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Original Article

Respiratory Medicine Section

Association Between Cycle Threshold Value of Cartridge-Based Nucleic Acid Amplification Test and Clinical Severity of Pulmonary Tuberculosis: A Cross-sectional Study

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ABSTRACT

Introduction: Tuberculosis (TB) is a major global health problem caused by *Mycobacterium tuberculosis* (MTB). The National Tuberculosis Elimination Programme (NTEP) emphasises early diagnosis and treatment of TB cases. The Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) is a semi-automated molecular assay that rapidly detects MTB Deoxyribonucleic Acid (DNA) and Rifampicin (RIF) resistance-associated mutations using Real-Time Polymerase Chain Reaction (RT-PCR).

Aim: This cross-sectional study aims to assess the association between the Cycle Threshold (Ct) value of CBNAAT and the clinical severity of Pulmonary Tuberculosis (PTB).

Materials and Methods: The study was conducted at the Department of Respiratory Medicine, Navodaya Medical College Hospital and Research Centre-Raichur, from January 2020 to July 2021. A sample size of 106 participants, aged ≥18 years and meeting the NTEP guidelines for presumptive PTB cases, were enrolled. Participants underwent chest radiography, sputum smear microscopy for Acid-Fast Bacilli (AFB), and CBNAAT testing. Clinical severity of PTB was categorised as mild, moderate, or severe using the Bandim TB score. Data was entered into Microsoft Excel 2017, and statistical analysis was performed using SPSS version 23. Descriptive statistics, frequencies, percentages, means, and standard deviations were calculated, and the chi-square test was utilised to assess associations between qualitative variables.

Results: Among the participants, the majority (55.7%, n=59) had moderate clinical severity. Of these, 12.3% (n=13) had high CBNAAT Ct values, 20.8% (n=22) had medium Ct values, 11.3% (n=12) had low Ct values, and 11.3% (n=12) had very low Ct values. A total of 31.1% (n=33) had severe clinical severity, with 13.2% (n=6) having high Ct values, 12.3% (n=13) having medium Ct values, and 6.6% (n=7) each having low and very low Ct values. Additionally, 13.2% (n=14) had mild clinical severity, with 0.9% (n=1) having high Ct values, 3.8% (n=4) having medium Ct values, 5.7% (n=6) having low Ct values, and 2.8% (n=3) having very low Ct values. The chi-square value was 4.697, with a p-value of 0.58.

Conclusion: There is no association between the Ct value of CBNAAT and the clinical severity of PTB.

Keywords: Clinical severity, *Mycobacterium tuberculosis*, Real time polymerase chain reaction

INTRODUCTION

TB is a communicable, preventable, and curable disease caused by MTB bacilli. India is the largest country in the Southeast Asian region, with an annual incidence of 210 TB cases per 100,000 population in 2021. Among the notified TB cases in 2021, 75.2% were PTB cases, and 24.8% were EPTB cases [1]. Microscopy has high specificity but low sensitivity. The gold standard test for diagnosing TB is culture on solid and liquid media. The main disadvantage of culture on solid media is the delay in detection (6-8 weeks). Liquid culture media such as BacT/ALERT system, Mycobacterial Growth Indicator Tube (MGIT), Microscopic Observation Drug Susceptibility Assay (MODS), and Bactec 460TB systems provide results in about 7 to 10 days, but they are costlier and require subculture on solid media [2]. To overcome these limitations, there is increased focus on Nucleic Acid Amplification Test (NAAT). Among NAAT, conventional PCR was the first to emerge [3].

The use of specific primers and targets plays an important role in determining the sensitivity and specificity of the assay. Primers such as repetitive insertion sequence IS6110 are commonly used. They are specific to the members of the MTB complex and are present in multiple copies in the MTBC genome. Various other gene targets have been used for TB detection. The decreased sensitivity of the test is due to the presence of PCR inhibitors in the samples. The decreased specificity is due to cross-contamination from heavily loaded samples, laboratory machinery, or work surfaces contaminated with amplicons. RT-PCR technology enables direct identification of MTB in clinical samples and provides faster results than other conventional methods. The advantage of this method is the absence of cross-contamination since no opening of tubes after amplification is required. In general, RT-PCR is an alternative to conventional PCR for MTB detection from biological samples, especially for species differentiation and drug resistance detection. CBNAAT, developed by the Foundation for Innovative New Diagnostics (FIND), utilises a single disposable cartridge containing a sample processing system and an automated heminested RT-PCR. It can simultaneously detect the presence of MTB and its resistance to RIF [4]. Different studies from India have reported varying sensitivity (61%-100%) using in-house PCR methods targeting different genes [4-6].

CBNAAT detects MTB and RIF resistance by amplifying the RIF Resistance Determining Region (RRDR) of the rpo-B gene and subsequently probing this region for RIF resistance mutations.

Several small studies on clinical samples have reported that the sensitivity of the test is 98% to 100%, and the specificity is 100%. In smear-negative cases, the sensitivity is 72% [7,8]. One study inferred that nearly 95% of Rifampicin Resistant (RR) TB cases have mutations in an 81-bp region [9].

CBNAAT requires less technical expertise, minimal laboratory facilities, and provides results within two hours. This makes it an efficient tool for early diagnosis and treatment of TB, particularly in

cases of EPTB, HIV-TB co-infection, and pediatric TB. Thus, it is more effective in paucibacillary cases [8].

CBNAAT is also known as Gene Xpert, so the CBNAAT Ct value is also referred to as Xpert Ct values. The interpretation of the CBNAAT result is based on the CBNAAT Ct value, categorised as high, medium, low, and very low [10]. The CBNAAT Ct value is a continuous variable and is inversely correlated with the concentration of the starting material. However, the clinical significance of the CBNAAT Ct value has not been extensively explored in previous studies. Therefore, the present study aims to assess the association between the Ct value and the clinical severity of PTB.

MATERIALS AND METHODS

An institutional-based cross-sectional study was conducted in the Department of Respiratory Medicine, Navodaya Medical College Hospital and Research Centre-Raichur, from January 2020 to July 2021, for a period of 19 months, with a sample size of 106, after obtaining Ethical Committee Clearance from the Institutional Ethical Committee (Letter No. ECC/24/2019).

Inclusion criteria: Patients of age ≥18 years who are newly diagnosed PTB cases on the basis of CBNAAT by MTB detection were included in the study after written informed consent.

Exclusion criteria: Those patients who were old diagnosed PTB cases and already on Anti-tubercular Therapy (ATT) were excluded from the study.

Procedure

Methodology: After obtaining informed consent, presumptive PTB cases as per NTEP guidelines, aged ≥18 years, were enrolled in the study. Patients with pulmonary symptoms of cough lasting more than two weeks, along with weight loss, night sweats, breathlessness, hemoptysis, chest pain, and fever, were clinically diagnosed as presumptive TB as per NTEP guidelines [11]. They were categorised into mild, moderate, and severe groups according to the Bandim TB score [12], which is based on signs, symptoms, and clinical findings. These include cough, hemoptysis, dyspnea, chest pain, night sweats, as well as signs like anemic conjunctiva, tachycardia, positive lung auscultation findings, axillary temperature >37.0°C, Body Mass Index (BMI) <18, and Middle Upper Arm Circumference (MUAC) <200 mm. X-ray examinations were performed on patients with suggestive findings, and sputum samples were collected for microscopy examination in both smear-positive and smear-negative cases. CBNAAT was conducted to confirm the presence of MTB bacilli and detect Rifampicin resistance. The details of each participant were recorded in a proforma.

Samples that tested positive for MTB, either rifampicin-sensitive or RR, were further subjected to first-line Line Probe Assay (LPA) to check for isoniazid resistance. Samples that were smear-negative but CBNAAT-positive, with a past history of PTB, rifampicin resistance, or isoniazid resistance, were processed for liquid culture. Among cases in whom MTB was detected in CBNAAT, the association between CBNAAT Ct value and clinical severity was correlated. CBNAAT Ct value was categorised as high, medium, low, or very low. MTB was considered detected when at least two of the five probes showed positive signals with a Ct of ≤38 cycles. The concentration of bacilli was semiquantitatively estimated based on the Ct range (Ct >28 indicates very low,

- 22-28 indicates low,
- 16-22 indicates medium,
- <16 indicates high) [10].

STATISTICAL ANALYSIS

The data was entered into Microsoft Excel 2017 version, and statistical analysis was performed using SPSS version 23. Descriptive statistics, such as frequencies and percentages, were calculated for qualitative data, while the mean and Standard Deviation (SD) were calculated for quantitative data. The chi-square test was used to determine the association between qualitative variables.

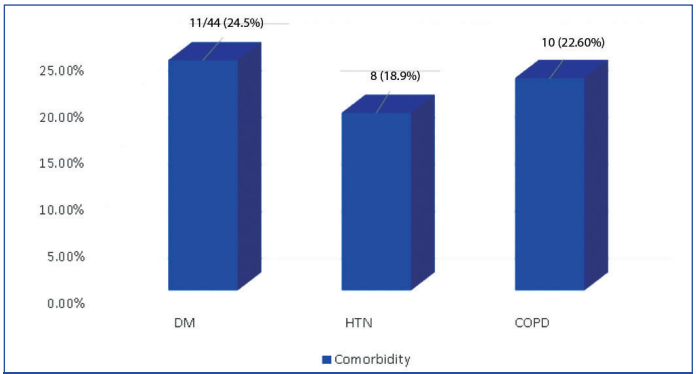
RESULTS

[Table/Fig-1] presents the age and gender distribution of the study subjects, showing that the most common age group affected in our study was 18 to 30 years, contributing to 34.9% of the cases. The mean age of the study participants was 41.73±14.78. The majority of the subjects were male (77/106), accounting for 72.64%.

Demographic data of all study participants	Number of patients n (N=106)	Percentage (%)
Age (years)		
18 to 30	37	34.9
31 to 40	21	19.8
41 to 50	24	22.6
51 to 60	18	17.0
≥61	6	5.7
Gender		
Male	77	72.64
Female	29	27.35

[Table/Fig-1]: Age and gender distribution of study subjects.

[Table/Fig-2] shows the comorbidities among the study subjects, with 41.5% of them having comorbidities, with diabetes mellitus being the most common comorbidity, observed in 24.50% of the cases.



[Table/Fig-2]: co-morbidities among the study subjects. n=44 (41.5%).
DM: Diabetes mellitus; HTN: Hypertension; COPD: Chronic obstructive pulmonary disorder

[Table/Fig-3] presents the Chest X-ray (CXR) findings among the study subjects, revealing that the majority of the patients (39.6%) had bilateral cavitatory lesions.

CXR findings	Number of patients (N=106)	Percentage (%)
Infiltration		
Bilateral	30	28.3
Unilateral	25	23.5
Cavitatory lesions		
Unilateral	04	3.7
Bilateral	42	39.6
No significant changes	05	4.7
Total	106	100

[Table/Fig-3]: Chest X-ray (CXR) findings among study subjects.

[Table/Fig-4] shows the zone of involvement in CXR among PTB patients, with the majority (35.6%) having involvement in all zones of the CXR. Five (4.7%) of them had no significant changes in their CXR.

[Table/Fig-5] displays the clinical severity of the disease among PTB cases, with the majority (55.7%) classified as having moderate clinical severity.

Zone of involvement	Number of patients n (N=101)	Percentage (%)
Upper zone	4	3.9
Mid zone	2	1.9
Lower zone	8	7.9
Upper and mid zone	22	21.7
Mid and lower zone	29	28.7
All zones	36	35.6
Total	101	100

[Table/Fig-4]: Zone of Involvement in CXR among PTB patients.
Five of total participants had no significant changes in CXR

Clinical severity	Number of patients	Percentage (%)
Mild	14	13.2
Moderate	59	55.7
Severe	33	31.1
Total	106	100

[Table/Fig-5]: Clinical severity of disease among PTB cases.

[Table/Fig-6] demonstrates the RIF resistance detected in CBNAAT among PTB patients, with the majority (77.4%) showing RIF resistance.

[Table/Fig-7] shows the Ct CBNAAT values of PTB patients, with the majority (36.8%) having a medium Ct CBNAAT value.

[Table/Fig-8] presents the association between CBNAAT Ct Value and clinical severity.

RIF resistance	Number of patients	Percentage (%)
RIF sensitive	24	22.6
RIF resistance	82	77.4
Total	106	100

[Table/Fig-6]: RIF Resistance detected in CBNAAT among PTB patients.

CBNAAT Ct value	Number of patients	Percentage (%)
Very low	22	20.8
Low	25	23.6
Medium	39	36.8
High	20	18.9
Total	106	100

[Table/Fig-7]: Ct CBNAAT value of PTB patients.

CBNAAT Ct value	Clinical severity			
	Mild	Moderate	Severe	Total
Very low count % of total	3 2.8%	12 11.3%	7 6.6%	22 20.8%
Low count % of total	6 5.7%	12 11.3%	7 6.6%	25 23.6%
Medium count % of total	4 3.8%	22 20.8%	13 12.3%	39 36.8%
High count % of total	1 0.9%	13 12.3%	6 5.7%	20 18.9%
Total count % of total	14 13.2%	59 55.7%	33 31.1%	106 100.0%

[Table/Fig-8]: Association between CBNAAT Ct Value and clinical severity.
Chi-square value: 4.697, p-value: 0.58

DISCUSSION

There are knowledge gaps in the in-depth analysis of the Ct value of CBNAAT and its clinical implications. Some studies have compared the Ct value of CBNAAT with smear microscopy [13-16], while others have explored the correlation of Ct categories with culture positivity and time to positivity [17]. However, the clinical correlation of CBNAAT Ct value has not been extensively explored. Therefore, this institutional-based study was conducted to evaluate the association between CBNAAT Ct value and the clinical severity of PTB in 106 subjects in whom MTB was detected in CBNAAT with various categories of Ct values. The study aimed to correlate the association between CBNAAT Ct value and the clinical severity of PTB. The p-value in the present study was 0.58, indicating no association between CBNAAT Ct value and the clinical severity of PTB.

The negative correlation between CBNAAT Ct values and smear grades of 0.55 falls within the range of correlations observed in South Africa. Hanrahan et al., reported correlations ranging from 0.54 to 0.74 in South Africa [13]. A study conducted by Najjingo et al., among TB patients from five referral hospitals in Uganda found that CBNAAT Ct values were minimally comparable to smear microscopy in assessing mycobacterial burden [14]. Another study by Prakash et al., on the clinical utility of Gene Xpert/MTB-RIF Ct values in diagnosing TB highlighted reduced sensitivity, particularly in samples with very low MTB bacillary load. In contrast, samples with high MTB bacillary loads showed a stronger correlation between Gene Xpert/MTB-RIF Ct values and MTB culture [17].

[Table/Fig-9] depicts the age distribution of the study participants in comparison with other studies. The majority (34.9%) belonged to the 18-30 years age group. The mean age of the study participants was 39.74±13.99 years. Similar findings were observed in a study conducted by Panda et al., [18]. Irene Najjingo et al., conducted a study in Uganda which showed that 51.2% of their participants were in the 18-32 years age group [14].

Age category (years)	Present study	Panda RK and Dash DJ [18]
18 to 30	34.9%	35.2%
31 to 40	19.8%	22%
41 to 50	22.6%	7.3%
51 to 60	17.0%	20.5%
≥61	5.7%	14.7%
Total	100	100

[Table/Fig-9]: Comparison of age distribution [18, present study].

In the present study, the majority of the subjects were males, accounting for approximately 72.64% as shown in Table/Figure 1. Similarly, in the study conducted by Irene Najjingo ID et al., the majority of the subjects were male, comprising about 67.4% [14].

In the present study, among 106 cases, the majority (24.5%) had Diabetes Mellitus, as shown in [Table/Fig-3]. A study conducted by Bhattacharya P et al., in 2017 on 173 selected patients reported that comorbidities were present in 92 (53.17%) patients, of whom 26.58% had diabetes mellitus and 17.34% had hypertension. In this study, the majority of the patients also had Diabetes Mellitus [19].

In the present study, the CXR findings are shown in [Table/Fig-4]. Among the 106 cases, the majority (39.6%) had Bilateral Cavitatory lesions. A study conducted by Panda RK et al., at JNM Medical College, Raipur on 68 selected patients revealed that 19 (27.9%) had consolidation on CXR, 16 (23.5%) had fibrocavitary lesions on CXR, 8 (11.7%) had nodules on CXR, 14 (20.5%) had infiltration on CXR, and 2 (2.9%) had miliary shadow on CXR [18].

In the present study, the majority (55.7%) of the cases had a moderate type of clinical severity. Due to a p-value greater than 0.05, there was no significant association between the clinical and radiological features of patients with CBNAAT Ct value. A study conducted by Bharadwaj AK et al., found that sputum positivity was significantly associated with cavitatory lesions on CXR [20].

In the present study, among the 106 cases with MTB detected in CBNAAT, the majority (36.8%) had a medium CBNAAT Ct value, as shown in Table/Figure 7. In a study conducted by Prakash AK et al., on the clinical utility of the cycle threshold value of GeneXpert MTB/RIF (CBNAAT) and its diagnostic accuracy in pulmonary and extra-pulmonary samples at a tertiary care centre in India, among 162 patients with MTB detected in CBNAAT, 13 had a high CBNAAT Ct

value, 52 had a medium CBNAAT Ct value, 63 had a low CBNAAT Ct value, and 34 had a very low CBNAAT Ct value [17].

Since the p-value was greater than 0.05, there was no significant association between CBNAAT Ct value and the clinical severity of PTB. Therefore, further studies with sufficient sample size should be conducted to assess the association. It is recommended to perform larger multicentric studies that include subjects with both PTB and EPTB to assess the association with CBNAAT Ct value.

Limitation(s)

Since the sample size is small, the results cannot be extrapolated to the general population. Additionally, it should be noted that the present study did not include cases of EPTB (Extra-Pulmonary Tuberculosis).

CONCLUSION(S)

CBNAAT is a robust diagnostic tool for the early detection of TB, and its results are interpreted based on CBNAAT Ct value. However, due to the small sample size in the present study, there was a weak correlation between the clinical severity of PTB and CBNAAT Ct value. As a result, the clinical significance of the CBNAAT value could not be assessed. Therefore, further studies with a larger sample size are needed to evaluate the clinical significance of the CBNAAT Ct value.

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