Scholars Journal of Applied Medical Sciences
Abbreviated Key Title: Sch J App Med Sci
ISSN 2347-954X (Print) | ISSN 2320-6691 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Pulmonary Medicine

To Study the Role of Gene Xpert in the Diagnosis of Tuberculous Cervical Lymphadenitis

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DOI: 10.36347/sjams.2021.v09i11.004 | **Received:** 30.09.2021 | **Accepted:** 03.11.2021 | **Published:** 08.11.2021

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Abstract **Original Research Article**

Role of Gene Xpert in the diagnosis of Tuberculous Cervical Lymphadenitis was determined and compared with FNAC. Female preponderance is seen in present study in the cases of cervical lymphadenopathy. Caseous material of the lymph nodes had a higher yield. CBNAAT with FNAC is highly effective in the diagnosis of Tuberculosis. CBNAAT has an important role in the diagnosis of Extra Pulmonary Tuberculosis.

Keywords: Gene Xpert, CBNAAT, Tuberculosis.

AIM

To determine the role of Gene Xpert in the diagnosis of tuberculous cervical lymphadenitis.

OBJECTIVES

To study the role of Gene Xpert in diagnosing tuberculous cervical lymphadenitis and to know the efficacy of Gene Xpert in comparison with FNAC and AFB culture

METHODOLOGY

PATIENTS

- Patients attending to Bhaskar general hospital with clinical features of tuberculous cervical lymphadenitis.

METHODS

- A written informed consent will be obtained from all patients.
- Patients presenting with signs and symptoms of tuberculous cervical lymphadenopathy are taken and FNAC is done.
- FNAC sample is sent for gene Xpert (CBNAAT) and AFB Culture and the results are analyzed.

Inclusion Criteria

- All patients above 10years Clinically presenting with tuberculous cervical lymphadenitis attending to Bhaskar general hospital.

Exclusion Criteria

- Patients who had received ATT already.
- Patients with terminal disease who might need emergency medical care.
- Patients who do not consent to be a part of the study.

STUDY DESIGN:

- Cross sectional study

SAMPLE SIZE: 50 patients fulfilling the inclusion criteria.

STUDY PERIOD: One and a half year from January 2019 to June 2020

ETHICAL IMPLICATIONS:

- The study subjects will be selected following inclusion and exclusion criteria.
- Written & informed consent will be taken.

- Every patient will be completely explained about the study and related procedures and their importance and complications in their own understandable language.

FINANCIAL IMPLICATIONS:

- Funding – none
- Expenses if any will be incurred by me if patients are not affordable.

INVESTIGATIONS

1. Thorough history taking and physical examination.
2. FNAC of the lymph node.
3. Gene Xpert of the aspirate from the lymph node.
4. AFB culture

DATA ANALYSIS

Statistical Methods

CBNAAT, FNAC AND CRS, AFB Culture was considered as primary outcome variable.

Age, gender was considered as primary explanatory variable. Rifampicin resistance, type of FNA aspirate were considered as other study relevant variable

Descriptive analysis: Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency and proportion for categorical variables. Data was also represented using appropriate diagrams like bar diagram and pie diagram.

All Quantitative variables were checked for normal distribution within each category of explanatory variable by using visual inspection of histograms and normality Q-Q plots. Shapiro-wilk test was also conducted to assess normal distribution. Shapiro wilk test p value of >0.05 was considered as normal distribution.

Categorical outcomes were compared between study groups using Chi square test /Fisher's Exact test (If the overall sample size was < 20 or if the expected number in any one of the cells is < 5, Fisher's exact test was used.)

FNAC, CRS AND AFC Culture was considered as gold standard. CBNAAT were considered as screening test. The sensitivity, specificity, predictive values and diagnostic accuracy of the screening test along with their 95% CI were presented.

P value < 0.05 was considered statistically significant. IBM SPSS version 22 was used for statistical analysis (1).

1. IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.

RESULTS

A total of 50 subjects were included in the final analysis.

Table 1: Descriptive analysis of age in study population (N=50)

Parameter	Mean ± SD	Minimum	Maximum
Age	29.22 ± 12.99	11.00	65.00

The mean age was 29.22 ± 12.99 in the study population, the ranged between 11 years and 65 years in the study population.

Table 2: Descriptive analysis of age group in the study population (N=50)

Age Group	Frequency	Percentages
Upto 20	14	28.00%
21 to 35	23	46.00%
36 to 50	10	20.00%
51 and above	3	6.00%

Among the study population, 14(28%) participants were aged upto 20 years, 23(46%) participants were aged between 21 to 35 years, 10(20%) participants were aged between 36 to 50 years and 3(6%) participants were aged 51years and above.

Table 3: Descriptive analysis of gender in the study population (N=50)

Gender	Frequency	Percentages
Male	14	28.00%
Female	36	72.00%

Among the study population, 14(28%) participants were remaining 36(72%) participants were female.

Table 4: Descriptive analysis of GeneXpert in the study population (N=50)

GeneXpert	Frequency	Percentages
MTB detected low	5	10.00%
MTB detected very low	7	14.00%
MTB not detected	38	76.00%

Among the study population, 5(10%) participants had MTB detected low, 7(14%) participants had MTB detected very low and 38(76%) participants had MTB Not detected.

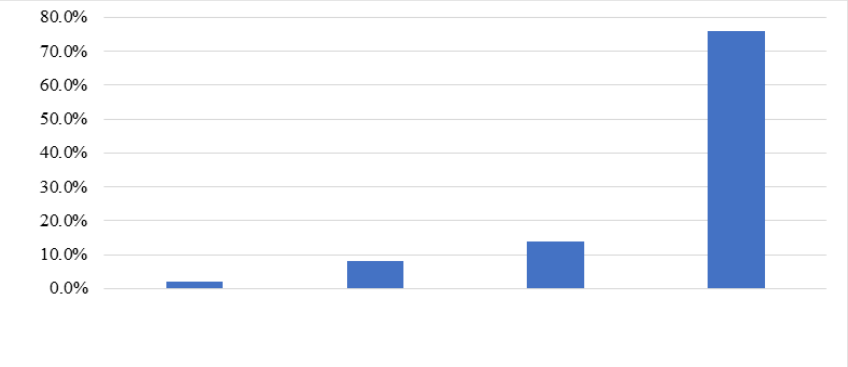

Figure 1: Bar chart of GeneXpert in the study population (N=50)

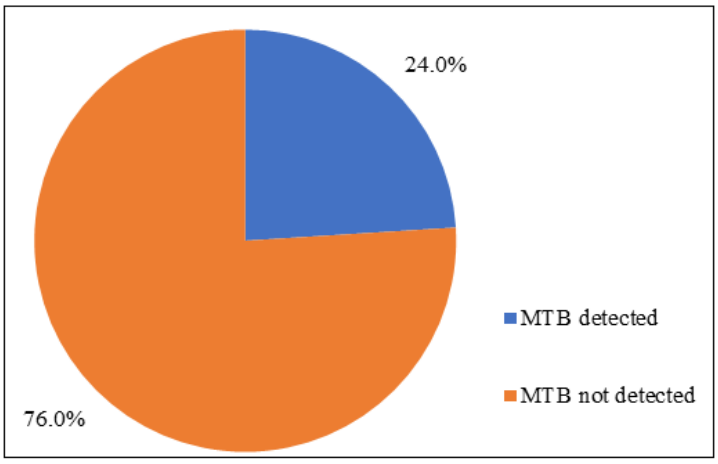

Figure 2: Pie chart of CBNAAT in the study population (N=50)

Table 5: Descriptive analysis of rifampicin resistance in the study population (N=12)

Rifampicin resistance	Frequency	Percentages
Not detected	12	100%

Among the study population, 12(100%) participants had rifampicin resistance not detected.

Table 6: Descriptive analysis of FNAC in the study population (N=50)

FNAC	Frequency	Percentages
Granulomas with caseous necrosis, Tuberculous Etiology	24	48.00%
Granulomas without caseous necrosis, Tuberculous Etiology	17	34.00%
Non specific lymphadenitis	3	6.00%
only caseous necrosis, Tuberculous Etiology	1	2.00%
Reactive lymphadenitis	5	10.00%

Among the study population, 24(48%) participants had granulomas with caseous necrosis, tuberculous etiology, 17(34%) participants had granulomas without caseous necrosis, tuberculous etiology, 3(6%) participants had nonspecific lymphadenitis, 1(2%) participant had only caseous necrosis, tuberculous etiology and 5(10%) participants had reactive lymphadenitis.

Table 7: Descriptive analysis of FNAC in the study population (N=50)

FNAC	Frequency	Percentages
Positive	42	84.00%
Negative	8	16.00%

Out of 50 participants, 42 (84%) participants had FNAC positive.

Table 8: Descriptive analysis of type of FNAC aspirate in the study population (N=50)

Type of FNAC aspirate	Frequency	Percentages
Blood mixed	9	18.00%
cheesy	2	4.00%
Purulent	39	78.00%

Among the study population, 9(18%) participants had blood mixed, 2(4%) participants had cheesy and 39(78%) participants had purulent.

Table 9: Descriptive analysis of AFB culture in the study population (N=50)

AFB culture	Frequency	Percentages
Positive	39	78.00%
Negative	11	22.00%

Out of 50 participants, 39(78%) participants had AFB culture positive.

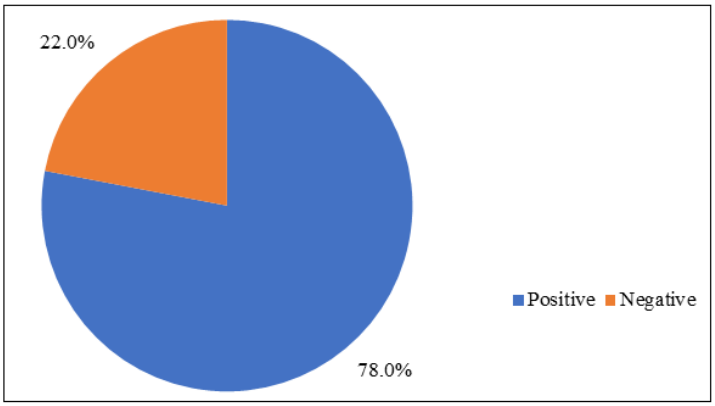

Figure 3: Pie chart of AFB culture in the study population (N=50)

Table 10: Descriptive analysis of rifampicin resistance in the study population (N=50)

CRS	Frequency	Percentages
Positive	50	100%

Out of 50 participants, all of them 50(100%) participants had CRS positive.

Table 11: Comparison of age group between CBNAAT (N=50)

Age Group	CBNAAT	
	MTB detected	MTB not detected
Upto 20 (N=14)	3 (21.43%)	11 (78.57%)
21 To 35 (N=23)	6 (26.09%)	17 (73.91%)
36 To 50 (N=10)	3 (30%)	7 (70%)
51 And Above (N=3)	0 (0%)	3 (100%)

**** No statistical test was applied-due to 0 subjects in the cell***

Out of 14 people with upto 20 years age group, 3 (21.43%) had MTB detected and another 11 (78.57%) had MTB not detected. Out of 23 people with 21 to 35 years were aged between,6 (26.09%) had MTB detected and another 17 (73.91%)had MTB not detected. Out of 10 people with 36 to 50 years were aged between, 3 (30%) had MTB detected and another 7 (70%) had MTB not detected. Out of 3 people with 51 years and above age group, 3 (100%) had MTB not detected.

Table 12: Comparison of gender between CBNAAT (N=50)

Gender	CBNAAT		Chi square	P value
	MTB detected	MTB not detected		
Male (N=14)	2 (14.29%)	12 (85.71%)	1.006	0.468
Female (N=36)	10 (27.78%)	26 (72.22%)		

Out of 14 people with male, 2 (14.29%) had MTB detected and another 12 (85.71%) had MTB not detected. Out of 36 people with female, 10 (27.78%) had MTB detected and another 26 (72.22%) had MTB not detected. The difference in the proportion of CBNAAT between gender was statistically not significant (P value 0.468)

Table 13: Comparison of age group between FNAC (N=50)

Age Group	FNAC	
	Positive	Negative
Upto 20 (N=14)	11 (78.57%)	3 (21.43%)
21 To 35 (N=23)	22 (95.65%)	1 (4.35%)
36 To 50 (N=10)	6 (60%)	4 (40%)
51 And Above (N=3)	3 (100%)	0 (0%)

**** No statistical test was applied-due to 0 subjects in the cell***

Out of 14 people with upto 20 years age group, 11 (78.57%) had FNAC positive and another 3 (21.43%) had FNAC negative. Out of 23 people with 21 to 35 years were aged between, 22 (95.65%) had FNAC positive and another 1 (4.35%) had FNAC negative. Out of 10 people with 36 to 50 years were aged between, 6 (60%) had FNAC positive and another 4 (40%) had FNAC negative. Out of 3 people with 51 years and above age group, 3 (100%) had FNAC positive.

Table 14: Comparison of gender between FNAC (N=50)

Gender	FNAC		Chi square	P value
	Positive	Negative		
Male (N=14)	10 (71.43%)	4 (28.57%)	2.286	0.197
Female (N=36)	32 (88.89%)	4 (11.11%)		

Out of 14 people with male, 10 (71.43%) had FNAC positive and another 4 (28.57%) had FNAC negative. Out of 36 people with female, 32 (88.89%) had FNAC positive and another 4(11.11%) had FNAC negative. The difference in the proportion of FNAC between gender was statistically not significant (P value 0.197)

Table 15: Comparison of type of FNAC aspirate between CBNAAT (N=50)

Type of FNAC aspirate	CBNAAT		Chi square	P value
	MTB detected (N=12)	MTB Not detected (N=38)		
Blood Mixed	5 (41.67%)	4 (10.53%)	7.242	0.027
Cheesy	1 (8.33%)	1 (2.63%)		
Purulent	6 (50%)	33 (86.84%)		

Among the people with MTB detected group, 5 (41.67%) people had blood mixed, 1(8.33%) people had cheesy and6 (50%) people had purulent. Among the people with MTB not detected group, 4 (10.53%) people had blood mixed, 1 (2.63%) people had cheesy and 33 (86.84%) people had purulent. Difference in the proportion of type of FNAC aspirate between CBNAAT was statistically significant (p value 0.027).

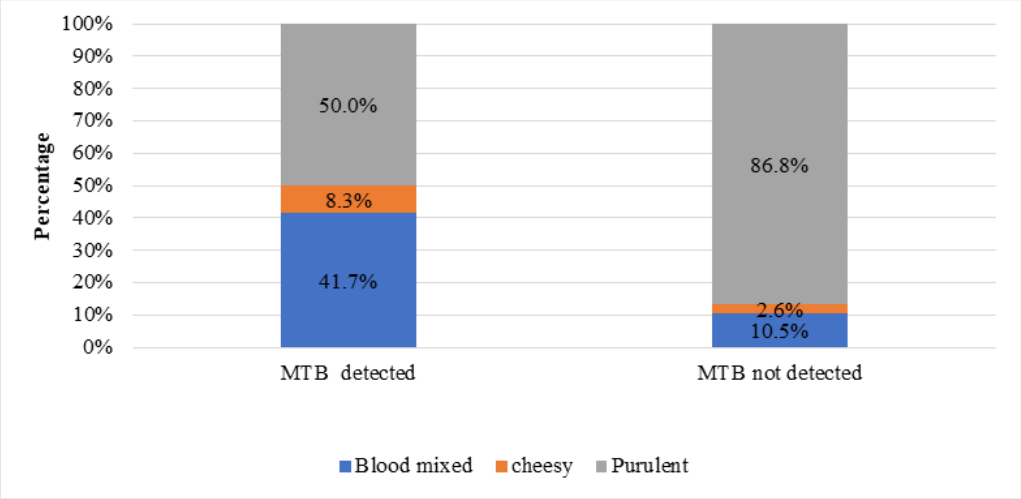

Figure 11: Staked bar chart of comparison of type of FNAC aspirate between CBNAAT (N=50)

Table 16: Comparison of FNAC with CBNAAT (N=50)

CBNATT	FNAC		Chi square	P value
	Positive (N=42)	Negative (N=8)		
MTB Detected	10 (23.81%)	2 (25%)	0.005	**0.942**
MTB Not Detected	32 (76.19%)	6 (75%)		

Among the people with FNAC positive group, 10 (23.81%) people had MTB detected, 32 (76.19%) people had MTB not detected. Among the people with FNAC negative group, 2 (25%) people had MTB detected, 6 (75%) people had MTB not detected. difference in the proportion of CBNAAT between FNAC was statistically not significant (p value 0.942).

Table 17: Predictive validity of CBNAAT in predicting FNAC (N=50)

Parameter	Value	95% CI	
		Lower	Upper
Sensitivity	23.81%	12.05%	39.45%
Specificity	75.00%	34.91%	96.81%
False positive rate	25.00%	3.19%	65.09%
False negative rate	76.19%	60.55%	87.95%
Positive predictive value	83.33%	51.59%	97.91%
Negative predictive value	15.79%	6.02%	31.25%
Diagnostic accuracy	32.00%	19.52%	46.70%

The diagnosis of CBNAAT had sensitivity of 23.81% (95% CI 12.05% to 39.45%) in predicting FNAC. specificity was 75.00% (95% CI 34.91%to 96.81%), false positive rate was 25.00% (95% CI 3.19%to 65.09%), false negative rate was 76.19% (95% CI 60.55% to 87.95%), positive predictive value was 83.33% (95% CI 51.59%to 97.91%), negative predictive value was 15.79%(95% CI 6.02%to 31.25%) and the total diagnostic accuracy was 32.00% (95% CI 19.52% to 46.70%).

Table 18: Comparison of AFB culture with CBNAAT (N=50)

CBNAAT	AFB culture	
	Positive (N=39)	Negative (N=11)
MTB Detected	12 (30.77%)	0 (0%)
MTB Not Detected	27 (69.23%)	11 (100%)

**** No statistical test was applied-due to 0 subjects in the cell***

Among the people with AFB culture positive group, 12 (30.77%) people had MTB detected, 27 (69.23%) people had MTB not detected. Among the people with AFB culture negative group, 11 (100%) people had MTB not detected.

Table 19: Predictive validity of CBNAAT in predicting AFB culture (N=50)

Parameter	Value	95% CI	
		Lower	Upper
Sensitivity	30.77%	17.02%	47.57%
Specificity	100.00%	71.51%	100.00%
False positive rate	0.00%	#NUM!	28.49%
False negative rate	69.23%	52.43%	82.98%
Positive predictive value	100.00%	73.54%	100.00%
Negative predictive value	28.95%	15.42%	45.90%
Diagnostic accuracy	46.00%	31.81%	60.68%

The diagnosis of CBNAAT had sensitivity of 30.77% (95% CI 17.02%to 47.57%) in predicting AFB culture. specificity was 100.00%(95% CI 71.51%to 100%), false positive rate was 0.00% (95% CI 0.00%to 28.49%), false negative rate was 69.23%(95% CI 52.43%to 82.98%), positive predictive value was 100%(95% CI 73.54%to 100%), negative predictive value was 28.95%(95% CI 15.42%to 45.90%) and the total diagnostic accuracy was 46.00%(95% CI 31.81%to 60.68%).

Table 20: Comparison of CRS with CBNAAT (N=50)

CBNAAT	CRS	
	Positive (N=50)	Negative (N=0)
MTB Detected	12 (24%)	0 (0%)
MTB Not Detected	38 (76%)	0 (0%)

Among the people with CRS positive group, 12 (24%) people had MTB detected, 38 (76%) people had MTB not detected.

Table 21: Predictive validity of CBNAAT in predicting CRS (N=50)

Parameter	Value	95% CI	
		Lower	Upper
Sensitivity	24.00%	13.06%	38.17%
Specificity	-	-	-
False positive rate	-	-	-
False negative rate	76.00%	61.83%	86.94%
Positive predictive value	100.00%	73.54%	100.00%
Negative predictive value	0.00%	-	9.25%
Diagnostic accuracy	24.00%	13.06%	38.17%

The diagnosis of CBNAAT had sensitivity of 24.00% (95% CI 13.06%to 38.17%) in predicting CRS. false negative rate was 76% (95% CI 61.83% to 86.94%), positive predictive value was 100% (95% CI 73.54%to 100%), negative predictive value was 0.00% (95% CI 0.00%to 9.25%) and the total diagnostic accuracy was 24.00% (95% CI 13.06% to 38.17%).

DISCUSSION

The present study is a hospital based cross sectional study on the role of Gene Xpert in diagnosing cervical TB lymphadenitis in comparison to FNAC and AFB culture.

In the present study, age and sex wise distribution of CBNAAT positive cases are compared with other studies where Younger age groups (25-35) were predominantly effected with Tb in all the studies including present study (Table 12). Female preponderance (78%) is seen in the present study which is correlated with other studies (Table 13) [14-18]. We also compared the distribution of type of FNA aspirate along with CBNAAT result which is not correlated with Mulualem *et al.*, study where caseous aspirates (69%) had more CBNAAT positivity compared to present study which has 50% cases were purulent aspirates with CBNAAT positivity.

In the present study non correlated cases of CBNAAT with FNAC were 42, out of which 19 cases were FNA- CBNAAT+ and other 23 cases were FNA +CBNAAT-.

Around 19 FNA-CBNAAT+ patients, 16 were grossly purulent aspirates. Out of 3 blood mixed aspirates. So, in our study the importance of CBNAAT lies in detecting 2 cases which were cytologically negative for Tb, who were surely benefitted by the CBNAAT.

Out of 32 (FNAC + CBNAAT-cases), majority of the cases were blood mixed (11/32). It is possible that in these cases representative sample might not be

obtained as bacterial load may have been too low for the GeneXpert to detect the DNA from MTB- complex. The possible cause for CBNAAT negativity in seven (7/50) cases of cheesy material might be solid nature of the cheesy material which usually have very low bacillary load in nature compared to liquid caseous material which have high bacillary load.

According to WHO Xpert guidelines all the patients had received Tb treatment as they were cytologically positive and clinically suspicious [22]. So, CBNAAT negative result can still have Tb. On comparison of CBNAAT diagnostic performance of present study (sensitivity 23.81%, specificity 75%) (CRS) with Singh KG *et al*., (sensitivity 91%, specificity 90%) Ligthelm *et al*., (sensitivity-96.7%, specificity 88.9%) showed less sensitivity and less specificity [23, 24]. In the present study rifampicin resistance on CBNAAT in cases of EPTB was nil.

CONCLUSION

1. CBNAAT has detected 4% of cases which were not detected by FNAC.
2. In cases with granulomatous lymphadenitis and purulent aspirates CBNAAT has an important role in diagnosing EPTB.
3. Rifampicin resistance detection by CBNAAT has greater advantage in the treatment of patients with shorter turn-around time which is not possible with FNA even though FNA is cost effective in the diagnosis of EPTB.
4. FNA in combination with CBNAAT has an advantage of detection of FNA missed cases and rifampicin resistance.
5. The study recommends integration of CBNAAT into a routine diagnostic protocol.

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