

A STUDY OF COMPARISON OF CONVENTIONAL TESTS WITH CBNAAT IN DIAGNOSIS OF TUBERCULOSIS IN PATIENTS WITH ASCITES AND CIRRHOSIS OF LIVER AND THEIR CLINICO-LABORATORY PROFILE

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ABSTRACT

BACKGROUND AND OBJECTIVES: Tuberculosis remains a major health problem, with an estimated 9.6 million deaths every year worldwide and approximately 1.5 million cases in India, currently on treatment. Diagnosis of extra pulmonary tuberculosis (EPTB) particularly tubercular ascites with lymphocytic predominance remained challenging over decades due to paucibacillary nature of ascitic fluid. The lack of explicit clinical features ascertaining the tubercular infection in cases of cirrhosis of liver, delays diagnosis and treatment of the tuberculosis leading to high mortality rate. The conventional diagnostic methods such as cytology, ADA, ZN staining and LJ culture done from ascitic fluid are not very sensitive for diagnosis of tuberculosis in ascites. Liquid culture methods adopted by most countries in the last ten years have improved the sensitivity to some extent but are time consuming, expensive and need sophisticated laboratory infrastructure. Detection of tuberculosis by CBNAAT in ascitic fluid has improved the sensitivity and specificity, especially when organisms are present in very small amounts. The objective is to diagnose secondary abdominal tuberculosis in patients with cirrhosis of liver and to compare conventional lab parameters with CBNAAT.

MATERIALS AND METHODOLOGY: Observational study of 50 patients attending a tertiary care hospital

Inclusion criteria:

- Adults > 19 years and willing to give informed consent
- Ascites due to cirrhosis of liver.

Exclusion criteria:

- Adults with Ascites due to other causes like Nephrotic syndrome, CCF, Hypoproteinemia, Pancreatitis.
- Primary abdominal tuberculosis

Patients were examined, investigated and evaluated for abdominal tuberculosis. Investigations included:

1. Ascitic fluid analysis for cell count, cell type, gram's stain, ZN stain for AFB, sugars, proteins, LDH, ADA, CBNAAT and culture.
2. USG abdomen.
3. CBC, LFT, PT, APTT, INR.

Collected data was entered into MS excel sheet and was analyzed using software version 24. Data was expressed as descriptive statistics using frequency and percentage. Comparison between the tests was done using chi-square test, p value < 0.05 was considered significant and diagnostic accuracy of CBNAAT test was Observed.

RESULTS: Among the 50 cases with liver cirrhosis taken for the study, 7 were CBNAAT TB positive, 2 were AFB positive and 3 were ADA positive. Based on the study conducted, CBNAAT showed significant difference in diagnosis of TB

compared to AFB and ADA. CBNAAT was able to detect significantly a greater number of abdominal TB compared to AFB and ADA ($p < 0.05$)

CONCLUSION: The outcome of the present study concludes that CBNAAT has a definite role in the diagnosis of tuberculosis. CBNAAT should be routinely utilized for rapid diagnosis of EPTB along with other conventional methods like AFB smear examination, ADA and culture for better overall results in the diagnosis of EPTB.

Keywords: Extra pulmonary tuberculosis, CBNAAT, Liver cirrhosis, ADA, Abdominal tuberculosis

INTRODUCTION

Tuberculosis is one of the most serious illnesses, affecting almost one-third of the global population. Even though excellent and reasonable treatments are available for the condition, it remains a hazardous infection. Tuberculosis remains a major public health issue, claiming an estimated 9.6 million lives each year worldwide ¹.

Although pulmonary tuberculosis accounts for around 80% of cases, the frequency of extrapulmonary tuberculosis has increased particularly since the HIV epidemic, since the 1980s. The lymph nodes, abdomen, spinal cord, brain, and pleura are the other most commonly affected areas of the body¹.

Abdominal tuberculosis is the sixth most common site for extra-pulmonary involvement and can affect any region of the gastrointestinal tract, peritoneum, or hepatobiliary system². The mycobacterium reaches the gastrointestinal tract via hematogenous spread, ingestion of infected sputum or direct spread from infected contiguous lymph nodes and fallopian tubes ³. The risk factors include HIV, Jejunio-ileal bypass, Intravenous drug abuse, Chronic renal failure, Immunocompromised (eg. Decompensated liver failure) and diabetes mellitus in descending order of relative risk⁴

The diagnosis of EPTB, particularly tubercular ascites with lymphocytic predominance, has remained difficult for decades due to the paucibacillary appearance of ascitic fluid. Ascites can be caused by a variety of clinical diseases, including both non-infectious and infectious etiology. Abdominal TB, when ascites is the outcome of tubercular infection per se. Other illnesses such as liver cirrhosis, CHF, nephrotic syndrome, and hypoproteinemia may be secondarily infected with tuberculous bacilli, particularly in high tuberculosis burden nations such as India⁵.

Cytology, ADA, ZN staining, and LJ culture performed on ascitic fluid are not very sensitive diagnostic techniques for detecting abdominal tuberculosis. Although, liquid culture methods have improved sensitivity, their time-consuming and expensive nature, as well as the need for sophisticated laboratory infrastructure, make them unsuitable for use in countries with limited resources, such as India.⁶

CBNAAT-based tuberculosis detection in ascitic fluid has enhanced sensitivity and specificity. The United States Food and Drug Administration approved two NAA tests in 2012, but only for use with sputum or respiratory secretions acquired during bronchoscopy. However, in 2014, the WHO standards authorized the CBNAAT for extrapulmonary tuberculosis as well⁷

So, routinely including CBNAAT of ascitic fluid in all patients with liver cirrhosis will aid in the detection of secondary tuberculosis, as well as reduce the use of empirical ATT, which is hepatotoxic and harmful to patients with liver cirrhosis. On analysis of laboratory parameters, Ascitic fluid analysis in TB peritonitis shows white blood cells (WBC) ranging between 500 and 1500 cells/cmm, predominantly lymphocytes⁸

And contains a high protein concentration (> 2.5 g/dL) with low glucose levels. The serum ascites albumin gradient (SAAG) provides a higher diagnostic yield than ascitic fluid total protein testing. SAAG levels in tuberculous peritonitis are modest (< 1.1 g/dL), but high levels (> 1.1 g/dL) indicate portal hypertension⁸

A high ADA value in any fluid compartment is not indicative of tuberculosis infection; rather, it reflects strong T-cell activation. An ADA value more than 32 IU/L in ascitic fluid has been found to have good sensitivity and specificity for the diagnosis of tubercular ascites⁹⁻¹²

While a positive smear requires at least 5000 bacilli/mL of samples, Ziehl-Neelsen staining of ascitic fluid for mycobacterial identification has a very poor yield (3% to 5%) even in proven tuberculous peritonitis patients. ¹³ Culture, while incredibly detailed and time-consuming, makes it difficult for clinicians to initiate early treatment.

Hence, the diagnosis of TB ascites is a common dilemma for the clinician.

CBNAAT is a cartridge-based nucleic acid amplification test with completely integrated and automated amplification and detection using real-time polymerase chain reaction that provides results within two hours. It is a very specific test since it uses three particular primers and five unique molecular probes to target the rpoB gene of MTB; no cross-reactions were seen with other bacterial species examined, so ruling out non-tubercular mycobacterium (NTM).¹³

In demonstration experiments the CBNAAT test's specificity in the diagnosis of pulmonary tuberculosis was found to be very high (97-100%)¹⁴

CBNAAT has been tested in laboratory trials in Germany, France, and Spain for the diagnosis of tuberculosis in non-respiratory samples such as pleural fluid, stomach fluid, tissue, cerebrospinal fluid, urine, and stool. 521 non-respiratory samples were discovered using the CBNAAT test, which had an overall sensitivity of 77.3% and specificity of 98.2%.¹⁵

The detection of tuberculosis using CBNAAT in ascitic fluid has increased sensitivity and specificity^{16,17} besides the added advantage of early reporting, allowing clinicians to begin therapy as soon as possible, and testing for rifampicin resistance in the organism¹⁸

Various studies have shown varying sensitivities of CB-NAAT in ascitic fluid versus ADA and other conventional methods resulting in confusion over the diagnostic test of choice. Hence we have undertaken this study to identify the diagnostic accuracy of CBNAAT versus other conventional methods especially in detecting secondary extrapulmonary tuberculosis. The study also analyses the implication of routine testing of all patients with ascites in liver cirrhosis with CBNAAT.

MATERIALS AND METHODS

A cross sectional observational study done in patients with ascites due to cirrhosis of liver above 19 years age attending a tertiary care hospital in Bangalore,

Over a period of 18 months, after obtaining informed consent from patients. Prior to the initiation of study, ethical & research committee approval was taken.

Inclusion criteria:

Adults with

- Age group: > 19 years
- Ascites in patients with cirrhosis of liver

Exclusion criteria:

Adults with

- Ascites due to other causes like Nephrotic syndrome, CCF, Hypo proteinemia, Pancreatitis.
- Primary abdominal tuberculosis

Sampling method: Purposive sampling

Subjects fulfilling inclusion criteria were selected for the study. Each patient's details such as age, sex, nationality, chief complaints, symptoms, risk factors were documented. Detailed history along with examination was recorded. Relevant investigations were sent. Patients were examined, investigated and evaluated for signs of abdominal tuberculosis.

Investigations included:

1. Ascitic fluid analysis in terms of cell count and cell type, gram's stain, ZN stain for AFB, sugar, protein, LDH, ADA, CBNAAT and culture/sensitivity
2. Ultrasonography of abdomen.
3. Routine blood tests like CBC, LFT, Coagulation profile.

Collected data was entered into MS excel sheet and was analyzed using software version 24. Data was expressed as descriptive statistics using frequency and percentage. Comparison between the tests was done using chi-square test, p value<0.05 was considered significant. and diagnostic accuracy of CBNAAT test was observed.

RESULTS

Liver cirrhosis was common in 41-50 years age group (23, 46%), followed by 51-60 years (14, 28%).

and commonly affected males (76%) compared to females (12, 24%). Majority in the study i.e., 34 (68%) belonged to rural area.

The clinical presentation of subjects was varied as depicted in Table 1 and etiology as depicted in Table 2

Table 1: Distribution of subjects according to presentation

SYMPTOM	FREQUENCY	PERCENTAGE
ABDOMINAL DISTENSION AND DISCOMFORT	44	88.0
MYALGIA	39	78.0
WEIGHT LOSS	28	56.0

NAUSEA AND VOMITING	24	48.0
PRURITUS	21	42.0
ANOREXIA	12	24.0
FEVER	11	22.0
DARK COLORED URINE	9	18.0

All the subjects with liver cirrhosis i.e., 50 (100%) had ascites, 26 (52%) had pallor, 24 (48%) had splenomegaly, 22 (44%) had icterus, 11 (22%) had leukonychia and 6 (12%) had hepatomegaly.

TABLE 2: DISTRIBUTION OF CIRRHOSIS PATIENTS ACCORDING TO ETIOLOGY

ETIOLOGY	FREQUENCY	PERCENTAGE
ALCOHOLIC LIVER DISEASE	23	46
NAFLD/MASLD	13	26
HEPATITIS B	6	12
HEPATITIS C	5	10
CRYPTOGENIC	2	4
AUTOIMMUNE	1	2
TOTAL	50	100

Among subjects with liver cirrhosis, total protein >2.5g/dl was found in 4 (36%), Glucose was low in 6 (50%) subjects with liver cirrhosis, cell count>250 in 6 (16%) and high LDH in 2 (40%).

AFB was positive in 2 (4%) of the subjects.

ADA was positive in 5 (10%) subjects.

CBNAAT was positive in 7 (14%) subjects as depicted in Table 3. There was significant difference found between the results on AFB and CBNAAT examination, CBNAAT was able to detect significantly more number of TB positive cases in comparison to AFB examination ($p<0.05$) as depicted in Table 4.

TABLE 3: DISTRIBUTION OF SUBJECTS ACCORDING TO ASCITIC FLUID CBNAAT STATUS

CBNAAT	FREQUENCY	PERCENTAGE
POSITIVE	7	14.0
NEGATIVE	43	86.0
TOTAL	50	100.0

TABLE 4: COMPARISON OF ASCITIC FLUID AFB AND CBNAAT REPORT AMONG STUDY SUBJECTS

AFB	CBNAAT		TOTAL	P VALUE
	POSITIVE	NEGATIVE		0.01714286*
POSITIVE	2	0	2	
NEGATIVE	5	43	48	
TOTAL	7	43	50	

TABLE 5: COMPARISON OF ASCITIC FLUID ADA AND CBNAAT REPORT AMONG STUDY SUBJECTS

ADA	CBNAAT		TOTAL	P VALUE
	POSITIVE	NEGATIVE		<0.00001*
POSITIVE	5	0	5	
NEGATIVE	2	43	45	
TOTAL	7	43	50	

There was significant difference found between the results on ADA and CBNAAT examination, CBNAAT was able to detect significantly a greater number of TB positive cases in comparison to ADA examination ($p<0.05$).

DISCUSSION

This study was conducted among patients with ascites due to cirrhosis of liver above 18 years age to Compare CBNAAT with other Traditional Methods for diagnosis of tuberculosis ascites in them.

EPTB is primarily a disease of the young. The greatest burden of disease occurs in 15–49 years old¹⁹. In this study, middle aged patients were most affected[41-50 years age group (23, 46%), followed by 51-60 years (14, 28%) which was consistent with world health statistics.

In the present study, there was a male preponderance for cirrhosis with a male to female ratio of 3.1: 1. In the studies done by Ervilla Das ²⁰(2018), Apurva S²¹ (2018) and Sudeshna Mallik ²²(2018) there was a similar male preponderance with a male to female ratio of 8:1, 5.4: 1 and 2.2: 1 respectively. This difference in incidence in sex ratios might be because of social habits of males at various stages of life.

In terms of presenting symptoms, 44 (88%) presented with abdominal distension and discomfort as the most common presenting symptom. This was consistent with the previous studies by Sudeshna Mallik et al²²(2018) and David Ce et al.²³ (2019) in which 80% and 75% of the subjects presented with abdominal distension and discomfort respectively, followed by myalgia(78%), which was in concordance to a study by David C et al.²³ (2019) in which 50% patients had the same symptom.

Other symptoms of weight loss, nausea vomiting, anorexia and dark coloured urine were also reported by Sudeshna Mallik et al²², David c et al.²² and Hemang Suthar et al²⁴.

In this study, 6 (12%) had hepatomegaly. This was much less compared to other studies like Sudeshna Mallik et al.²² in which 72.61%, in a study by Behera and Dash et al.²⁵ (2020), 71.43% had hepatomegaly.

In the present study, 24 (48%) had splenomegaly also much lesser than similar studies by Joshi, et al.²⁶, Sudeshna Mallik et al.²² and Behera and Dash et al²⁵, 76.19%, 74% and 50.72% observed splenomegaly. In this study, 26 (52%) had pallor. In other studies, like Joshi et al. ²⁶ and Behera and Dash et al. ²⁵ 71.48% and 60.87% had pallor respectively which was in accordance to this study.

A comparison of observed clinical signs is depicted in the Table 6.

TABLE 6: COMPARISON OF SIGNS WITH OTHER STUDIES

Signs	Joshi et al ⁶⁴	Sudeshna Mallik et al. ⁶⁰	Behera and Dash et al ⁶³	Present study
Pallor	71.48 %	-	60.87%	45.16%
Hepatomegaly	72.61 %	71.43%	55 %	06.45%
Icterus	26.19 %	42.86%	20.29%	38.70%
Splenomegaly	76.19 %	74%	50.72%	45.16%
Leukonychia	38.09	-	-	19.35%

Lab parameters:

In this study 48% patients had anemia. which was similar to a study by Hegde et al.²⁷ who also found severe anemia in 43% of liver cirrhosis cases.

Also in accordance with a study by Kujovich MD et al.⁶⁷ which reported thrombocytopenia in 49–64% patients with decompensated CLD, In the present study, 54% had thrombocytopenia. Elevated serum creatinine >1.3mg/dl was found in 15 (30%) patients in this study which is comparable to a study by Pathak et al.²⁹ in which 39.4% and with a study by Hegde et al.²⁷ in which 30% had increased serum creatinine levels.

In this study, 44 (88%) had total bilirubin >1.3 mg/dl, 76% had S.G.O.T >50 IU/L. This was comparable with a study by Pathak et al.²⁹ in which 89% showed similar reading and 74% had S.G.P.T >50 IU/L, which was comparable with Mendelhall et al.³⁰ (91% showed increase in SGPT).

In this study, 38 (76%) had hypoalbuminemia comparable to Mendelhall et al.³⁰ in which 81.47% showed similar findings. Among subjects with liver cirrhosis, total protein >2.5g/dl was found in 4 (36%), Glucose was low in 6 (50%) subjects with liver cirrhosis, cell count >250 in 6 (16%) and high LDH in 2 (40%). Staining for acid fast bacilli was positive in 2 (4%) of the subjects. In previous study conducted by Bhargava DK et al.³¹ staining for acid fast bacilli was positive in less than 3% of cases. Similarly in a study done by Dr Reddy GS³² et al, only 3% of patients were positive for AFB.

In this study, ADA was positive in 5 (10%) subjects. Ascitic fluid ADA level above 36 U/L and an ascitic fluid to serum ADA ratio more than 0.98 is suggestive of tuberculosis.³¹ In a study by Soni et al.³³ 50% of the tubercular peritonitis cases were ADA positive.

In this study, CBNAAT was positive in 7 (14%) subjects. Same percentage was observed in other studies like Ashi Singh et al.³⁴ in which CBNAAT gave positive result in 92% of the subjects. And another study conducted by Komanapalli SK et al.³⁵(2018) observed 84.34% positive CBNAAT reports.

There was significant difference found between AFB and CBNAAT examination ($p < 0.05$), implying superiority of CBNAAT over AFB. This was similar to a study by Jaykumar et al.³⁶

There was significant difference found between ADA and CBNAAT examination ($p < 0.05$), implying superiority over ADA in picking up TB Ascites. This was similar to a study by Soni et al.³³

CONCLUSION

The outcomes of present study concluded that liver cirrhosis is commonly seen in males compared to females, with most patients with abdominal distension and discomfort being the most common presentation. CBNAAT was able to detect a significantly greater number of tuberculosis positive cases compared to ADA and AFB examination in Ascitic Fluid on analysis.

Hence, from this study results, we conclude that CBNAAT is superior to ADA and AFB examination in detection of tuberculosis giving quicker results even when compared to culture and helps in the timely treatment of extrapulmonary TB.

We conclude that CBNAAT can be routinely utilized for rapid diagnosis of EPTB in conjunction with other conventional methods like AFB smear and ADA for better diagnostic efficacy

STUDY LIMITATIONS:

1. There may be limitations in directly comparing these methods due to variations in sensitivity and specificity, which can be influenced by factors such as sample quality and operator proficiency.
2. Relatively small sample size.

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CONFLICTS OF INTEREST: None

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