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Original Research Paper

Microbiology

IMPORTANCE OF CHANGING TRENDS IN DIAGNOSTIC YIELD OF TUBERCULOSIS- A STUDY DONE UNDER NATIONAL TUBERCULOSIS ELIMINATION PROGRAMME

Dr. Smita Mohod	Assistant Professor.
Dr. Swati Bhise	Professor and Head.
Dr. Prasanna Raut*	Senior Resident. *Corresponding Author
Dr. Bhoomika Choudhary	Junior Resident.

ABSTRACT

Introduction: In underdeveloped countries, Tuberculosis (TB) is a major source of morbidity & mortality. For clinical microbiologists, identifying and detecting *Mycobacterium tuberculosis* (MTB) constitutes as an important hurdle. Thus, to eliminate the disease early and lower morbidity, early diagnosis of TB and drug resistance is crucial. When there is insufficient, inadequate sputum and paucity of bacilli, diagnosing TB can be difficult. Our study aimed to compare Ziehl-Neelsen's (ZN) stain, Fluorescent stain, and CBNAAT (Cartridge based nucleic acid amplification assay) in accordance with National Tuberculosis Elimination Programme (NTEP) guidelines. **Methods:** A total of 500 Respiratory samples of clinically suspected TB cases were tested using ZN stain, Fluorescent stain & CBNAAT. This prospective Cross-sectional study was conducted in Department of Microbiology at tertiary care hospital, Nagpur over a period of 6 months. The results were recorded and analysed. **Results:** In our study, out of 500 cases, 372 were males and 128 were females with TB positivity rate more in rural area than in urban area. Overall, 30 (6%) were found positive on ZN staining, 50 (10%) were positive on Fluorescent staining & CBNAAT had positivity rate of 80 (16%). **Conclusion:** The sensitivity of CBNAAT is more than Microscopic staining methods. While sensitivity of Fluorescent staining (62.5%) is more as compared to ZN staining (37.5%). For smear-negative cases, the CBNAAT assay is an effective & reliable method. When diagnosing TB, this technique is highly appealing due to its simplicity, sensitivity, speed, and automation. It also offers the benefit of detecting patients that are Multi-drug resistant (MDR).

KEYWORDS : Tuberculosis (TB), ZN staining, Fluorescent staining, CBNAAT (Cartridge based nucleic acid amplification assay), Multi-drug resistant (MDR).

INTRODUCTION:

Tuberculosis (TB) is caused by bacilli *Mycobacterium tuberculosis* (MTB), one of the most prevalent infectious diseases in the world. Moreover, TB was the most common infectious agent-related cause of death globally until the COVID-19 pandemic (it was higher than HIV/AIDS) [1]. An estimated 2 billion people (~29% of the world's population) are exposed to MTB each year, and 8 million new cases of TB are diagnosed, leading to 2 million deaths. As a result, TB continues to be a public health concern (WHO, 2006) [2]. Lungs are the organ/site most frequently affected by MTB, accounting for 90% of cases. Chest pain, fever, and expectoration in the cough are typical symptoms. The emergence of resistant strains, including Multi drug resistant (MDR) and Extensively drug resistant (XDR) strains, has presented a significant challenge, and the inability of traditional sputum microscopy to identify resistance has contributed significantly to the spread of the illness [3].

The primary method of diagnosis in low- and middle-income / underdeveloped countries is microscopic examination. However, this method only has a sensitivity of 50–60% in cases of confirmed (bacillary) pulmonary TB, and an even lower sensitivity (<30%) in patients who are HIV-positive, immunosuppressed, or in children [4]. Auramine was first used as a fluorescent marker in the 1940s. By concentrating sputum in a sediment and using auramine-O fluorescence staining, direct microscopy's sensitivity can be increased, but this method is insufficient to differentiate MTB from other mycobacteria. Microscopy can also help us assess the severity of the disease as well as treatment outcome. For the analysis of patient sputum from pulmonary TB patients, *Kivihya-Ndugga et al*. (2003) compared the efficacy and cost-effectiveness of FM with those of the ZN approach. It has been demonstrated that FM has more sensitivity when evaluating cost-effectiveness (78 % vs 60 % for ZN) [5]. The current gold standard for the quick identification of MDR TB is the liquid culture technique with molecular line probe, according to the World Health Organisation (WHO). However, growth needs a highly specialised, regulated laboratory setup, as well as highly skilled personnel, and it takes two to six weeks to yield results [6]. The sole advantage of the rapid diagnosis is that different specimens can be used to identify even minute genomic copies of MTB using these methods. The WHO most recently approved the CBNAAT or GenXpert for the diagnosis of tuberculosis. The introduction and expansion of CBNAAT was implemented by National Tuberculosis Elimination Programme (NTEP) under the National Strategic Plan 2012-17. It has been reported that CBNAAT may accurately diagnose both pulmonary and extrapulmonary tuberculosis. It's the first fully automated, quick, easy benchtop CBNAAT TB detection test that needs minimum technical knowledge [7]. It takes about 10,000 AFB/mL of sputum for standard microscopy to detect them. GeneXpert (CBNAAT) has a detection limit of approximately 131 bacilli/mL of specimen. GeneXpert (Xpert Ultra) detects 16 bacilli/mL, making it more sensitive and selective than GeneXpert [8]. Xpert MTB/RIF uses three distinct primers & unique molecular probes to achieve a high degree of specificity in the detection of MTB and mutations that confer rifampicin resistance. In less than two hours, the assay delivers results directly from the sample [9].

METHODS

The prospective Cross-sectional study was conducted in Department of Microbiology of a tertiary care hospital in Nagpur. A total of 500 samples of suspected symptomatic patients of Pulmonary TB from TB Chest Medicine OPD were collected over a period of 6 months i.e. from November 2023 to April 2024 under NTEP. All extrapulmonary samples, Patients not giving consent, Patients on/previously taken Antitubercular treatment were excluded.

Sample Collection & Storage:

Sputum samples (Spot and early morning) of these patients were collected in wide mouth screw capped sterile container. Other samples included Bronchoalveolar lavage (BAL),

Gastric lavage (GL). Sample was divided into 2 parts: one part used for CBNAAT and rest was divided for direct microscopy.

Microscopy:

All the samples were subjected to staining. Mucopurulent portion of the sputum was picked with a piece of clean wooden stick and an oval shaped uniform smear measuring 2 x 3 cm in size was prepared. 2 such smears on different slides were made from each sample. The uniformity of smear was assessed by placing the slide over a printed paper. It should be able to read the print. The slides were subjected to ZN-staining & fluorescent staining and screened under light microscope and LED microscope respectively. The interpretation was confirmed based on grading given by NTEP guidelines [10,11]. Acid fast bacilli (AFB) were seen as red, beaded rods when assessed under oil immersion (X 100) lens (fig no- 1.1). Whereas for Fluorescent staining the tubercle bacilli appeared bright brilliant greenish yellow against dark background using 40 X lens using LED microscope (fig no- 1.2).

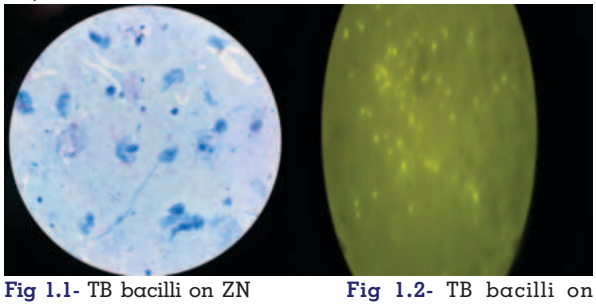

Fig 1.1- TB bacilli on ZN stain.

Fig 1.2- TB bacilli on Fluorescent stain

Cartridge Based NAAT:

All the samples were tested by CBNAAT as per manufacturer's guidelines and results were interpreted as MTB Detected/ MTB Not detected and RIF resistance detected/not detected.

A comparison of findings from all three diagnostic modalities was done, results were statistically analyzed and P value calculated.

RESULTS

1) Gender wise distribution: Out of 500 TB suspected cases, 372 (74%) were males and 128 (25%) were females showing male preponderance. TB positivity was seen in 80 cases, among which 61 (76%) were males and 19 (24%) were females as shown in fig. no 2.

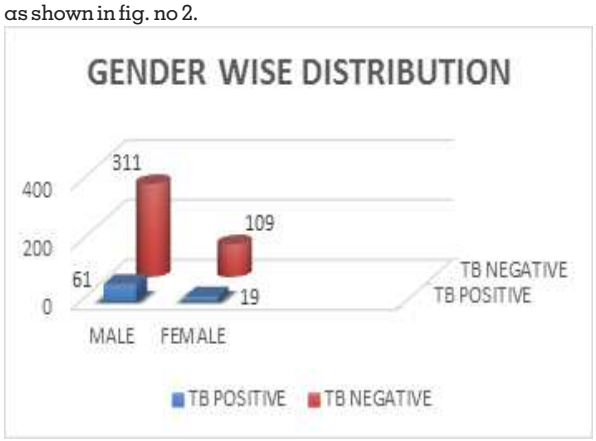

Fig no 2- Gender wise distribution

2) Geographical distribution: Among the 500 cases, 327 cases were from Rural area while 173 were from Urban area. Out of these TB positives were 49 (61%) & 31 (39%) from rural and urban areas respectively which showed higher rate of TB cases from Rural areas as compared to Urban area as shown in Fig no. 3.

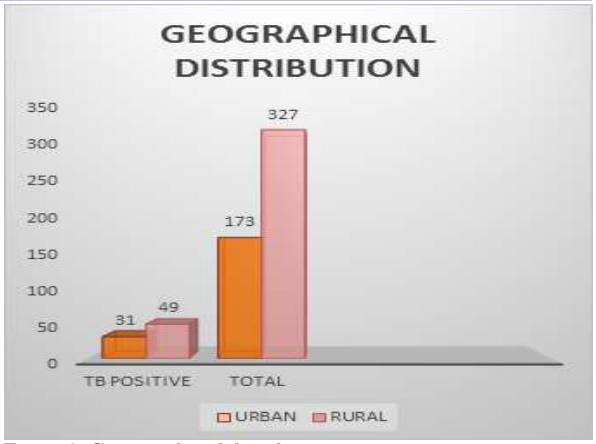

Fig no 3- Geographical distribution

3) Age Wise Distribution: The number of TB cases were found to be maximum in the age group of 31-45 years which is about 65%, followed by age group of 15-30 years showing 20% of total positive cases. Those with age of >50 years showed 14% positive rate while lowest rate of about 1% was seen in below 15 years of age as shown in Table no. 1.

Table No. 1- Age Wise Distribution

AGE	NO OF TB SUSPECTED CASES	TB POSITIVE CASES
<15	14	1 (1%)
15-30	132	16 (20%)
31-45	292	52 (65%)
>50	62	11 (14%)
TOTAL	500	80 (100%)

4) HIV-TB Co-infection: Out of the 500 cases tested, 444 cases were HIV negative while 56 cases had HIV positive status. TB positive results were seen in 21 cases among HIV positive patients and 59 cases among HIV negative patients showing positivity rate of 38% and 13% respectively as shown in fig. no 4. Also, the HIV positivity was higher in TB negative cases (62%) than in TB positive cases (38%).

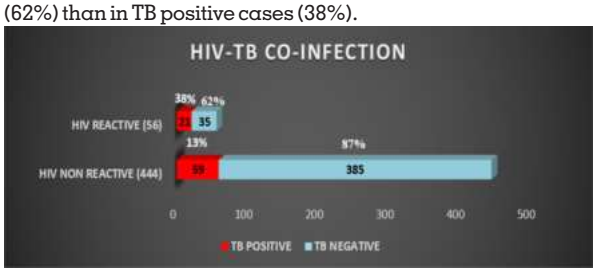

Fig no. 4- HIV-TB Co-infection

5) Diagnostic Tools:

Microscopy: The samples from all 500 cases were subjected to microscopy by doing ZN staining and Fluorescent staining. Out of these, 30 (6%) were found positive on ZN staining while 50 (10%) were found positive on Fluorescent staining as shown in Table no. 2.

CBNAAT: All these samples were again tested on CBNAAT and results were recorded. 80 (16%) cases among the total 500 tested were found to be positive. This was higher than those tested by microscopic methods. Out of the 80 positive samples, Rifampicin resistance was detected in 15 patients as shown in Table no. 2.

Thus, it was found that sensitivity of CBNAAT (100%) is much more than Microscopy. While sensitivity of Fluorescent staining (62.5%) was more compared to ZN staining (37.5%). Since culture was inaccessible, CBNAAT was used as the gold standard to compare test sensitivities (table no- 3).

Table No. 2- Comparison Of Diagnostic Findings Of TB Cases

DIAGNOSTIC TOOLS FOR TB	ZN STAINING		FLUORESCENT STAINING		CBNAAT	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
TOTAL NO OF SAMPLES (500)	30 (6%)	470 (94%)	50 (10%)	450 (90%)	80 (16%)	420 (84%)
RIFAMPICIN RESISTANT	NA		NA		15 (19%)	

Table No.3- Sensitivity & Specificity Of Diagnostic Tests

	ZN STAINING	FLOURESCENT STAINING	CBNAAT
TB POSITIVE	30	50	80
TB NEGATIVE	470	450	420
TOTAL SAMPLES	500	500	500
SENSITIVITY	37.5%	62.5%	100%
SPECIFICITY	100%	100%	100%

DISCUSSION:

The present study showed that, out of 500 cases tested, 372 (74%) were males and 128 (25%) were females showing male preponderance. Also, the positivity rate was higher in males (76%) than females (24%) (figure 2). A similar study was conducted by *Ashwini BS et al* (2020) [12], showing similar results with male preponderance. Men tend to have higher rates of proximal risk factors for TB, such as drinking and smoking. Also, men often stay out later than women, which increases the chance of acquiring an infection. Men may be more vulnerable to *M. tuberculosis* due to their larger lung capacities because the disease is unusual in that it is genuinely airborne rather than disseminated by respiratory droplets.

In this study, out of 500 cases, majority cases were from Rural area (327) while others from Urban area (173). Also, TB positivity rate was similar showing higher prevalence in Rural area 49 (61%) than Urban area 31 (39%) (fig no 3). A similar study conducted by Surpreet kaur et al (2018) [13] & *Mukul Singh et al* (2023) [14], showed higher prevalence of TB cases in rural areas. Information regarding community management of TB and treatment-seeking patterns in rural area is rare, poor socio-economic status, overcrowding, lack of education also contributes to higher prevalence of cases from Rural areas. Since private labs are primarily used by the urban population, there is no record of them being entered, resulting in fewer cases overall.

Out of 500 cases, maximum number was found to be in age group of 31-45 years (292) followed by age group of 15-30 years (132). Lowest number was from below 15 years age (14). Also, the positive cases were found to be maximum in 31-45 years of age (65%), followed by 20%, 14% & 1% in age groups 15-30, >50 & <15 years respectively (table 1). Similar findings were seen in study conducted by *Sriram Selvaraju et al* (2023) [15]. As people age, a higher prevalence of tuberculosis (TB) may be caused by more frequent social interactions and increasing usage of public transportation, which might increase exposure.

The HIV-TB co-infection rate in our study was 38% while HIV positivity rate in TB negative case was 62% (fig 4) and 13% of TB positive cases were HIV negative. A study conducted by *Tiewsoh.et al* (2023) [16] showed co-infection rate of 42%. In HIV positive patients, the chances of developing Extra-pulmonary TB are more than Pulmonary TB[17]. This fact coincides with our study as we found HIV positive rate less in Pulmonary TB positive cases (38%) than pulmonary TB negative cases (62%). The findings were statistically significant (P<0.0001).

Diagnostic tools: In our study all the samples were tested by ZN staining, Fluorescent staining & CBNAAT. TB positivity rate by all these methods was, 30 (6%) were found positive on ZN staining while 50 (10%) were found positive on Fluorescent staining. CBNAAT reported 80 (16%) cases which were much higher than above two methods (table no- 2). All the differences between yields of smears verses CBNAAT were highly statistically significant (P<0.0001). *Loveena Oberoi et al* (2021) [18] conducted a study showing similar findings with TB positive cases detection rate higher with CBNAAT. *Rathod UD et al* (2016) [19] also demonstrated that sensitivity of Fluorescent staining was more than ZN staining.

Thus, it concludes that sensitivity of CBNAAT (100%) is higher followed by Fluorescent staining (62.5%) and lowest sensitivity is that of ZN staining (37.5%) (Table no- 3). Also, CBNAAT could detect Rifampicin resistance found in 15 (19%) TB positive cases which could not be done by staining methods. In a study by *Jain G et al* (2019) [20], they showed 15% Rifampicin resistance on CBNAAT.

The COVID-19 pandemic still negatively affects both the burden of TB disease and access to TB diagnosis and treatment. Reductions in the reported number of cases of TB in 2020 and 2021 indicate that more people may have undiagnosed and untreated TB [21]. The early identification of cases is crucial to the control of the disease, even with the abundance of very sensitive diagnostic technologies at our disposal. This process is particularly dependent on the identification of acid-fast bacilli in clinical samples. Thus, CBNAAT are the go-to option for improving turnaround times because of their quick results, great sensitivity, and low technical knowledge.

CONCLUSION:

Smear negative TB cases forms majority of these undiagnosed cases that leads to morbidity and mortality. CBNAAT's introduction for peripheral sites is a game-changer in the fight against tuberculosis, offering fast, real-time results that benefit the patient, as well as the added benefit of lowering testing personnel risks. With the capacity to detect even those cases that could be missed due to restrictions such as paucity of Bacilli, person-to-person variance in observation, defective staining procedures, inappropriate reagents, insufficient sputum the greater positivity rate of CBNAAT over ZN staining is the cherry on top. Additionally, it can be used to test for MDR-TB and detect rifampicin resistance. In MDR-TB era, this study highlights the high sensitivity of CBNAAT technique for proper management, better treatment outcome and successful TB control, lowering the rates of morbidity and mortality. Thus, now there is a shift in approach to diagnosis of TB from conventional microscopy to molecular techniques (NAAT) in NTEP.

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