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Prospective Study to Find Out the Role of Cartridge Based Nucleic Acid Amplification Test (Cb-Naat) On Pleural Fluid as a Diagnostic Method in Childhood Tuberculosis

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Abstract

Tuberculosis in children has been relatively neglected due to lack of pathognomonic clinical presentation and sensitive diagnostic tools. The introduction of CBNAAT has significantly transformed the diagnosis of tuberculosis in adults and its application in Pediatric Tuberculosis is under evaluation. Therefore, we conducted a study on role of pleural fluid CBNAAT in the diagnosis of childhood Tuberculosis.

Methodology: We did prospective hospital based study from June 2018 to July 2019 on 100 randomly selected patients suspected of tuberculous pleural effusion who had their pleural fluid aspirate tested for CBNAAT, culture sensitivity and ZN stain for AFB with Mantoux test and other investigations. Chi square test was used.

Results: The sensitivity, specificity, positive predictive value and negative predictive value for CBNAAT were 81.81%, 82.08%, 69.23% and 90.16%. CBNAAT detected in extra 26 tuberculosis cases compared with smear microscopy and was more sensitive than it. Positive contact history, reactive Mantoux test (p value 0.0015) and low socioeconomic status were independently associated with positive CBNAAT result.

Conclusion: Analysis of pleural fluid aspirate with CBNAAT is a sensitive and specific method for rapid diagnosis of tuberculous effusion in children. Compared with microscopy, CBNAAT offers better sensitivity and its scale up will improve access to tuberculosis diagnosis in children. A negative CBNAAT result does not rule out tuberculosis.

Keywords: CBNAAT, ZN staining

Introduction

Tuberculosis is one of the most widespread infections in the world especially in developing countries. World Health Organization (WHO) estimated the global burden of tuberculosis (TB) at 10 million new cases and 1.3 million deaths in 2017[(1)]. India is 17th among 22 high burden countries in terms of overall TB incidence. Pleural effusion is one of the common complication of primary tuberculosis or in conjunction with pulmonary infiltrate typical of post primary tuberculosis.

The recent introduction of Cartridge based nucleic acid amplification test has significantly transformed the diagnostics of tuberculosis in adults but its application for the diagnosis of Pediatric Tuberculosis is under evaluation. World Health Organization (WHO) in 2011, recommended the use of Cartridge based nucleic acid amplification test as a preliminary diagnostic tool among children[(2)].

Aims & Objectives

1. To evaluate the role of CBNAAT on pleural fluid specimen in children suspected to be suffering from TB
2. To evaluate the correlation between Monteux test and positivity of CBNAAT on pleural fluid
3. To evaluate the correlation between results of culture and sensitivity and CBNAAT on pleural fluid

Material & Methods

The study is a prospective hospital based study conducted from July 2018 to June 2019 in the Department of Paediatrics, JLN Medical College and Associated group of Hospitals Ajmer.

The methodology of the study consisted of following steps:

Study group comprised of 100 randomly selected patients ≤ 17 years of age of either sex who met the following criteria

All Patients who were diagnosed to have pleural effusion using digital chest radiographs including standard Postero-anterior and lateral views with symptoms suggestive of tuberculosis like :-

- Fever and or cough for >more than two weeks
- Fever with weight loss or no weight gain
- History of contact in last two years
- Hilar lymphadenopathy on chest skiagram
- Tuberculin skin test positivity
- Co-existence of precipitating illness
- Hemoptysis
- Significant decrease in appetite

All the selected patients were subjected to the following:

- Detailed history
- Good general physical examination
- Systemic examination
- Routine investigations

A. Hematological investigations
   i. Level of hemoglobin
   ii. TLC, DLC,ESR
B. Mantoux test
C. Chest X-ray

Special Investigations: -

Pleural Fluid For Cb-Naat

Thoracentesis

Interpretation of Results of Cb-Naat

This is DNA based test meant for new TB suspect, ensure don't enroll 'follow up' patient. The results are interpreted by the system from measured fluorescent signals and embedded calculation algorithms and will be displayed in the "view results" window.

- MTB detected
- MTB not detected
- INVALID

Culture And Sensitivity Test Of Pleural Fluid Lowenstein-Jensen (LJ)[(3)] is the selective medium which is used for the cultivation and isolation of Mycobacterium species.

Other relevant Investigations will be done where ever necessary to support the diagnosis

1. USG Thorax
2. Chest CT scan/MRI.

Observation S & Results

In our study, patients in the age group of 0-5 years were 13, 29 in the age group 5- 10 years and 58 in the age group 10-17 years. 55% of cases in our study were male while 45% of cases were females.

Table 1: Distribution of Cases: Socioeconomic Status

Class	Status	No. of Patients	Percentage
I	Upper	4	4.0
II	Upper Middle	16	16.0
III	Lower Middle	42	42.0
IV	Upper Lower	25	25.0
V	Lower	13	13.0
Total		100	100

Table no 1 shows maximum no. of cases belong to lower middle class with 42%, 25% were in upper lower and only 4% cases were of upper class.

Table 2: Distribution of Cases: Type of Tuberculous effusion

S. N.	Status	No. of Patients	Percentage
1.	Only Unilateral pleural effusion	53	53
2.	Only Bilateral pleural Effusion	5	5
3.	Bilateral pleural Effusion with parenchymal disease	2	2
4.	Pleural Effusion with parenchymal disease	29	29
5.	Tuberculous empyema	2	2
6.	Pleural effusion with mediastinal lymphadenopathy	9	9
7.	Pleural effusion with military tuberculosis	0	0
Total		100	100

Table no. 2 shows 53% cases presented with only unilateral pleural effusion, 29% had pleural effusion with parenchymal disease, 9% had Pleural effusion with mediastinal lymphadenopathy, 5% with Only Bilateral pleural Effusion while 2% presented with Bilateral pleural Effusion with parenchymal disease and 2% were Tuberculous empyema.

In our study, history of contact was present in 56% cases while absent in 44% of cases, majority of cases (73%) were having BCG scar present and cases with a reactive mantoux test were 42% while 58% cases had non-reactive mantoux test.

Table 3: Distribution of Cases: Presenting Complaints

S. N	Complaints	Status	No. of Patients	Percentage
1.	Fever>2 weeks	Yes	86	86.0
		No	14	14.0
2.	Cough>2weeks	Yes	72	72.0
		No	28	28.0
3.	Respiratory difficulty	Yes	64	64.0
		No	36	36.0
4.	Hemoptysis	Yes	4	4.0
		No	96	96.0
		No	95	95.0
5.	Decreased appetite	Yes	52	52.0
		No	48	48.0
6.	Weight loss / no weight gain	Yes	54	54.0
		No	46	46.0

Table no 3 shows 86% of cases presented with complain of fever>2 weeks, 72% of cases with cough> 2 weeks, 64% of cases with respiratory difficulty.

Table No. 4

		Culture*		Total
		Positive	Negative	
CBNAAT	Positive	27	12	39
	Negative	7	55	61
Total		33	67	100

P value = 0.0001

Table 5: Distribution of cartridge based nucleic acid amplification test and age

			Age(Years)			Total
			0-5	5-10	10-17	
CBNAAT	Not detected	N	12	16	33	61
		%	19.67	26.22	54.09	100
	Detected	N	1	13	25	39
		%	2.56	33.33	64.10	100
Total		N	13	29	58	100
		%	13	29	58	100

P value=0.0454

Table no 5 shows rpoB gene of Mycobacterium tuberculosis was detected by Cartridge based nucleic acid amplification test in 2.56% of children in 0-5 yrs age group and 33.33% 5-10 years of age group and in 10-17 years 64.10% which was statistically significant.

Graph 1: Distribution of CBNAAT result with gender

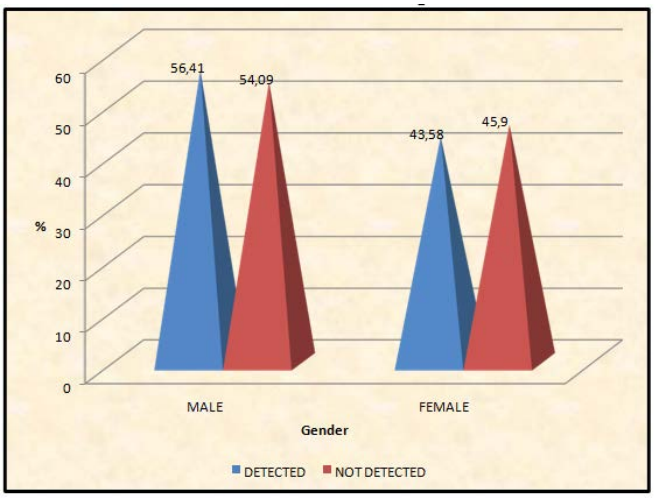


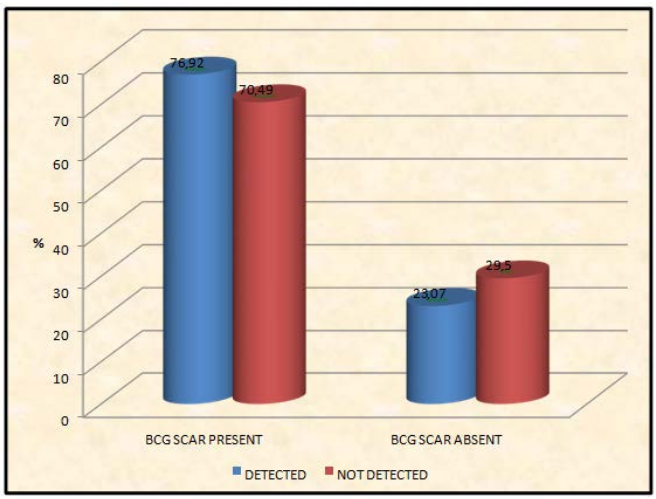
Table 6: Distribution of Cartridge Based Nucleic Acid Amplification Test And Socioeconomic Status

			Socioeconomic Status					Total
			CLASS I	CLASS II	CLASS III	CLASS IV	CLASS V	
CBNAAT	Not detected	N	2	10	28	18	3	61
		%	3.27	16.39	45.90	29.5	4.91	100
	Detected	N	2	6	14	7	10	39
		%	5.12	15.38	35.89	17.94	25.64	100
Total		N	4	16	42	25	13	100
		%	4.0%	16.0%	42.0%	25.0%	13.0%	100.0%

P value=0.041

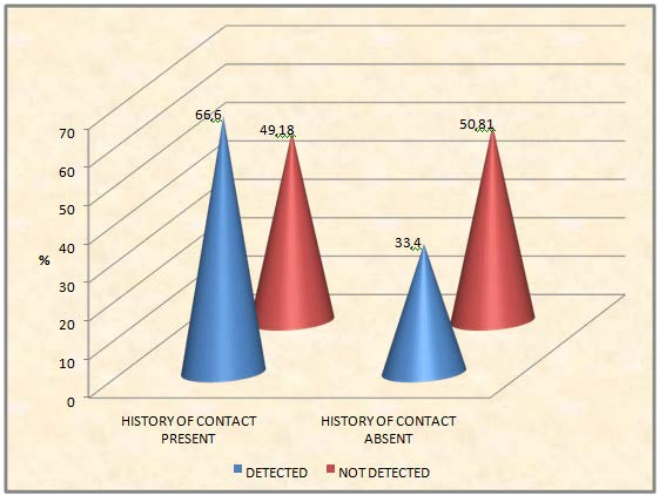
Table no 6 shows rpoB gene was detected by Cartridge based nucleic acid amplification test maximum in both class III and class IV children with a percentage of 35.89% and 17.94%.

Graph 2: Distribution of CBNAAT result with BCG scar



P value 0.47

Graph 3: Distribution of CBNAAT result with history of contact



P value 0.08

Table 7: Association of cartridge based nucleic acid amplification test and Mantoux test

		Mantoux test		Total
		Negative	Positive	
CBNAAT	Detected	15	24	39
	Not detected	43	18	61
Total		58	42	100
P Value		0.0015 (S)		

Table no. 7 shows rpoB gene was detected by Cartridge based nucleic acid amplification test in 15 children with positive Mantoux test and 24 children with negative Mantoux test.

Table 8: Association of Cartridge Based Nucleic Acid Amplification Test, Ziehl Neelsen Staining and Mantoux test

		ZIEHL NEELSEN staining		CBNAAT		Culture		Total
		-ve	+ve	Detected	Not detected	-ve	+ve	
Mantoux Test	-ve	57	1	15	43	44	14	58
	+ve	36	6	24	18	23	19	42
Total		93	7	39	61	67	33	100
P Value		0.015 (S)		0.0015 (S)		0.026 (S)		

Table 9: Correlation among the study variables

		ZN staining	Culture and Sensitivity	CBNAAT
Mantoux test	Pearson Correlation	0.163*	0.221	0.671**
	P Value	0.015	0.026	0.0015

Table no 9 shows Pearson correlation for Mantoux test and Ziehl Neelsen staining is 0.163 while for Mantoux test and CBNNAT it is 0.671 and mantoux test and culture and sensitivity is 0.221 which shows positive association that is if the positivity of Mantoux test increases so does the positivity of Ziehl-Neelsen, Cartridge based nucleic acid amplification test and culture and sensitivity and increases but Ziehl Neelsen staining shows more variation around the line of best fit as compared to Cartridge based nucleic acid amplification test.

P value for Mantoux test and Cartridge based nucleic acid amplification test is 0.0015 which is statistically SIGNIFICANT.

Discussion

Out of the selected group of 100 cases, 57 were in the age group of 0-5 years, 27 in the age group of 5-10 years and 16 in the age group of 10-12 years.

Sangeeta Sharma et al (2009)[4] in their study showed that 16% were in age group 0-5 years,32% in 5-10 years, 58% in 10-14 years.

In our study of 100 patients, 55% were males and 45% were females. Similarly,

Bayhan et al (2018)[5] showed that 71.4% cases were males and the rest 28.6% were females.

Tuberculosis is high in children of low socio-economic groups. This is because of high prevalence of chronic pulmonary tuberculosis in adults, overcrowding, close contact with sputum positive cases, poverty and related

disease, poor ventilation and unhygienic living conditions.

According to Kuppuswamy Scale to grade socioeconomic status, 42% belonged to grade III. Patients belonged to grade V, IV, II & I were 9%, 23%, 22%, and 4% respectively. Similarly, P.M. Udani and Saroj Mehta (1994)[6] in their study observed that prevalence of tuberculosis is very high in children of low socioeconomic groups.

In our study, 53% cases were having only Unilateral pleural effusion, 5% cases having Only Bilateral pleural Effusion, 29% cases were of Pleural

Effusion with parenchymal disease and Pleural effusion with mediastinal lymphadenopathy, Tuberculous empyema, Bilateral pleural Effusion with parenchymal disease were 9% , 2% and 2% respectively.

Bayhan, et al (2018)[5] in their study showed that Unilateral hemithorax involvement and pleural involvement without lung involvement is seen in 41%–53.6% of the cases.

In the selected group of 100 patients, history of contact with tuberculosis was positive in 41% and negative in 59%. In contrast to our study, Sangeeta Sharma et al (2009)[4] showed that 67% case had family history of contact.

BCG scar mark was present in 81% cases while scar mark was lacking in 19% cases. Similarly, Kakrani V.A. et al (1992)[7] in their study of 120 cases of pulmonary tuberculosis observed that BCG scar mark was present in 72 (60%) cases.

Tuberculin skin test was reactive in 34% cases and non-reactive in 66% cases. Similarly, Boloursaz MR et al (2010)[8] in their study observed positive tuberculin skin test in 33.33% cases.

Among the symptoms with which the patients presented, majority (86%) cases were running fever of more than 2 weeks duration. Cough for more than 2 weeks duration was complained by 72% cases. Likewise, anorexia was present in 52%, weight loss / no weight gain in 50%, respiratory distress in 35%, GI disturbances in 11%, seizures in 5% cases, hemoptysis in 4% cases.

In a study Sangeeta Sharma et al (2009)[4] that fever and cough were present in 98% and 70% cases respectively, loss of weight in 23% and chest pain in55% cases.

Chih young chiu et al (2007)[9] in their study showed Fever (92%), cough

(69%) and malaise (46%) were the most common symptoms.

Although Cartridge based nucleic acid amplification test helps to provide rapid confirmation of disease, its sensitivity remains suboptimum compared with culture results. The paucibacillary nature of samples from young children partly explains the lower sensitivity of Cartridge based nucleic acid amplification test than that observed in adults.

A negative Cartridge based nucleic acid amplification test does not exclude a diagnosis of pulmonary tuberculosis given the fact that the test was unable to identify 7% of children with culture confirmed pulmonary tuberculosis. A clinical decision in the context of the patient is therefore important in initiating anti-tuberculous therapy in a child who has a negative Cartridge based nucleic acid amplification test.

A Cartridge based nucleic acid amplification test was found positive maximum in both 5-10 years and 10-17 years of age group which showed statistically

significant results (p value 0.045). Sekadde et al(2013)[10] in their study 'Evaluation of the Xpert MTB/RIF test for the diagnosis of childhood pulmonary tuberculosis in Uganda' concluded that age more than 5 years (p value 0.03) had statistical significant association with positive Xpert.

There was no significant difference (p value 0.82) in positivity of Cartridge based nucleic acid amplification test with respect to gender (Graph 1). Similarly, Qing-Qin Yin et al (2014)[11] demonstrated no significant difference in the positivity of Xpert on the basis of gender (p value 0.741).

We obtained that Cartridge based nucleic acid amplification test was found positive in 76.92% of cases with BCG scar present and in 23.08% of cases with absent BCG scar (p value 0.47) which shows no statistical difference. (Graph 2)

Qing-Qin Yin et al(2014)[11] demonstrated that positive rate of Xpert MTB/RIF assay in patients with no BCG scar was significantly higher than with BCG scar (p value 0.001).

We report positive rate of Cartridge based nucleic acid amplification test in patients with history of tuberculosis contact was significantly higher

(p value 0.049) than that with no history of contact (Graph 3). Also the positivity of Cartridge based nucleic acid amplification test was higher in lower socioeconomic groups (class III and IV). Similarly, Sekadde et al (2013)[10] obtained significant association between positive history of contact and positive Xpert (p value 0.03).

We have shown a statistically significant association (p value = 0.0015 and pearson correlation of 0.671) between positive rates of Cartridge based nucleic acid amplification test and reactivity of tuberculin sensitivity test.

Sekadde et al (2013)[10] obtained significant association between positive tuberculin sensitivity test and positive Xpert (p value 0.002) which is in accordance to our study.

Conclusion

Thus we can safely conclude that Cartridge based nucleic acid amplification test on one pleural fluid aspirate sample rapidly and correctly identified the majority of children with culture confirmed tuberculosis with a high specificity. Compared with microscopy, CBNAAT offers better sensitivity and its scale up will improve access to tuberculosis diagnosis in children.

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