Diagnostic Accuracy of CBNAAT in Spinal Tuberculosis

Original Research Article

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ABSTRACT

Cartridge-Based Nucleic Acid Amplification Testing (CB-NAAT) is a rapid molecular test which is endorsed by Indian RNTCP for the diagnosis of extrapulmonary tuberculosis. However, Indian RNTCP has not specified its use in diagnosis of bone and joint TB. CBNAAT was used to test its accuracy in 42 patients with suspected spinal tuberculosis. Samples taken were split and simultaneously sent for CBNAAT, liquid culture and histopathology. The sensitivity and specificity of CBNAAT against liquid culture was 81.25% and 90.9% respectively. The sensitivity and specificity of CBNAAT against histopathology was 81.25% and 83.3% respectively. Hence, CBNAAT has fairly high sensitivity and specificity in spinal tuberculosis. CBNAAT can be considered as an initial test in the diagnosis of spinal tuberculosis.

KEY WORDS: CBNAAT, histopathology, liquid culture, spinal tuberculosis

INTRODUCTION:

Spinal tuberculosis is an important health problem in the low and middle income countries. At present there is no such test that can rapidly and accurately diagnose spinal tuberculosis. Any delay in diagnosis can lead to progression of the disease and may also lead to neurological complications[1]. Although MRI is not very specific, it is still being used for diagnosis of spinal tuberculosis. A combination of smear microscopy, biopsy (histopathology) and culture of *Mycobacterium tuberculosis* are being used currently as gold standard test for diagnosing spinal tuberculosis. To culture MTB, it takes a long time (around 4-6weeks)[2]. Hence, there is a need for a rapid diagnostic test. Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) is a rapid molecular beacon based assay technology. It is also endorsed by the Indian RNTCP as a rapid molecular diagnostic tool for extrapulmonary tuberculosis but does not specify its use in spinal tuberculosis[3]. This study aimed to assess the accuracy of CBNAAT against liquid culture and histopathology in diagnosing spinal tuberculosis.

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MATERIAL AND METHODS:

This was a prospective, non-randomized, cross sectional study conducted over a period of one year six months (January 2016 to June 2017). The study was approved by Research Advisory Committee and Institutional Ethics Committee of People's College of Medical Sciences & Research Centre, Bhopal. Samples were taken from patients attending OPD/ admitted at People's Hospital with suspected spinal tuberculosis. People's Hospital Bhanpur, Bhopal, is a tertiary care and referral center in central India. Clinical suspicion of spinal tuberculosis was made on basis of a chronic history of back pain beyond three months, constitutional symptoms such as low-grade fever, stiffness, night sweats, decreased appetite, weight loss and signs of neurological deficits such as tingling and weakness in lower limbs.

Sampling procedure: Forty two patients were included in the study. All new cases with suspected spinal tuberculosis in the age group of 10 to 80 years presenting to People's Hospital and Research Centre, Bhopal, were included. All patients with past history of spinal tuberculosis, patients with other spinal diseases, patients with active or suspected malignancy, patients with terminal disease not likely to survive six weeks were excluded from the study.

Patients suspected to have spinal tuberculosis underwent X-ray and MRI of the particular region of the spine. The X-ray findings associated with spinal tuberculosis are rarefaction of the vertebral endplates,

Loss of disk height, osseous destruction and Soft tissue swelling. MRI findings are loss of disk height, paraspinal soft-tissue masses and thick rim of enhancement around paraspinal and intraosseous abscesses. Patients with any positive findings suggestive of spinal tuberculosis on X-Ray/MRI were then subjected to CT guided biopsy or FNAC. We used Siemen's Samtron Sensation 40 Slice CT machine and sampling was done using 18 gauge coaxial needle for CT guided core needle biopsy samples and FNAC through 18 gauge lumber puncher needle[4,5].

We used GE Voluson S6 Pro USG machine, using high-resolution convex transducer of 6-16 MHz for USG guided aspiration of pus in cases of Psoas abscess. In patients who underwent surgery, samples were also taken during the time of surgery for spinal tuberculosis.

CBNAAT, also known as Gene Xpert MTB/Rif assay which was developed by Cepheid, Sunnyvale, California, USA. CBNAAT is recommended by WHO as WRD (World Health Organization– Recommended Rapid Diagnostic) in selected specimens of extrapulmonary tuberculosis [6]. It is recommended by RNTCP for diagnosis of extrapulmonary tuberculosis however, RNTCP does not specify its use in bone and joint tuberculosis [3].

CBNAAT simultaneously detects DNA of *Mycobacterium tuberculosis* (MTB) and resistance to rifampicin (RIF) (i.e. mutation of the rpoB gene) using real-time PCR, in less than two hours. It has single-use disposable Xpert MTB/RIF cartridges. Sample extraction, amplification, and detection are all carried out within this self-contained cartridge.

The samples we took were sent to RNTCP accredited laboratory for liquid culture. BACTEC MGIT 960 System was used for liquid culture. It works on the fluorescent indicator which detects oxygen level. As bacteria grow, oxygen gets depleted and fluorescence occurs indicating bacterial growth.

Statistical analysis: Statistical analysis was done using Statistical Package of Social Science (SPSS Version 20) and data comparison was done by applying *Fisher's exact test* to find out the statistical significance of the comparisons. Continuous variable were summarized as frequency, mean, standard deviation, sensitivity and specificity.

RESULTS:

A total of 42 patients between the ages of 10-80 years were enrolled from January 2016 to June 2017. We suspected spinal tuberculosis in 42 patients. However, only 14 out of 42 patients were confirmed TB i.e. positive by liquid culture, rest 28 out of 42 patients were categorized as probable TB and started on antitubercular drugs upon clinical, radiological and histopathological evidence. All 42 patients responded well to antitubercular drugs. There was one case which was positive by liquid culture but negative on CBNAAT, similarly there were two such cases which were positive by histopathology but negative by CBNAAT. The sensitivity and specificity of CBNAAT against liquit culture was 81.2% and 90.9% respectively and sensitivity and specificity of CBNAAT against histopathology was 81.2% and 83.3% respectively. There were 24 (57%) men and 18 women (43%). There were only 4 (9.5%) diabetic cases.

DISCUSSION:

Spinal tuberculosis is a common problem. There is a need for rapid and accurate diagnostic test for spinal tuberculosis. The aim of this hospital-based prospective cross-sectional study was to access the accuracy of CBNAAT against liquid culture and histopathology in diagnosing spinal tuberculosis. It is recommended by RNTCP for diagnosis of extrapulmonary tuberculosis however, RNTCP does not specify its use in bone and joint tuberculosis[3].

In our study, the sensitivity and specificity of CBNAAT against liquid culture was 81.2% and 90.9% respectively and the positive predictive value was 92.8% and the negative predictive value was 76.9%. Similarly, sensitivity and specificity of CBNAAT against histopathology was 81.2% and 83.3% respectively and the positive predictive value was 86.6% and the negative predictive value was 76.9%.

In a similar study, M. Held et al found the sensitivity and specificity of CBNAAT against liquid culture were 92.6% and 79.5% respectively with a positive predictive value of 73.5% and a negative predictive value of 94.6%. Sensitivity and specificity of CBNAAT against combined histopathology and culture was 95.6% and 96.2% with a positive predictive value of 97.7% and a negative predictive value of 92.6% as shown in table 2 and 3[1].

Monni T, et al in 2012 in their published article mentioned that sensitivity of PCR in 29 adult patients with spinal tuberculosis was 72% in HIV negative and 82 % in HIV positive patients but they did not use the culture and histopathology as the reference standard[7].

Yunting Gu et al in the year 2015 evaluated

Table 1: Characteristics of study population (n = 42).

Variables	Number of Patients (n = 42)	Percent
AGE (39.17±19.36, Range 10 to 64)		
10 to 18 yr	1	2.4
18 to 30 yr	14	33.3
31 to 40 yr	11	26.2
41 to 50 yr	14	33.3
51 yr & above	2	4.8
SOCIOECONOMIC STATUS		
Lower	5	11.9
Lower middle	17	40.5
Middle	15	35.7
Upper middle	5	11.9
SAMPLE TAKEN		
CT guided aspiration	19	45.2
Surgery	12	28.6
USG guided aspiration	10	23.8
Not done	1	2.4
VERTEBRAE INVOLVED		
Cervical	1	2
Lumbar	32	63
Sacrum	5	10
Thoracic	13	25
CBNAAT		
Negative	18	45
Positive	22	55
Total	40	100
LIQUID CULTURE		
Negative	13	48.1
Positive	14	51.9
Total	27	100
HISTOPATHOLOGY		
Negative	5	17.8
Positive	23	82.2
Total	28	100
DIABETES		
Absent	38	90.5
Present	4	9.5
HIV serology		
Negative	42	100
Positive	0	0
SYMPTOMS		
Fever	18	42.9
Back Pain/Stiffness	41	97.6
Night Sweats	5	11.9
Decreased Appetite	6	14.3
Monoparesis	*4*	*9.5*
Paraparesis	*12*	*28.6*
Quadriparesis	*0*	*0*

Table 2: Comparison of results of CBNAAT with Liquid Culture.

Liquid culture	CBNAAT		
	Positive	Negative	Total
Positive	13	1	14
Negative	3	10	13
Total	16	11	27
Sensitivity and specificity of CBNAAT against liquid culture			
Sensitivity	Specificity	PPV	NPV
81.25%	90.90%	92.85%	76.92%
Results of M Held study			
Sensitivity	Specificity	PPV	NPV
92.6	79.5	73.5	94.6

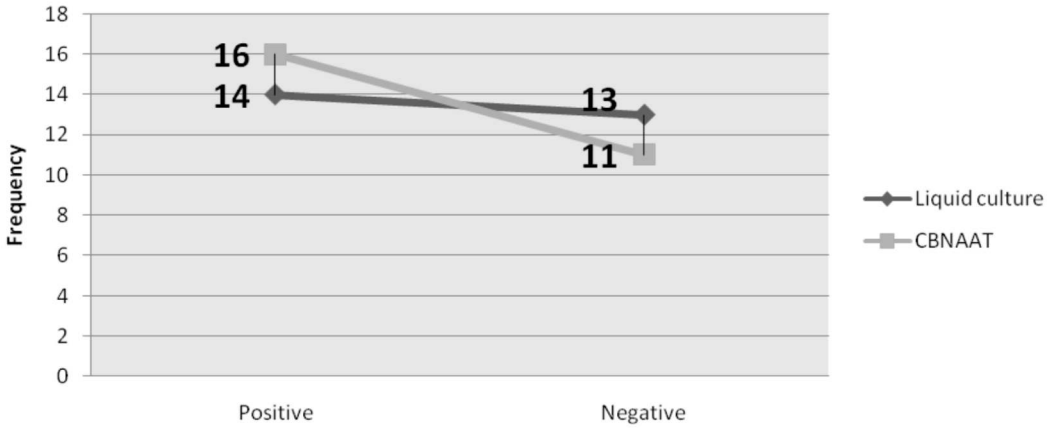

Figure 1: Showing comparison of results of CBNAAT with Liquid Culture.

Table 3: Comparison of results of CBNAAT with Histopathology.

Histopathology	CBNAAT		
	Positive	Negative	Total
Positive	13	2	15
Negative	3	10	13
Total	16	12	28
Sensitivity and specificity of CBNAAT against histopathology			
Sensitivity	Specificity	PPV	NPV
81.25%	83.33%	86.66%	76.92%
Results of M Held study			
Sensitivity	Specificity	PPV	NPV
95.6	96.2	97.7	92.6

the accuracy of CBNAAT in bone and joint tuberculosis. They took 50 patients with bone and joint tuberculosis, out of which 37 patients had a suspicion of spinal tuberculosis, rest 13 had Tuberculosis of some other site, 24/37 had Spinal tuberculosis confirmed via liquid culture. The overall sensitivity and specificity of CBNAAT in all 50 patients were 82% and 100% respectively[8]. The authors did not publish the yield of CBNAAT in spinal tuberculosis.

M. Held et al in another study in 2016 evaluated the diagnostic accuracy of CBNAAT for

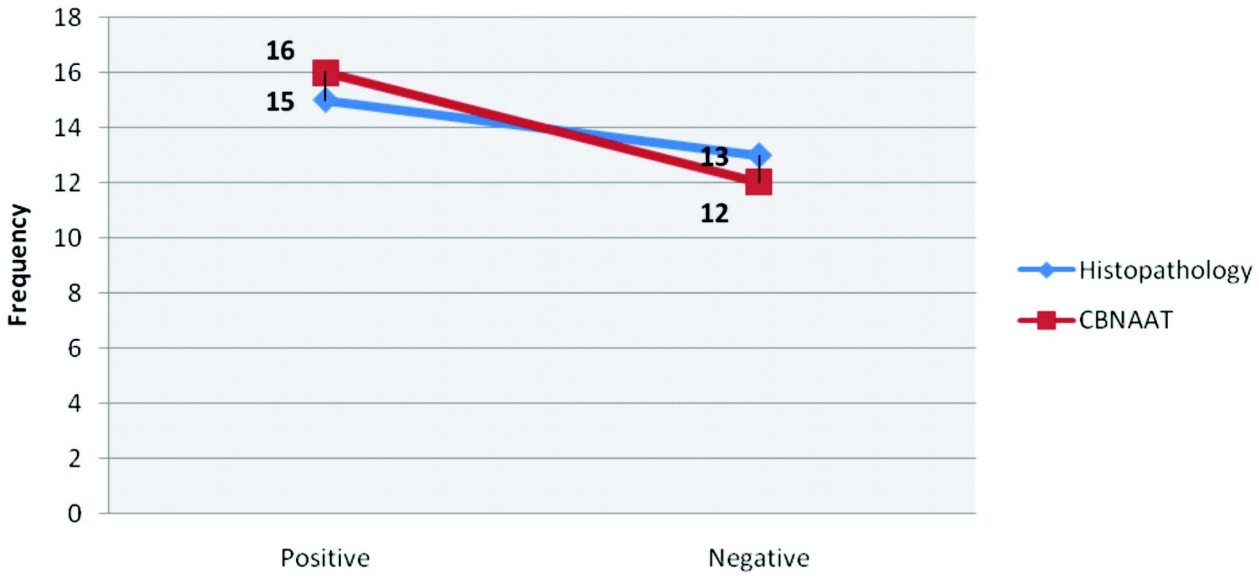

Figure 2: Showing comparison of results of CBNAAT with Histopathology

extrapulmonary tuberculosis in children with musculoskeletal infections. They evaluated a total 109 samples out which 14 were spinal samples; there were 23 / 109 samples with confirmed TB by culture or histology (21.1%). Histology was positive in all 23 cases, while culture was positive in 14 samples (12.8%). CBNAAT was positive in 17 samples (15.6%). Sensitivity and specificity in all samples was 73.9% and 100%[9].

We did not come across any HIV and spinal tuberculosis co-infection. Hence we could not comment on the accuracy of CBNAAT in HIV infected individuals.

The limitations of our study are: we took only spinal tuberculosis patients and therefore false positivity of CBNAAT could not be assessed. The duration of our study was 1year and 6 months hence; follow up for the full duration of anti tubercular therapy could not be done. However, during the duration of study patients on antitubercular drugs responded well to anti tubercular treatment indicating that those patients in whom the histopathology, liquid culture and CBAAT were negative for MTB but radiology suggestive of spinal tuberculosis in fact had spinal tuberculosis.

The disparity in the sensitivity and specificity of our study with other studies could be because of sampling technique, as we have used CT guided needle aspiration as the most common method of sampling. The amount of the sample was often very less in quantity i.e. it was not possible to send it for all the three investigations at the same time namely CBNAAT, liquid culture and histopathology. The reason for low sensitivity and specificity in our study could also be the low bacterial load ($<10^5$) in osteoarticular tuberculosis[10]. However, Singh KK, et al in their study mentioned that PCR techniques have a detection limit of 130 CFU/ml.

CONCLUSION:

Our study has demonstrated that CBNAAT has fairly high sensitivity and result of which are available earlier than those of liquid culture and histopathology. We recommend that CBNAAT should be considered as an initial test in diagnosis of spinal tuberculosis.

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