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Research Article

TO DETECT MYCOBACTERIUM TUBERCULOSIS AND ITS SENSITIVITY TO RIFAMPICIN USING CBNAAT OF SPUTUM NEGATIVE PATIENT WHO HAVE SYMPTOMS OF TB IN DISTRICT HOSPITAL GONDA

Adarsh Srivastava[1*], Vipul Kumar[2], Farhat Aziz[3]

1MMLT, Student, Dept. of Medical Laboratory Science, SCPM College of Nursing & Paramedical Sciences, Gonda, U.P. India.
2 Associate professor, SCPM College of Nursing and Paramedical Sciences, Gonda,U.P. India.
3 Assistant Professor, SCPM College of Nursing and Paramedical Sciences, Gonda, U.P. India.
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Abstract

Aim:

To detect Mycobacterium tuberculosis and evaluate its Rifampicin resistance using CBNAAT in patients at District, Hospital Gonda who exhibit TB symptoms but have negative sputum test results.

Material and Method:

In the Department of Pulmonary Medicine at District Hospital, Gonda, we performed a retrospective study to assess the utility of CBNAAT over the period from March 2022 to February 2023. The study encompassed all patients who underwent CBNAAT testing during this timeframe. Prior to CBNAAT/RIF testing, sputum samples were first examined using Acid Fast Bacilli (AFB) smear microscopy. These tests were conducted at diagnostic centers equipped for CBNAAT/RIF testing. Data collection and organization were carried out using Microsoft Excel, and the analysis was performed with SPSS 20.0.

Results:

During the study period, 120 sputum samples underwent CBNAAT testing. Out of these, 98 cases (81.7%) yielded negative results and 22 cases (18.3%) were positive. Among the suspected TB instances, 14 (63.6%) were male and 8 (36.4%) were female. A resistance rate of 13.6% (3 cases) was observed for rifampicin.

Conclusion:

CBNAAT has established itself as a trustworthy diagnostic method for individuals suspected of having tuberculosis despite negative sputum results, offering notable sensitivity, specificity, and prompt result generation. Essential in the present scenario marked by a growing burden of pulmonary tuberculosis infections is the imperative need to address the escalating rates of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis.

Keywords: Cartridge-Based Nucleic Acid Amplification Test**,** Mycobacterium tuberculosis, Rifappicin, Multidrug-resistant tuberculosis.

1. Introduction

According to a 2019 WHO report, approximately 10 million individuals globally are infected with TB, yet only seven million have access to TB care, leaving three million undiagnosed. The WHO's Global TB Report indicates that in 2018, TB claimed the lives of 1.5 million people. That same year, India was among the 30 countries with the highest TB burden, accounting for 87% of new TB cases. Eight countries are responsible for two-thirds of the total cases, with India at the forefront, followed by China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa[1].One of the most persistent diagnostic challenges in tuberculosis control arises from sputum smear-negative cases, which constitute a significant proportion of the overall TB burden[2].

In India, known for its substantial tuberculosis prevalence, the Revised National Tuberculosis Control Program (RNTCP) has embraced the use of CBNAAT for the detection of tuberculosis in cases where sputum is negative or the infection is extra pulmonary[3]. There arises a need for rapid and more accurate diagnostic test for TB for prompt treatment and global TB control. In December 2010, World Health Organization (WHO) has endorsed Gene Xpert® MTB/RIF, a Cartridge based nucleic acid amplification test (CBNAAT) which can diagnose active TB disease and multidrug-resistant (MDR)

*Adarsh Srivastava,MMLT Student, SCPM, College of Nursing and Paramedical Sciences Gonda U.P. India.
E mail: asadarsh.clj@gmail.com
ORCID ID: 0009-0000-0000-0000
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TB in less than 2 hour[4]. Majority of mutations manifest at codons 516, 526, and 531 within the Rifampicin Resistance Determining Region (RRDR), commonly referred to as the hotspot area[5,6].

MATERIALS AND METHODS

This retrospective investigation was conducted at the Department of Pulmonary Medicine within Gonda District Hospital between March 2022 and February 2023. The study focused on analyzing sputum samples from 120 tuberculosis suspects who tested negative for acid-fast bacilli (AFB) using smear microscopy. Data was collected from CBNAAT Centre.Data collection and organization were carried out using Microsoft Excel, and the analysis was performed with SPSS 20.0.

Samples for analysis were collected in sterile 50 ml containers according to the guidelines of the Revised National Tuberculosis Control Program (RNTCP). Subsequently, the samples were refrigerated at a temperature ranging from 2 to 8 ^{0}C. The purulent section of the specimen was utilized to prepare a direct smear on a clean slide measuring 3X1 cm. After air drying, the smears were heat-fixed and subjected to Ziehl-Neelsen staining (ZN stain) using Ziehlcarbol-fuchsin and heat, followed by decolorization and staining with methylene blue. The grading of the AFB-stained sputum smear was conducted according to the guidelines provided by the Revised National Tuberculosis Control Program (RNTCP). During the CBNAAT analysis on the Xpert/MTB/RIF assay, a sample of 1 ml was mixed with 2 ml of sample reagent in a falcon tube and stirred thoroughly. This mixture was allowed to sit at room temperature for about 10 to 15 minutes before being transferred into the sample cartridge chamber using a sterile graduated pipette. The cartridge was then inserted into the GeneXpart (CBNAAT) device. The GeneXpartDx System software, which uses an algorithm to assess fluorescent signals, was employed to complete the data analysis and interpretation[7-12].

RESULTS

Total 120 sputum samples collected, first staining with Acid Fast Bacilli stain (AFB) for reconfirming that the samples are AFB negative, then samples are further processed and tested with CBNAAT/RIF assay for detection of organism and sensitivity of organism to Rifampicin. In our study of the 120 sputum tested for CBNAAT. 22 cases (18.3%) were found positive for Mycobacterium Tuberculosis with result MTB Detected, and remaining 98 cases (81.7%) were found negative for the CBNAAT Assay [Figure No. 1, 2 and 3]. A total of 22 MTB Positive of sputum were collected from 14 cases (63.6%) males and 8 cases (36.4%) females who have symptoms of Mycobacterium Tuberculosis [Figure No. 4]. The patients were between 15 years and 60 years of age. Maximum number of CBNAAT detected Mycobacterium Tuberculosis patients 8 (36.4%) were in the age group of 36–45 years followed by 6 (27.3%) in the age group of 26-35 years, 5 (22.7%) in the age group 46–60 years, and 3 (13.6%) in the age group of 15–25 years [Figure No. 5]. Mycobacterium Tuberculosis was observed in 22 cases; out of which 16 (72.7%) were Rural area and 6 (27.3%) cases were Urban area [Figure No. 6]. Out of the total 22 RIF Assay tested by detected Rifampicin resistance. 19 cases (86.4%) were Rifampicin sensitive and 3 cases (13.6%) were Rifampicin resistance. GENEXPERT being prime molecular technique in the detection of bacterial DNA in lesser time and have added advantage of RIF resistance detection which helps in early detection of resistant infection and better treatment [Figure No. 1, 2 and 7

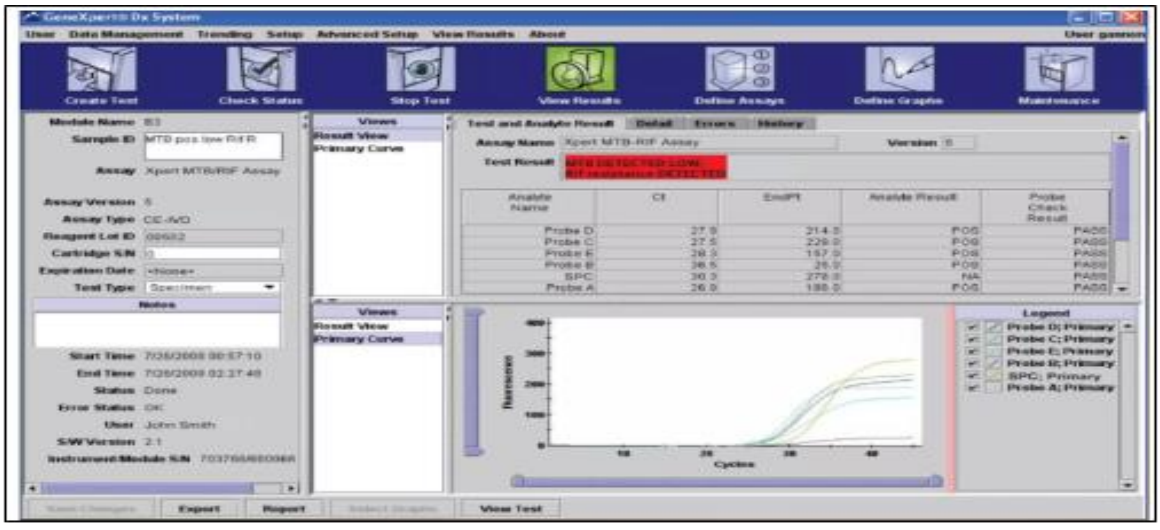

Figure 1:- MTB Positive and Rifampicin Resistance detected by Xpart MTB/RIF Assay.

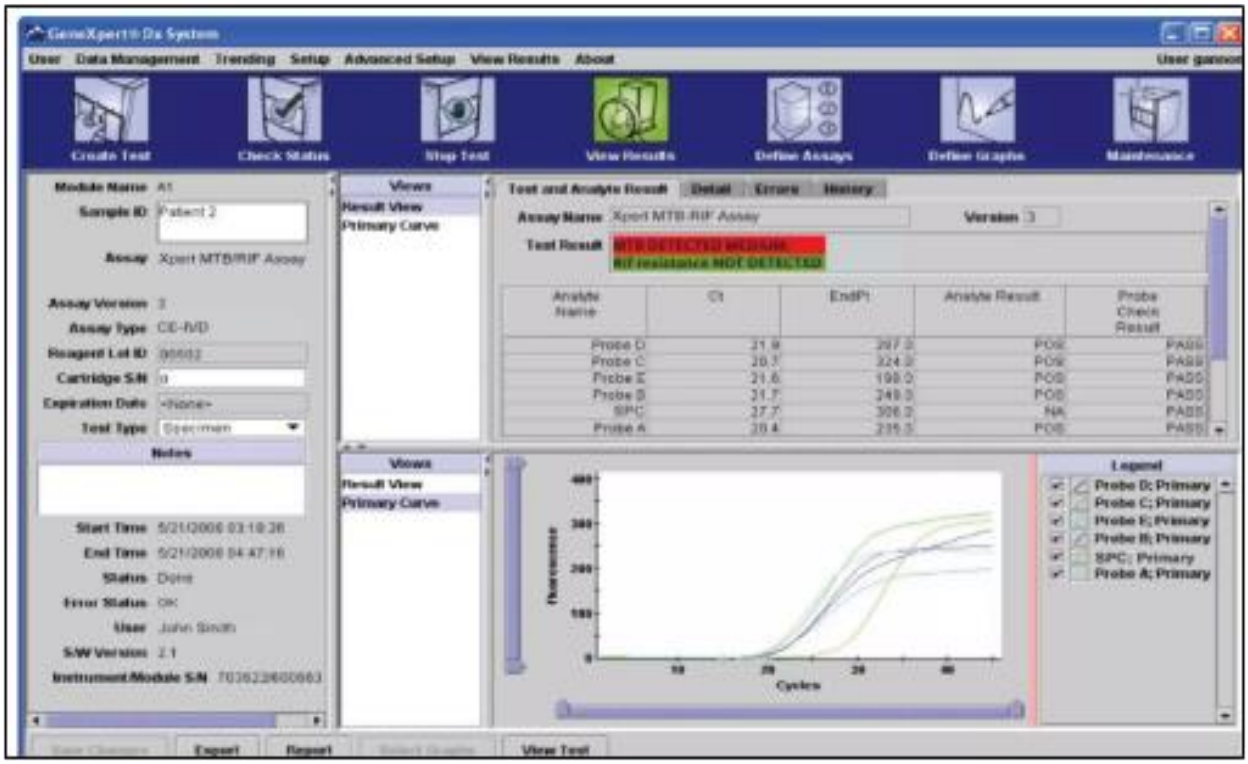

Figure 2:- MTB Positive and Rifampicin Sensitive detected by Xpart MTB/RIF Assay.

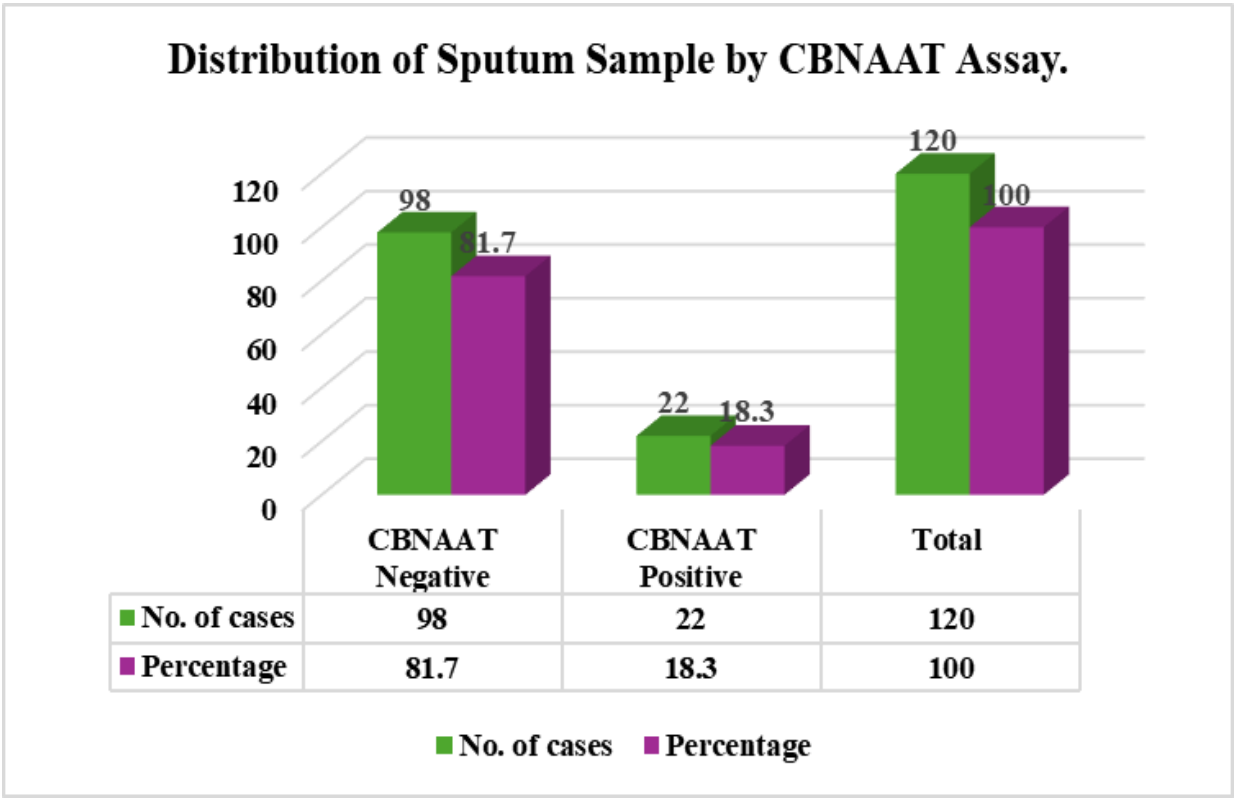

Figure 3:- Distribution of Sputum Sample by CBNAAT Assay.

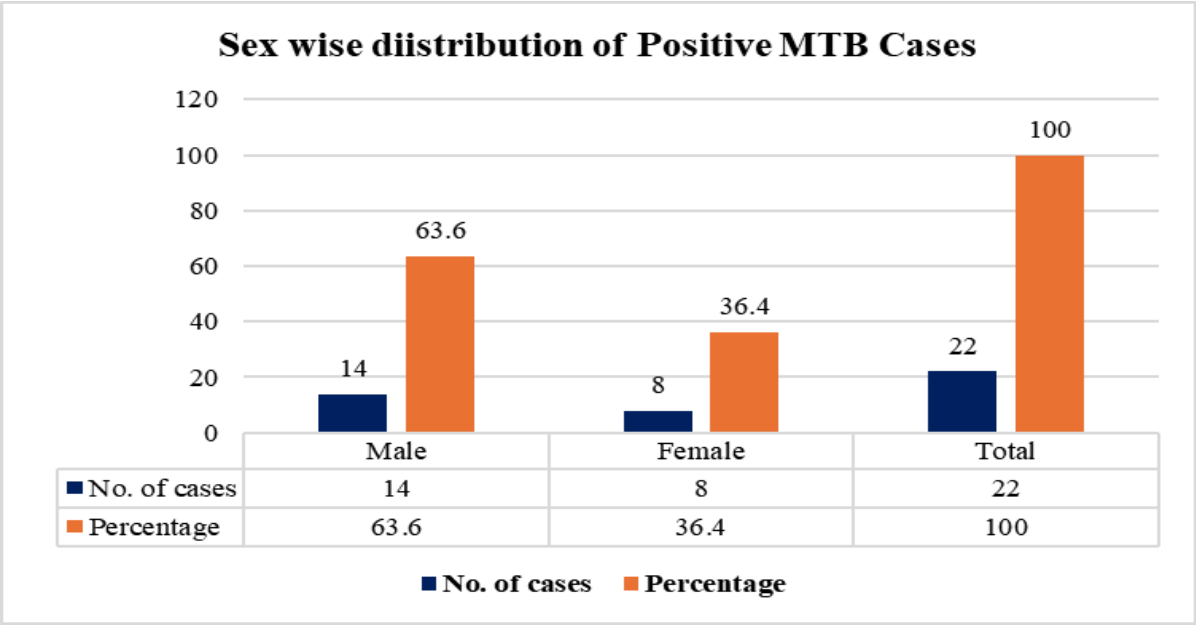

Figure 4:- Sex wise distribution of Positive MTB Cases

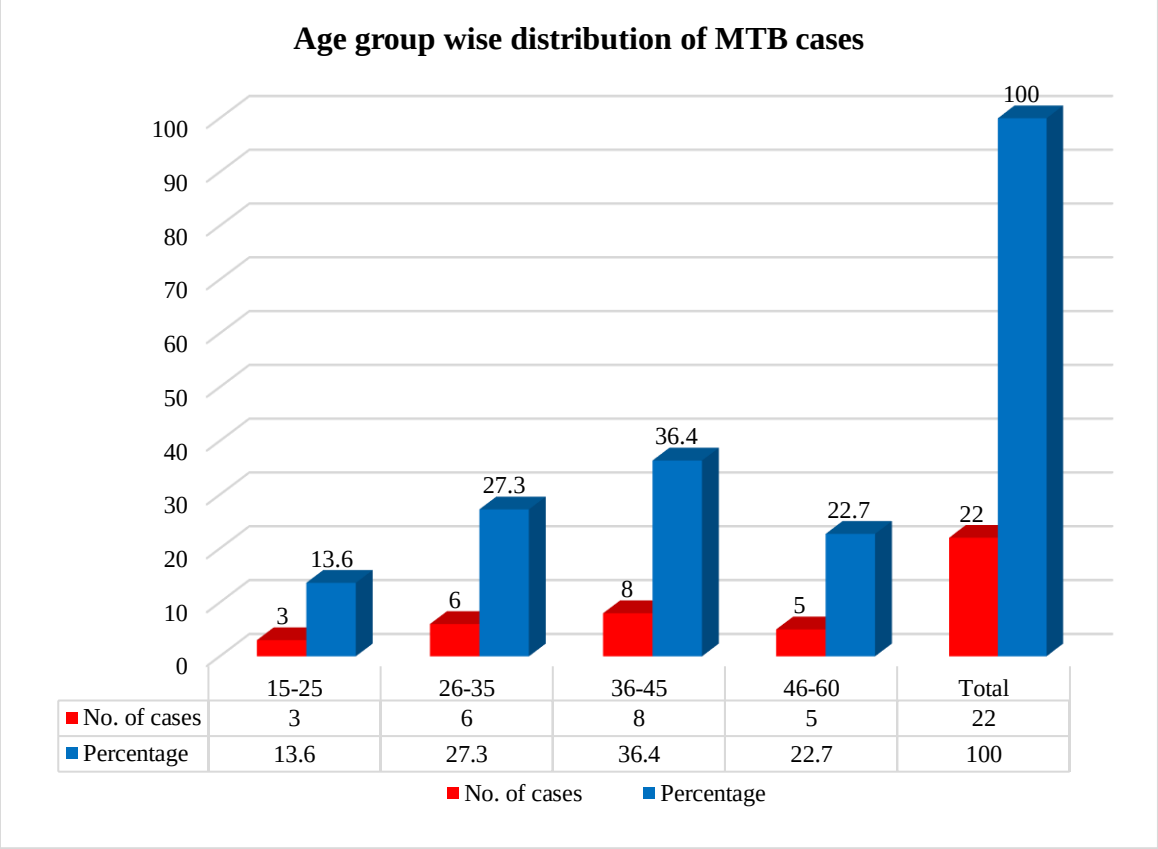

Figure 5: Age group wise distribution of MTB cases.

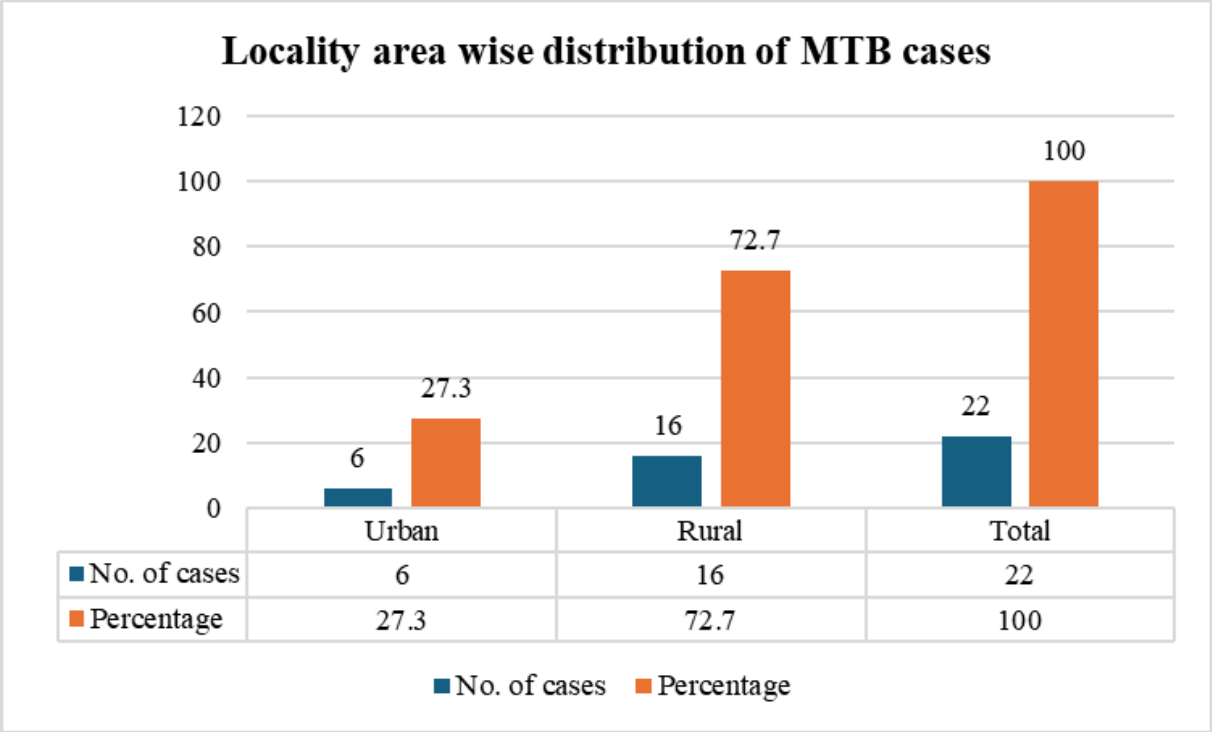

Figure 6:- Locality area wise distribution of MTB cases.

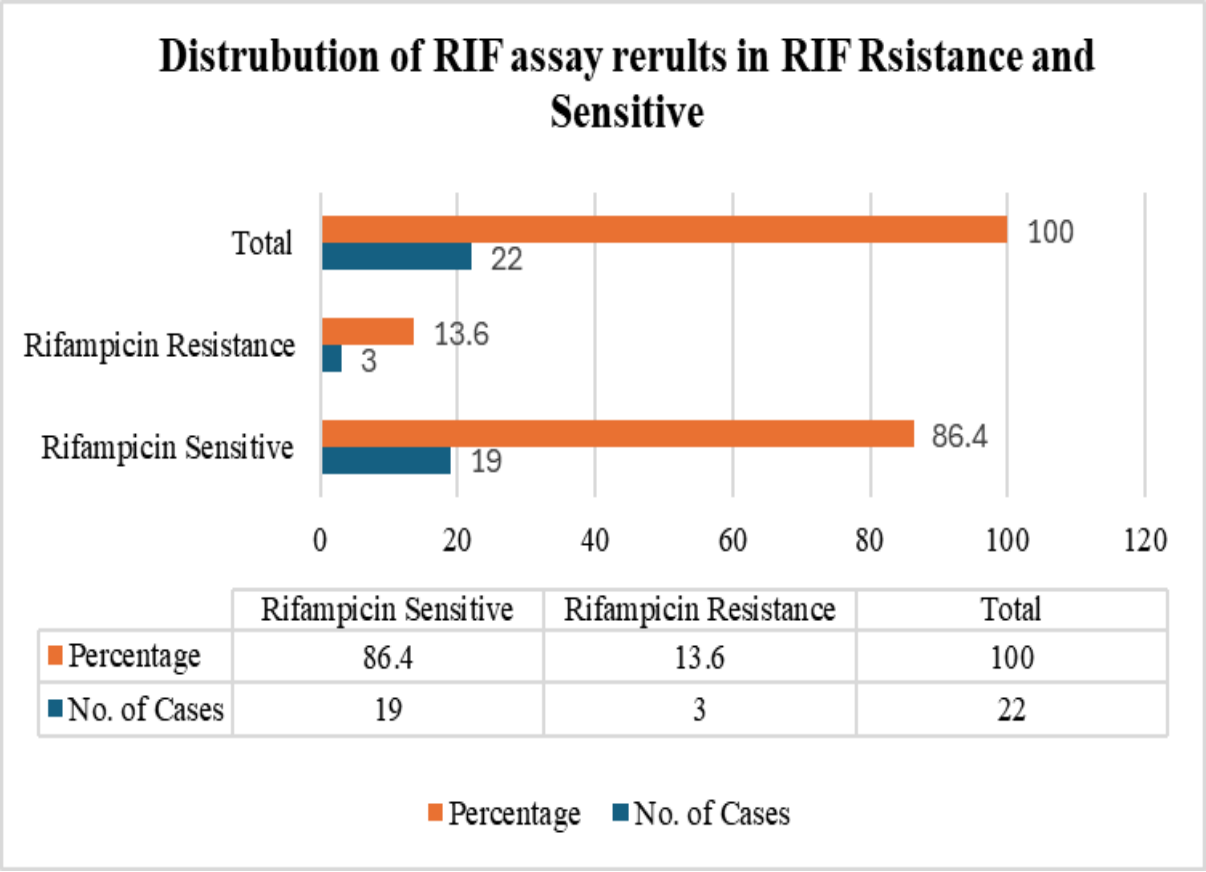

Figure 7:- Distribution of RIF assay results in RIF Resistance and Sensitive.

DISCUSSION

Identifying and treating TB at an early stage is essential to reduce its spread. Recently, the WHO has approved CBNAAT as a diagnostic tool for TB. Our research revealed that CBNAAT identified approximately 22 cases (18.3%) of patients who tested negative on smear tests. Additionally, the CBNAAT/RIF Assay detected rifampicin-resistant TB in 3 cases (13.6%).

The 2010 Global Burden of Disease estimates show that 57% of all tuberculosis deaths globally occurred among people older than 50, with more than half of these deaths in those aged 65 and above. 8 In our study maximum positivity for MTB was found in age group 35-45 years age range. This may be ascribed to increased life expectancy and waning immunity in adults. Prompt identification of TB cases is vital for breaking the chain of transmission of the disease. It is well-established that untreated TB patients who are AFB smear microscopy positive are the primary source of infection to healthy individuals. Studies have also shown that about 17% of TB transmission is linked to individuals suspected of having TB but who are AFB smear microscopy negative. Therefore, the possibility of disease spread from AFB-negative cases to healthy individuals should not be underestimated [13]. A study conducted by Dewan et al. in Delhi reported a 25% resistance rate to rifampicin. This elevated resistance level compared to our findings might be attributed to the higher incidence of MDR TB in northern India and referrals from various states.[14] Research by Virupakshappa V et al. in South India identified a 2.4% resistance rate to rifampicin. The lower resistance level compared to our results could be due to the higher prevalence of MDR TB in northern India and patient referrals from different states [15]. A mutation in the rpoB gene, specifically at the 531 codons, was identified in 58% of patients as Probe E mutation. This was the most common, followed by Probe D at 21%, Probe A and Probe B Each at 10%, and Probe C at 1%. These findings align with a study conducted in Malawi, Africa [16]. In research carried out by Vijay et al, 425 samples were analyzed, revealing a male predominance of 68% compared to females at 32%, with participants aged between 18 and 65 years on average. Our own investigation found that among 22 individuals testing positive for MTB, males were more prevalent with 14 (63.6%) cases compared to females with 8 (36.4%), with the majority of MTB detections (36.8%) occurring in individuals aged 36 to 45 years [17]. The investigation determined that the GeneXpert assay demonstrates notably enhanced sensitivity in detecting Mycobacterium tuberculosis (MTB) compared to Ziehl-Neelsen (ZN) smear microscopy in pulmonary specimens. Although conventional culturing remains the benchmark for MTB diagnosis, it is time-consuming to cultivate MTB colonies and lacks the capacity for simultaneous identification of rifampicin-resistant/drug-resistant (RIF/DR) strains. Nonetheless, the GeneXpert assay serves as a valuable diagnostic instrument for prompt MTB identification in acid-fast bacilli (AFB) smear-positive and smear-negative tuberculosis (TB) suspects, enabling concurrent detection of multidrug-resistant TB (MDR-TB). Such early detection aids in curbing disease transmission, promptly initiating TB treatment, and consequently leads to a substantial decrease in MDR-TB incidences.

CONCLUSION

The purpose of the present study was to Detect Mycobacterium tuberculosis and its sensitivity to Rifampicin using CBNAAT of sputum Negative patient who have symptoms of TB in District Hospital Gonda. On completion of the study, in which the samples from patient having tuberculosis-like symptoms were collected and examined first with AFB/ZN technique and after found negative samples were further treated and tested with Cartridge Based Nucleic Acid Amplification Test [CBNAAT] for detection of latent infection which can't be diagnosed with AFB smear microscopy. The use of CBNAAT assay will help in early detection of infection and RIF resistance detection is helpful in providing proper treatment, which is a must requirement in current situation where load of pulmonary tuberculosis infection is rising day by day with an increase in MDR TB and XDR TB. Techniques like CBNAAT are boon in treatment of infection as it provides reliable results in lesser time than conventional methods like culture and others. Implementation of CBNAAT assay in small laboratories and hospital will have a great result in early detection and treatment. Testing the patient's sputum which is found AFB negative with CBNAAT/RIF assay.

8. Conflict of Interest: Nil

9. References

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