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CHANGING DYNAMICS IN EXTRAPULONARY TUBERCULOSIS DETECTION AND ROLE OF CARTRIDGE-BASED NUCLEIC ACID AMPLIFICATION TEST IN ITS DIAGNOSIS

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ABSTRACT

Introduction: Extrapulmonary Tuberculosis (EPTB) accounts for 15%–53% of all TB cases. Diagnosis is challenging due to its paucibacillary nature. In recent years, the Cartridge-based Nucleic Acid Amplification (CBNAAT) has emerged as a diagnostic tool with higher sensitivity. Literature on CBNAAT for an extra pulmonary sample is limited. Hence the study was conducted to evaluate the role of CBNAAT in early diagnosis of EPTB, and also to seek the spectrum of extrapulmonary tubercular infection and determine Rifampicin resistance among them from this region. **Material And Methods:** In this cross-sectional, laboratory-based comparative study of 1 year duration, 246 samples from suspected EPTB cases were subjected to ZN stain microscopy and CBNAAT, and diagnostic methods were compared (ZN smear, CBNAAT and liquid Culture). Thorough clinical corelation were made. For all CBNAAT positive samples rifampicin resistance were noted as marker for MDR TB. **Results:** The positivity rate of CBNAAT (19.9%) was higher than that of ZN smear (4%) and Liquid culture (18%). Most common EPTB in our study was found in tubercular lymphadenopathy followed by tubercular meningitis. Rifampicin resistance was found to be 8.16% among EPTB cases. Results was entered into Microsoft excel format and diagnostic test were evaluated by SPSS version-22 using liquid culture as reference. **Conclusion:** CBNAAT was found to be more sensitive than liquid culture for suspected EPTB cases. It also has added advantage of early detection, significantly reduced turnaround time, and ability to indicate MDR-TB (RIF resistance) for early initiation of appropriate treatment.

KEYWORDS

Extra-pulmonary tuberculosis (EPTB), Cartridge based nucleic acid amplification test (CBNAAT), Ziehl Neelsen (ZN) staining, Rifampicin resistance.

INTRODUCTION

An extra pulmonary tubercular infection (EPTB) describes the isolated occurrence of TB at body sites other than the lung. EPTB are paucibacillary, hence more often smear-negative than the pulmonary cases, making the diagnosis difficult despite the high global burden, which a significant hurdle for tuberculosis elimination(1-3,4). EPTB presents with varied clinical manifestations (5-7), necessitating confirmation of diagnosis before initiating anti-TB treatment. WHO recommends use of molecular tools for rapid diagnosis of EPTB(8) to achieve its goal of eliminating TB by 2030. Several studies have shown that molecular diagnosis are better in low-TB burden settings. However, further evaluation is needed in high-TB burden countries to confirm its effectiveness (9,10). To achieve the 90% notification rate for TB, the National Tuberculosis Elimination Programme requires diagnostic tests with high sensitivity and specificity. The sensitivity and specificity are low for Ziehl-Neelsen (ZN) stain in EPTB giving higher false-negative results (11,12). The GeneXpert MTB/RIF assay (cartridge-based nucleic acid amplification test) is an advanced developed, automated diagnostic molecular test. This assay works on nested real-time PCR and molecular beacon technology. Patients with a high risk of tuberculosis, such as presumptive HIV associated tuberculosis patients and paediatric patients, smear negative EPTB cases mainly benefit from GeneXpert assay (13-15).

Use in detecting Mycobacterium tuberculosis in extra pulmonary samples is considered "off label" use because its effectiveness in diagnosing extra pulmonary tuberculosis has not been demonstrated extensively(16). It is necessary to evaluate the GeneXpert MTB/RIF assay to rapidly diagnose extra pulmonary tuberculosis in routine laboratory use. In India, literature on CBNAAT for an extra pulmonary sample is limited. Thus this study aims to determine the performance of CBNAAT assay for the detection of Mycobacterium tuberculosis in extra pulmonary samples and to compare it with other conventional methods. The study also aims to understand the spectrum of extrapulmonary tubercular infections and determine the RIF resistance status of positive samples from this region, addressing the lack of published data to support the local epidemiology.

MATERIAL AND METHODS

This is a cross-sectional, laboratory-based study done in department of Microbiology, from a tertiary care centre. Patients with suspected extra pulmonary tuberculosis were included in the study. Research approval and ethical clearance were obtained from the respective institutional committee. Informed consent and relevant clinical data were obtained from the patients before enrolment into the study. A total of 246 samples collected and subjected to ZN stain microscopy and CBNAAT after digestion, liquefaction and decontamination process in a Type II A2 Biosafety cabinet. Among which 61 randomly selected samples were subjected to liquid culture in the BacT/Alert 3D system. Test results were collected & data was entered into Microsoft excels and was analysed using SPSS version-22. The percentage was calculated for categorical variables. Mean/median was calculated for continuous variables. Logistic regression was applied to classify the ZN and CBNAAT samples according to the liquid culture results and ROC analysis was done.

RESULTS:

In this study, 246 patient suspected to be suffering from EPTB samples of extra pulmonary origin were received and processed as per protocol: 128(52%) were male, and 118(48%) were female. Mean age of the study population was 35 years with age ranging from 3 months to 82 years.

Table 1: Spectrum Of EPTB And Positivity With Respect To ZN And CBNAAT

Sr. No.	Clinical Diagnosis	EPTB specimen	Total no. of Specimen	ZN smear positivity	CBNAAT
1	Meningitis	CSF	50	0 (0.0%)	7 (2.8%)
2	*	Pus	42	6 (2.4%)	19 (7.7%)
3	Ascites	Ascitic fluid	39	0 (0.0%)	0 (0)
4	Plural effusion	Pleural fluid	36	2 (0.8%)	4 (1.6%)
5	*	Tissue	35	0 (0%)	2 (0.8%)
6	Lymphadenitis	LN Aspirate	23	2 (0.81%)	14 (5.6%)

7	Synovitis	Synovial fluid	16	0 (0.0%)	3 (1.2%)
8	*	Endometrial tissue	3	0 (0%)	0 (0)
9	Pericardial effusion	Pericardial fluid	2	0 (0%)	0 (0)
		Total	246	10 (4%)	49 (19.9%)

***Pus was sent in view of Abdominal, Genitourinary infections and cold abscess. Tissue was sent in view of Bone and joint infection. Endometrial tissue was sent in view of Primary infertility and DUB.**

The maximum number of samples received was CSF 50 (20%). This was followed by pus 42(17%), ascitic fluid 39(16%), pleural fluid 36 (15%), tissue 35(14%), lymph node aspirate 23(9%), synovial fluid 16 (7%), menstrual blood 3(1%) and last 2(~1%) samples were pericardial fluid.

All the sample were processed for smear microscopy by ZN stain as well as CBNAAT and the result is depicted in table1. As seen, rate of positivity by CBNAAT (19.9%) was found to be more in comparison to ZN stain (4%), and most common sample was CSF collected in view of Tubercular meningitis, followed by pus collected from different anatomical sites (Cold abases aspirate, Abdominal and others). Positivity rate by CBNAAT was found to be highest in lymphadenitis.

Table 2: Diagnostic Yield Of CBNAAT, ZN Stain Against Liquid Culture

Result	CBNAAT	ZN stain	liquid culture
Positive	22	04	11
Negative	39	57	50
Total	61	61	61

Using logistic regression, we found ZN stain sensitivity to be 36.36%, specificity to be 100%, positive predictive value to be 100% and negative predictive value to be 99.85% and accuracy to be 88.52%. Similar analysis also for CBNAAT revealed the sensitivity to be 100%, specificity to be 78.04%, positive predictive value to be 50% and negative predictive value to be 100%, accuracy to be 81.97% by using table 2.

Among 246 samples, 49 were CBNAAT positive, out of which 43 were rifampicin sensitive, four were rifampicin-resistant, and 2 were Rifampicin indeterminate. The most common comorbidity observed in the study population was diabetes mellitus (19.51%); 14.4% of the study population had a smoking history and 16.3% have given history of alcohol intake.

DISCUSSION

India, as a signatory of the United Nations Sustainable Development Goals (SDGs), is committed to end the TB epidemic by 2025 through National Tuberculosis Elimination Programme —five years ahead of the 2030 target outlined under Goal 3. Diagnosing Extra pulmonary TB is a challenge because of varied clinical presentations, problem with sample collection and paucity in the amount of sample and a low bacterial load. CBNAAT being an amplification test with less turnaround time seems to be an ideal method for early diagnosis of EPTB. Hence the study was formulated to evaluate the role of CBNAAT in diagnosis of Extrapulmonary tuberculosis.

In the present study, maximum number of suspected EPTB cases were found to be belonging to the age group 19 to 39 years in 44.71% (110/246) followed by age group 40-59 years in 19.51% (48/246). The reasons may be because of the fact that these age groups are socially more active and are therefore more exposed to open cases of TB than any other age groups. Because of the huge prevalence of the organism most of the Indians are exposed to the organism in their early part of life , but do not manifest as active tuberculosis due to intact immunity. Because of various reasons e.g. weaning of immunity, widespread malnutrition, large proportion of diabetes and secondary infections due to virulent strains many a time a large proportion of people manifest with extrapulmonary symptoms of tuberculosis. This finding with regards to age predilection among patients with extrapulmonary tuberculosis appears to be in accordance with various other studies by Jagadevi *et al.* (17), Prakasha *etal.* (18), Jain *et al(19),* Raval *et al (20),* Nishal *et al (21)who have reported maximum age predilection of* 44.39% for age group 30-49 yrs, 50.95% for age group 15-44yrs, 30% for age group 18-29yrs, 26.47% for age group 30-39 yrs, 51.0% for age group 25-30yrs respectively.

Out of 246 specimens, ZN staining detected acid fast bacilli in 10/246 (4%) and CBNAAT could detect Mycobacterium tuberculosis 49/246 (19.9%). Purohit *et al.* have reported a sensitivity ranging from 0-40%for ZN Staining in majority of pauci-bacillary EPTB samples in their study (22). In our study positivity by CBNAAT is more than that of ZN staining which is in concordance with many other similar studies by Bagdia *et al.*(23), Jagadevi *et al.*(17), Avashia *et al.* (24), Jain G *et al.* (19) who have demonstrated ZN staining positivity V/s CBNAAT positivity as 5.17% V/s 15.38%; 0% V/s 9.54%; 13.33% V/s 37%;7.33% V/s 25.33% respectively.

Considering culture as the gold standard the sensitivity, specificity, positive predictive value, negative predictive value of ZN staining and CBNAAT was found to be reliable than ZN staining. The figures are in concordance with that of other similar studies by Singh P *et al*. (25) and Bagdia *et al*. (23). However few other studies have reported different sensitivities and specificities as depicted in table 3. For CBNAAT, the figures are discordant from other studies given below in table 3, which may be due to variation in the type and number of extra pulmonary samples and difference in the sample size in different studies.

Table 3: Comparison Of Various Studies.

Author	Year	Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)	
		ZN	CBNAAT	ZN	CBNAAT	ZN	CBNAAT	ZN	CBNAAT
Bagdia *et al.*(23)	2018	47.83	85.71	98	97.36	78.57	75	92.65	98.67
Singh p *et al.* (25)	2020	37.84	82.4	98.2	100	-	-	-	-
Siddiqui *et al.* (31)	2013	5	-	100	-	-	-	-	-
Zeka *et al.* (32)	2011	9.1	52.1	100	100	100	100	74.4	74.4
Present study	2021	36.36	100	100	78.04	100	50	89.1	100

The recovery rate of liquid culture from extra pulmonary specimens was 11/61 (18.03%) in the present study, which is almost similar to that of another study by Scott LE *et al*. (26), in which the culture yield was 23.5%. Among culture positivity in extra pulmonary specimens in the present study, lymph node aspirate alone contributed in 5 cases followed by pus (3), pleural fluid (2) and CSF (1). Multidrug-resistant tuberculosis (MDR-TB) is a type of TB that doesn't respond to the two most important drugs Isoniazid and Rifampicin(27). Since Rifampicin resistance is a strong sign of MDR-TB and since about 90% of strains resistant to it are also resistant to isoniazid, hence testing for rifampicin resistance is a key way to detect MDR-TB early. This resistance usually comes from changes in a specific part of the rpo B gene, and these changes can be detected quickly with advanced techniques like Real-Time PCR (28). In our study, we found a 8.16% rifampicin resistance rate (4 out of 49 cases) using CBNAAT. This included 2 cases of suspected pleuritis, 1 case of meningitis, and 1 case of lymphadenitis. Traditional resistance tests, like Line Probe Assay and liquid culture (MGIT), are time-consuming, expensive, and labour-intensive. By using rifampicin resistance as a marker with CBNAAT, MDR cases may be treated effectively or at least,early treatment may be initiated till confirmatory report arrives. Authors like Avashia *et al.* 5.4% (6/111) and Gupta S *et al.* 5.8% (5/85) however have reported lower rifampicin resistance (24,29). Rising trend of drug-resistant EPTB (DR-EPTB) cases have been reported in the last decade, posing significant challenges to diagnose and treatment(30). Thus with this rising trend, it is not only complicating efforts towards diagnosis and management of individual cases, but also threatening to derail both the global SDG goal of ending the TB cases by 2030 and India's ambitious target of eliminating TB by 2025 through the National Tuberculosis Elimination Programme.

Limitations Of The Study:-

Culture could not be performed on all samples as the quantity was not sufficient in majority of EPTB samples that is required for culture. The other limitation of this study lies in comparing with various other

diagnostic techniques, including fluorescent microscopy, and Line Probe Assays (LPA) etc.

Implication Of Future Studies

The future of research in the diagnosis of extrapulmonary tuberculosis (EPTB) holds significant promise for improving both the accuracy and speed of diagnosis, with other diagnostic innovations like chip based nucleic acid amplification, LPA etc in diagnosing atypical EPTB .

CONCLUSION

Culture, the classical gold standard for the diagnosis of tuberculosis, suffers from increased technical and logistical constraints in EPTB cases. Emerging EPTB situation globally calls for significant developments in rapid and accurate diagnosis and better therapeutic interventions are urgently needed. CBNAAT is a novel test that has revolutionized faster identification tuberculosis (TB) disease and drug resistance. We conclude from our study that CBNAAT can be a faster alternative to time taking methods like culture and at the same time a more efficient alternative to other rapid methods like AFB smear examination particularly in extra pulmonary specimens with Sensitivity of 100% and specificity 78%.

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