagnosing MTB complex, endorsed by WHO [8]. It uses PCR to test specimens for genetic material specific to MTB and simultainously detects a gene which confers resistance to Rifampicin , Rpo B. It is a fully automated test which gives results within 2 hours . The reported sensitivity of a single X-pert MTB/RIF test in smear-negative/culture-positive patients was 72.5% and increased to 90.2% when three samples were tested. Specificity of X-pert MTB/RIF was 99%. X-pert MTB/RIF detected rifampicin resistance with 99.1% sensitivity and excluded resistance with 100% specificity[9].

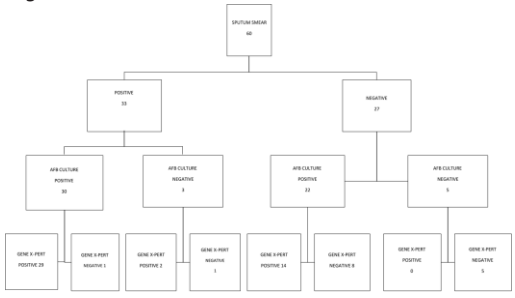
According to WHO 2013 policy recommendations X-pert MTB/RIF is recommended in suspected MDR-TB or HIV associated TB (strong recommendation), conditional recommendation acknowledging resource implications but high quality evidence in all patients suspected of having TB, and also used as follow-on test to microscopy in suspected cases of TB who are at a risk of MDR-TB or HIV associated TB specially when further testing of smear negative specimens is necessary [10].

However, currently in the Indian scenario, only a small percentage of patients which includes all failures of 1st line anti tuberculous regimen, contacts of MDR TB, smear positive re-treatment cases at diagnosis or any smear positive follow-up cases, smear negative re-treatment cases and HIV associated new TB are subjected to investigations to rule out drug resistance[11].

This study was carried out with aim of determining sensitivity and specificity of Gene X-pert for detection of tuberculosis in pulmonary specimens and compare it with both smears & TB cultures, to determine if the molecular diagnostic tests should be employed in all primary cases in order to improve the diagnostic yield, which would have been missed had we relied only on the basis of smear results for diagnosis of pulmonary tuberculosis.

MATERIALS AND METHODS - In this study, sputum samples of 60 TB patients obtained during the clinical routine sent for ZNCF staining, Gene X-pert and AFB culture and DST were reviewed. The study included patients who were diagnosed as Pulmonary tuberculosis on the basis of the above investigations or started anti-TB treatment based on clinico-radiological findings. Patients were grouped in the following categories:

1. Smear positive, Culture positive, Gene X-pert positive or negative



RESULTS -

Out of 60 sputum specimens studied 33 were found to be smear positive by ZNCF staining. Out of these 30 were found to be culture positive. 29 of the 30 smear positive and culture positive samples

were found to be Gene X-pert positive (96.7%). Out of the 3 smear positive but culture negative samples, 2 were Gene X-pert positive.Out of 27 smear negative samples, 5 were culture negative and were also Gene X-pert negative (100%). Out of 27 smear negative samples, 22 were culture positive, out of which 14 were Gene X-pert positive and 8 were Gene X-pert negative.

DISCUSSION:

Sputum smear examination is a relatively less costly test performed routinely in suspected PTB patients however the reported sensitivity of smear microscopy is low, varying between 22 and 80 % [12]. Culture is considered as gold standard test for diagnosing pulmonary tuberculosis but it takes 6-8 weeks which can result in delayed diagnosis of drug resistant pulmonary tuberculosis.

X-pert MTB/RIF is a commercially available diagnostic test for Mycobacterium tuberculosis complex, which uses polymerase chain reaction (PCR) to test specimens for genetic material specific to Mycobacterium tuberculosis, and simultaneously detects a gene which confers resistance to rifampicin, rpoB [13].In general, the routine application of nucleic acid amplification techniques for detection of *Mycobacterium tuberculosis* results in accurate diagnosis of tuberculosis [14,15,16,17,18] but requires laborious processing time and dedicated bio-safety conditions. The recently introduced X-pert MTB/RIF assay is a fully automated, walk-away real-time PCR-based assay with a time to result of approximately 2 h. To detect *M. tuberculosis* and mutations associated with resistance to rifampicin (RIF), the 81-bp core region of the *rpoB* gene is amplified and probed with five overlapping molecular beacons[19].The objective of the present study was to evaluate the feasibility of the X-pert MTB/RIF assay to replace direct smear microscopy as a primary screening test for urgent clinical specimens in a setting of low TB prevalence.

In a retrospective study we evaluated the diagnostic yield of Gene X-pert in comparison with sputum smear and culture reports. Mycobacterium culture had been processed in an accredited lab using MGIT 960 liquid culture medium as per standard protocol.Smears were studied using the recommended protocol for ZNFC staining and reporting .Gene X-pert MTB/RIF assay was an automated cartridge based test performed as per WHO guidelines. Previous studies of MTB/RIF assay have reported sensitivity of 57 % to 76.9% in smear negative but culture positive PTB and 98 to 100 % in cases of smear positive, culture positive pulmonary tuberculosis while test specificity remained at 99 to 100 % [20,21,22,23,24]. A previous study by Narute et al has reported sensitivity of Gene X-pert to be 96.9 % and specificity to be 87% in pulmonary samples [25]. In our study the sensitivity of Gene X-pert was found to be 96.67 % in smear positive culture positive specimens and 63.6 % in smear negative culture positive specimen which was in congruence with the previous studies. Overall specificity was found to be 100% with 100% positive predictive value and negative predictive value of 83.3%

Sputum Smear and culture				
Gene X-pert		positive	negative	total
	positive	29	0	29
	negative	1	5	6
total		30	5	35
sensitivity=29/30 (96.67%) ,specificity = 5/5 (100%) , PPV = 29/29 (100%) , NPV = 5/6 (83.33%)				

In a study conducted by Zeka et al the overall sensitivity, specificity, PPV and NPV of Gene X-pert were 86.8%, 93.1%, 78.5% and 96% respectively3. In the mid 1990's when WHO declared as a global emergency the impact of HIV epidemic on dynamics of TB were lesser known and the scenario of drug resistance being a rare event, smear microscopy was deemed as an ideal modality for rapid diagnosis of TB, However in the current scenario with increasing evidence of the growing proportion of drug resistant TB specially primary drug resistant TB rapid diagnostic test to rule out drug resistance have become the need of the day.

Stagnation in TB control and MDR-TB care delivery has severe consequences for TB patients, who often belong to the most vulnerable and neglected sector of society. Scientific breakthroughs such as the X-pert MTB/RIF assay (and hopefully additional new diagnostics, drugs and vaccines coming to use in the next few years) should not be withheld from these marginalized groups but deployed without undue delay, optimizing patient and public health benefits.X-pert MTB/RIF is useful for rapid and early detection of Tuberculosis, however it must be followed by culture & DST to rule out XDR-TB.

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