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COMPARATIVE STUDY DEPICTING EFFICACY OF GENE EXPERT, FLUORESCENT AFB AND ZN STAINING IN TUBERCULOSIS PATIENTS IN A TERTIARY CARE CENTRE

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Conflicts of Interest: Nil

ABSTRACT:

Background: The comparative study was conducted at Bombay Hospital in the Department of Pathology and Microbiology over a period from December 2017 to September 2018. Both in- patients and out- patients of all age groups of both sexes presenting with cough and expectoration for 2 or >2 weeks or lymphadenopathy or cold abscess with or without symptoms like loss of appetite, loss of weight, chest pain and heamoptysis.

Conclusion: Tuberculosis is a menace in developing nations. Timely diagnosis and treatment remains the major pillars to fight against this disease**.** Amongst the various diagnostic modalities available, PCR is highly sensitive and specific method as compared to ZN and fluorescent staining.

Keywords: Gene Expert, Fluorescent AFB, ZN Staining & TB.

Introduction

Tuberculosis, caused by Mycobacterium tuberculosis remains the major cause of mortality and morbidity in developing nations. According to World Health Organization (WHO), tubercular infections are currently spreading at the rate of one person per second per million people. With three millions dying from it, TB continues to be a major health trouble in our country & is the single largest cause of loss of working hours in the productive age group[1].

Despite longstanding effort to conquer tuberculosis, this disease remains an expanding global health crisis with 1.86 billion infected people[2].

Early diagnosis is essential for early patient management & successful patient outcomes. False-negative results & misdiagnosis of TB suspects are common in developing nations, as most TB control programmes use Ziehl-Neelsen (ZN) smear microscopy, which has poor sensitivity & multiple visits are required that leads to higher default. Mycobacterial culture, even though considered as the gold standard but is slow & usually takes 2-5 weeks time to yield a final result and requires proper infrastructure and technical expertise [3,4].

Fluorescent staining method although require more investment and precision is more sensitive than ZN staining is less tedious as screening is done on 40X.

Methods for the diagnosis of tuberculosis have improved in recent years and several molecular techniques for its diagnosis have been introduced for clinical use. Molecular methods provide several advantages, including confirmation of the presence of Mycobacterium tuberculosis within 1 to 3 day. Furthermore molecular techniques can differentiate MTB from NMT with precision which is not possible with conventional methods. With the rise of NMT in immunocompromised individuals better techniques need to be devised and established.

Material & Method

The comparative study was conducted at Bombay Hospital in the Department of Pathology and Microbiology over a period from December 2017 to September 2018. Both in- patients and out- patients of all age groups of both sexes presenting with cough and expectoration for 2 or >2 weeks or lymphadenopathy or cold abscess with or

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without symptoms like loss of appetite, loss of weight, chest pain and heamoptysis.

The study includes total 300 patients of both pulmonary and extra pulmonary tuberculosis

Samples included sputum sample (80), Bronchioalveolar lavage fluid (50), pleural fluid (70), pus(60), lymph node aspirated (30), and CSF (10).

Samples were collected from each patient in two clean, sterile, heat proof wide mouth containers. The processing of samples was carried out in a bio safety cabinet. Fluid was centrifuged and slides were prepared from sediment.

Each sample was then subjected to Z-N staining and Fluorescence Auramine rhodamine stain

Ziehl–Neelsen stain: The reagents used are Ziehl–Neelsen carbol fuchsin, acid alcohol, and methylene blue.

2.2 Fluorescence (Auramine o) staining: Reagents used - Auramine-Phenol solution, 1% Acid alcohol, 0.1% Potassium permanganate solution

For all the samples a nested PCR technique was applied (Gene Xpert). A nested PCR technique used to detect Mycobacterium tuberculosis and results were then compared to both ZN staining and Fluoroscent staining keeping PCR as gold standard.

Results:

Table 1: **Comparison of Zn Fluorescence Staining and PCR Reports**

Staining method used	No. of Positive smears	No. of Negative smears
ZN stain	90 (41.6%)	210
Fluorescence stain	200 (66%)	100
Gene Xpert	158(52.6)	142

Table 2: Sensitivity and specificity of Microscopy

	ZN Saining	Fluoroscent staining
Sensitivity	54%	94%
Specificity	75%	47%

Table 3: Common presenting features in suspected cases of tuberculosis

Symptoms	Male %	Female %	P
Cough	63	37	0.809
Fever	48	52	0.291
Loss of Weight/Loss of Appetite	41	59	0.642
Breathlessness	59	41	0.446
Hemoptysis	56	44	0.537
Chest Pain	62	38	0.619
Expectoration	28	72	0.439

The most common presenting symptom was cough, that is, 63%.

Discussion

TB unlike its other forms lacks systemic symptoms and has long latency periods leading to a delay in evaluation. Extra pulmonary tuberculosis patient suffer from delay in diagnosis owing to paucibacillary nature of the disease. Rapid diagnosis is critical as timely initiation of antitubercular therapy may limit the severity. Several of the rapid tests for tuberculosis have had limited clinical utility, especially in endemic countries and in paucibacillary settings. For over a century, AFB smear microscopy was the only available rapid, reliable and cost effective modality in developing nations.

The commonest presenting symptom among the suspected cases of TB was cough that is followed by cases with fever and with hemoptysis

Other commonly seen symptoms are weight loss, fatigue and in case of extrapulmonary TB: Lymphadenopathy and cold abscesses were noticed.

In the present study we compared the results of light microscopy by Z N stain smear with that by fluorescent microscopy of auramine rhodamine stain smear and Nested PCR (GENE XPERT) for detection of AFB. These results shows fluorescence staining technique is more sensitive (94% sensitivity) in detection of AFB in sputum as well as extra pulmonary samples compared to ZN stain (54% sensitivity). Although being more sensitive Fluoroscent staining lacks specificity as low as 47%. Several studies have shown fluorescent microscopy is more sensitive as compared to ZN staining.

As compared to both ZN staining and Fluorescent method PCR offers a more sensitive and highly specific diagnostic modality.

Smear result obtained by Githui et al.[5] had shown 80% by florescent microscopy & 65% by Z N staining; Ulukanligil et al. also demonstrated 85.2% positivity by fluorescence microscopy and 67.6% by Z N method. Similar result obtained by S J Murry et al. [6] had shown 9positivibyFI microscopy and 73% by Z N staining. Jain et al. [7] had shown 41% by FI and 32% by Z N stain.

Our study shows that fluorochrome staining method is more sensitive as compared to Z N staining method for demonstration of AFB. This may be due to appearance of brilliant yellow colored bacilli against more dark back ground which is easily observable.[8]

Sputum smear microscopy is a simple, rapid and inexpensive technique which is highly specific in areas with a very high prevalence of tuberculosis. It also identifies the most infectious patients and is widely applicable in various populations with different socioeconomic levels. Hence, it has been an integral part of the global strategy for TB control.[9]

In comparison with Z N stain fluorescence microscopy which was introduce in 1930, requires the fluorochrome dye, halogen or high pressure mercury lamp to excite the dye. It is more sensitive in screening the low grade smear positive sputum samples and also extra pulmonary samples and samples from HIV patients as compared to Z N staining. As it screens the smear in low power objectives, time require to screen the smear is less as compared to Z N stain which requires at least 10 - 15 minutes screening one negative smear, which is important in high burden countries like India with high work load. Cost constraints are major issues with fluorescent microscopy. sitivity of fluorescence microscopy id 10 percent higher Although senthan Z N stain, specificity is same as Z N stain and chances of false positivity are more in flurochrome stain while chances of false negativity are more with Z N stain. Over all microscopy method results are affected by type of sample, quality and quantity of sample, thickness of smear, experience of observer and sensitivity is low in comparison to culture method and molecular method[10].

Polymerase chain reaction (GENE XPERT) on the other hand overcomes the disadvantage of low sensitivity of ZN staining and low specificity of Fluoroscent staining. With the advent of Non mycobacterium tuberculosis, which has a different treatment modality we need more specific technique as both staining methods do not differentiate between MT and NMT. Although the cost involved with the set up and test procedures are more, it has added advantage of detecting rifampicin resistance in hours[11]

At our institute we meticulously use both fluorescent microscopy and Nested PCR technique supported by culture to offer a better

diagnosis to help in patient care. Few (42) cases that were positive on Fluoroscent study were found to be negative on PCR, depicting low specificity of Fluoroscent staining.

Conclusion:

Tuberculosis is a menace in developing nations. Timely diagnosis and treatment remains the major pillars to fight against this disease**.** Amongst the various diagnostic modalities available, PCR is highly sensitive and specific method as compared to ZN and fluorescent staining. The main advantages of PCR are, for diagnosis, reliability when compared to microscopy and the speed of getting the result when compared with the culture test. For diagnosis of TB, although microscopy is both quick and cheap, it is often unreliable. It is particularly unreliable when people are HIV positive. Although culture gives a definitive diagnosis, to get the result usually takes weeks rather than the hours of the Genexpert test[12].

The main advantage in respect of identifying rifampicin resistance is again the matter of speed. Normally to get any drug resistance result takes weeks rather than hours.

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