

Comparison of GeneXpert with ZN Stain in FNA samples of suspected extrapulmonary tuberculosis

Dr. Gundrajakuppam Lavanya¹, Dr. C. Sujatha², Dr. Khader Faheem³,
Dr. B. Anuradha⁴

1,2,3- Assistant professor, department of pathology, S.V. Medical College, Tirupati.

4- Professor & Head department of pathology, S.V. Medical College, Tirupati

Corresponding Author: Dr. C. Sujatha

Abstract: Background: Extrapulmonary tuberculosis is challenging because of its paucibacillary nature. As per WHO recommendations, Gene Xpert is an initial diagnostic test for these cases. Aim of this study to compare the FNAC findings of extrapulmonary tuberculosis with ZN stain and GeneXpert.

Methodology: It's a prospective study with 151 cases of clinical suspicion of tuberculosis over a period of 12 months. FNA was done and material sent to TBCD Department for Gene Xpert and microscopy, ZN Stain was done in all cases, results were obtained.

Results: Out of 151 cases, presumptive tuberculosis was diagnosed in 80 cases morphologically. Thirty-one cases were Gene Xpert positive, and 12 cases were ZN stain positive with the sensitivity of 83.3% and specificity of 84.8%. Rifampicin resistance was noted 6.4% of gene xpert positive cases.

Conclusion: Till the day FNA has been the cheapest method of diagnosing the EPTB. However, in suppurative lesions gene xpert has an important role. Besides, it offers rifampicin resistance, making it a better diagnostic tool.

Keywords: EPTB, FNA, Gene Xpert, ZN Stain

Date of Submission: 10-07-2019

Date of acceptance: 25-07-2019

I. Introduction

Tuberculosis (TB) is one of the world's deadliest communicable diseases according to the Global Tuberculosis report 2014 of World Health Organization (WHO) that is caused by the Bacterium *Mycobacterium tuberculosis* (MTB) (1). This disease primarily affects the lungs called pulmonary TB and spread by air (2). India is the country with the highest burden of TB. As per the Global TB report 2017, the estimated incidence of TB in India was approximately 28,00,000, accounting for one-fourth of the world's TB cases. (3). It is estimated that about 40% of the Indian population is infected with TB bacteria; the vast majority have latent TB rather than TB disease. (4)

Extrapulmonary tuberculosis is *Mycobacterium tuberculosis* infection of organs and tissues other than lungs. The burden of EPTB is 15-20% of all TB cases in HIV negative patients, while it is 40-50% in HIV positive cases. (5) Diagnosing EPTB remains challenging because clinical samples obtained from relatively inaccessible sites may be paucibacillary, thus decreasing the sensitivity of diagnostic tests. Early diagnosis and management are crucial in managing these patients, which can cause less morbidity and communicability. The most common challenges faced by developing nations like India are misdiagnosis of suspected TB cases and false-negative results. Most TB control programs use Ziehl-Neelsen (ZN) smear microscopy, which has poor sensitivity and multiple visits are warranted that leads to higher default. Another mode of diagnosing the TB is culture, which is a gold standard technique, but its time taking, and it needs technical expertise. (1,4,6).

With the recent advances in gene therapy in diagnosing and managing the cases, NAA (Nucleic acid amplification) method developed for early detection of MTB in both pulmonary and extrapulmonary TB cases. In recent times WHO has been endorsing the usage of NAA method in diagnosing these cases called as GeneXpert/Xpert® MTB/Rif assay (7). The further advantage of this technique is it can detect the least MTB genomic copies available in the sample. (7-8). The GeneXpert utilizes a DNA-PCR technique for simultaneous detection of *Mycobacterium tuberculosis* and Rifampicin resistance-related mutations. It is fully automated benchtop cartridge-based nucleic acid amplification (CB-NAAT) assay for TB detection that includes all the necessary steps of DNA PCR and gives results within 2 hours. The diagnostic accuracy of GeneXpert for pulmonary TB has been reported high [9,10]. Patients with a high risk of tuberculosis-like HIV-associated TB patients and extrapulmonary cases in whom ZN Stain smear examination is usually negative are the most likely to be benefited from GeneXpert [10].

Aims and objectives of this study were to compare the FNAC findings of extrapulmonary tuberculosis with ZN stain and GeneXpert and to assess the difference in results.

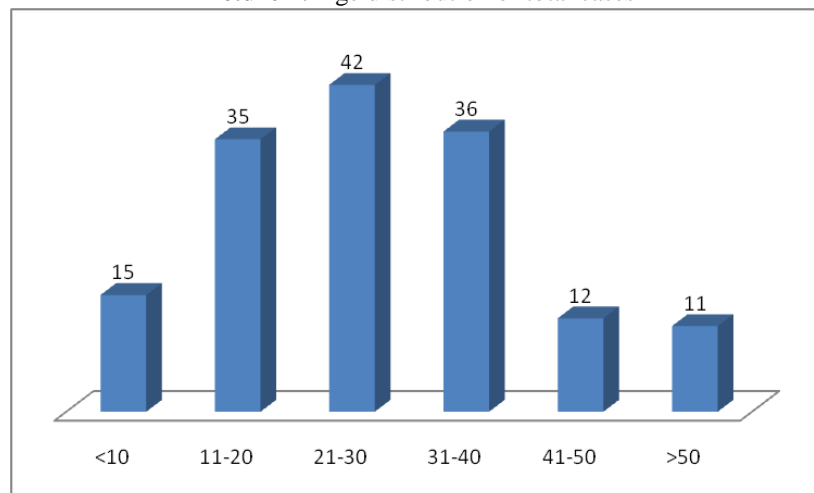
II. Materials and Methodology

Patients with clinical suspicion of tuberculosis presenting with lymphadenopathy, Purulent aspirates on FNA were included in the study. A prospective study conducted with 151 cases of clinical suspicion of tuberculosis presenting with lymphadenopathy in the department of pathology, SV Medical College, Tirupathi for a period of 12 months (April 2018 to March 2019). FNA samples were collected by aspirating with 23-25 gauge needles attached to 10 ml syringe. A total of 3 smears prepared, two of these fixed with alcohol, and H&E staining was done and reported, the other smear reported after ZN stain. Report result is mentioned as positive/negative only. The remaining aspirated material was sent to TBCD department, SVVRRGGH Hospital for Gene Xpert. The sample was added with buffer at 1:2 ratio and incubated at room temperature for 30 mins. Then 2 ml of the material is transferred to cartridge and loaded onto the machine. The final results reported as positive/negative. The semi-quantitative estimate is given based on the Ct range. High (ct<16), Medium (16-22), Low, (22-28), and very low (<28). Rifampicin resistance reported as susceptible, resistant, and indeterminate.

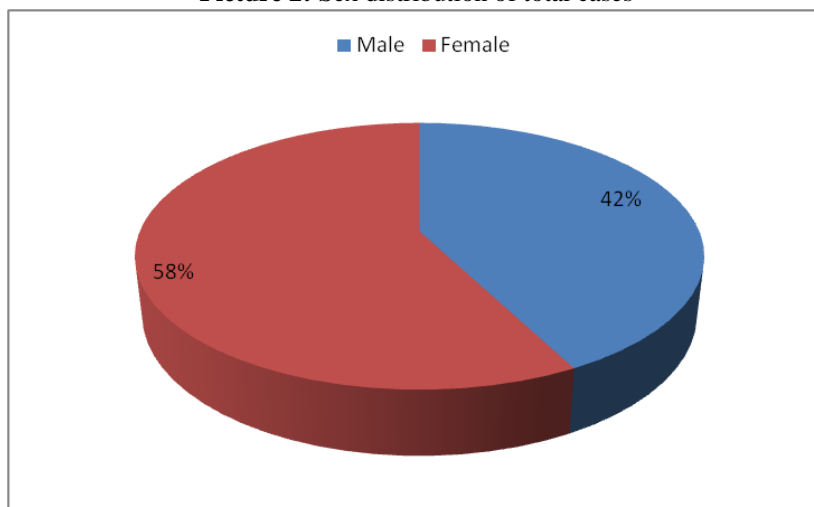
III. Results

One hundred fifty-one cases with clinical suspicion of tuberculosis with lymphadenopathy subjected to FNAC, ZN Stain, and gene Xpert. Most of the cases were laying in the age group of 11-30 years 50.9% (77/151) as in picture 1, and most of the cases were females with 57.6% (87/151), as in picture 2. The predominant site involved was cervical with 81% (121/151) followed by axillary and inguinal region as in picture 3.

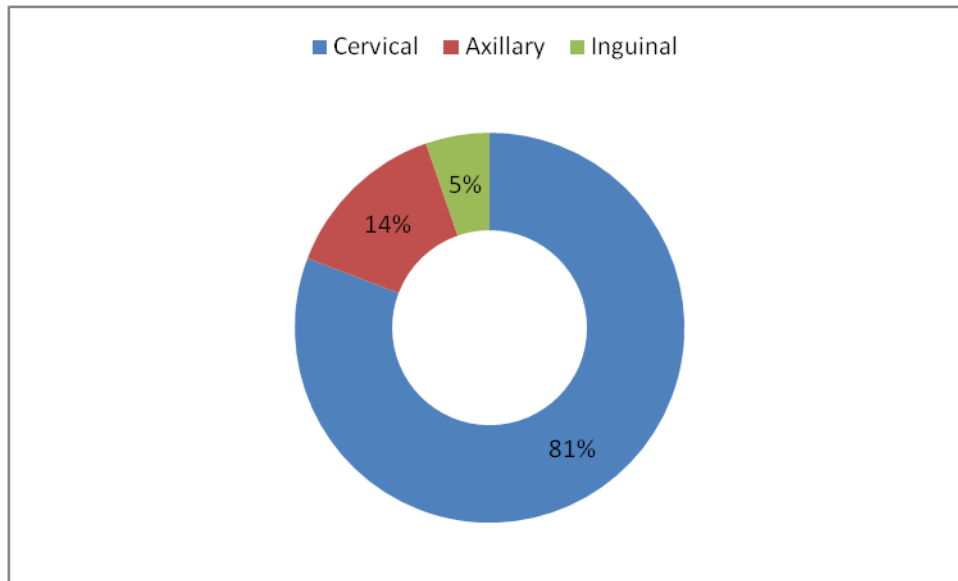
Picture 1: Age distribution of total cases



Picture 2: Sex distribution of total cases



Picture 3: Site wise distribution of total cases



Out of 151 cases, 80 were cytomorphologically tuberculosis and among these 55 cases (36.4%) were Chronic Granulomatous lymphadenitis (CGLN), and 25 cases (16.5%) were necrotising lymphadenitis (NL) without granulomas. In these 80 cases, 23 (28.7%) were Xpert positive, and 10 (12.5%) were ZN Stain positive. Surprisingly in a total of 27 suppurative lymphadenitis (SL) cases, 6 (22.2%) were Xpert positive, which indicates suppurative lesions cannot exclude tuberculosis, and 2(7.4%) were ZN Stain positive. In all other cases, Xpert and ZN Stain were negative except non-specific lymphadenitis with 2 (11.1) case of Xpert positive as in table 1.

Table 1:Comparison of cytomorphological diagnosis with Gene Xpert and ZN Stain

S.No.	FNA diagnosis	No.of cases	Xpert Positive	ZN Stain positive
1	Tuberculosis	80		
	CGLN	55(36.4%)	15	6
	NL	25(16.5%)	8	4
2.	SL	27(17.8%)	6	2
3.	NSL	18(11.9%)	2	-
4.	RL	13(8.6%)	-	-
5.	Epidermoid cyst	3(0.6%)	-	-
6.	Fungal abscess	1(1.9%)	-	-
7.	Metastasis	6(3.9%)	-	-
8.	Non specific sialadenitis	2(1.9%)	-	-

Out of 31 Gene Xpert positive cases on comparison with FNA, 23 were both positive, eight were only Xpert positive, which includes 6 cases of SL and 2 cases of NSL. Out of 120 Xpert negative cases, 57 were positive according to FNA and 63 were both negative, which constitutes RL, Epidermoid cyst, fungal abscess, and Metastasis as in table 2.

Table 2:Diagnostic performance of Gene Xpert with Fine Needle Aspiration (FNA)

Gene expert	FNA		Total
	Positive	Negative	
Positive	23	08	31
Negative	57	63	120
Total	80	78	151

Out of 12 ZN Stain positive cases on comparison with Fine Needle Aspiration (FNA) ten were both positive, two were only ZN Stain positive, which was seen in SL. Out of 139 ZN Stain negative cases, 70 were positive according to FNA and 69 were both negative, which constitutes NSL, RL, Epidermoid cyst, fungal abscess, and Metastasis as in table 3.

Table 3: Diagnostic performance of ZN Stain with Fine Needle Aspiration (FNA)

ZN Stain	FNA		Total
	Positive	Negative	
Positive	10	02	12
Negative	70	69	139
Total	80	71	151

Out of 151 cases, 6.6% (10/151) were both Xpert and ZN Stain positive, 78.1 % (118/151) were both negative. 13.9% (21/151) were Xpert positive and ZN Stain negative. 1.3% (2/151) were ZN Stain positive and Xpert negative, which could be due to inadequate sample size/nonmycobacterial tuberculosis as in table 4.

Table 4: Diagnostic performance of the Gene Xpert versus ZN Stain was done

Xpert	ZN Stain		Total
	Positive	Negative	
Positive	10	21	31
Negative	2	118	120
Total	12	139	151

Sensitivity and specificity of gene Xpert with FNA was 28.75% and 88.7% respectively, and the ZN Stain with FNA was 12.5% and 97.1% respectively. However, when both gene Xpert and ZN Stain compared, the sensitivity was 83.3%, and specificity was 84.8% as in table 5.

Table 5: Diagnostic performance of the Gene Xpert versus ZN Stain versus FNA

Reference standard	Sensitivity	Specificity	Positive predictive value	Negative Predictive value
Gene Expert vsFNA	28.75%	88.7%	74.1%	52.5%
ZN StainvsFNA	12.5%	97.1%	83.3%	49.6%
Gene Expert vsZN Stain	83.3%	84.8%	32.3%	84.7%

Out of 31 Xpert positive cases, 11 were very low (ct>28), 10 were low (ct 22-28), 5 were medium (ct16-22) and 2 were high (ct<16). The mean ct values were lower for smear positive cases. In Xpert semi-quantitative result, most of the cases were very low and low (24/31) that indicates the paucibacillary nature of extrapulmonary tuberculosis as in table 6.

Table 6: Comparison of Xpert semi-quantitative result (Ct-value) and ZN STAIN

Xpert Result	ZN Stain Grade	
	Negative	Positive
Very low (13)	11	2
Low(11)	10	1
Medium (5)	-	5
High(2)	-	2

In 31 xpert positive cases, 2 (6.4%) cases of rifampicin-resistant detected, which was seen in 1 very low and one high. 1 (3.2%) case was indeterminant, which was very low as in table 7.

Table 7: Comparison of Xpert semi-quantitative result (Ct-value) and Rifampicin resistance

Rifampicin Resistant	xpert				Total
	Very low	Low	Medium	High	
Sensitive	11	11	5	1	28
Resistant	1	-	-	1	2
Indeterminant	1	-	-	-	1

IV. Discussion:

The current study is a hospital-based prospective study on the diagnosis of extrapulmonary Tuberculosis by Gene Xpert and ZN Stain in comparison to FNA.

In the present study, we compared the age and sex wise distribution of Gene expert positive cases with other studies where Younger age groups were predominantly affected with Tb in all the studies including present study (Table 8) and female preponderance is seen in the present study which is correlated with other studies (Table 9). (11-16).

Table 8: Comparison of age wise distribution of gene xpert positive cases with other studies

Study	Age group	% of cases
Present study	11-30	50.9%
Yassin et al10	15-24	30.7%
Aroravk et al11	15-24	38%
Brayn et al12	15-24	43%
Muluaalem et al13	16-30	58 %

Table 9: Comparison of sex wise distribution of gene xpert positive cases with other studies

Study	Male	Female
Present study	42.4 %	57.6%
Brain et al 12	46%	54%
Muluaalem et al 13	67%	76%
Poojasingh et al 14	31%	69%

In concordance with other studies, the major site of involvement was cervical, followed by axillary region and the inguinal region as in Komanapalli et al. (16). Gene Xpert assay had an overall sensitivity of 28.7%, which is superior to that of ZN Stain smear microscopy with a sensitivity of 12.5% in the current study. In agreement with other studies, Xpert has higher sensitivity than ZN Stain smear microscopy (17, 19). However, Specificity was more with ZN Stain smear study with 97% when compared to the Gene Xpert assay (88%).

The sensitivity of the current study (83.3%) is less when compared to what was found in Singh KG et al., (sensitivity 91%) and Ligthelm et al. (sensitivity-96.7%). However, the specificity of the current study (88.7%) was found to be consistent with previous studies reported by Singh KG et al., (specificity 90%) and Ligthelm et al. (specificity 88.9%).(18, 19). In the current study, rifampicin resistance on gene xpert in cases of Extrapulmonary tuberculosis (EPTB) was 6.4%.

V. Conclusion:

Gene Xpert has an important role in diagnosing EPTB. The sensitivity of Gene Xpert is more compared to ZN Stain smear microscopy in the present study. In addition, gene Xpert helps in detection of Rifampicin resistance which is not possible with FNA and ZN Stain even though FNA is cost effective in the diagnosis of EPTB, combining with Gene Xpert has an advantage of detection of FNA missed cases, especially in a suppurative abscess. Moreover, it has a quicker turnaround time (2hours) compared to culture, which is a gold standard.

Abbreviations:

EPTB-Extrapulmonary tuberculosis
 FNA-Fine needle aspiration cytology
 CGLN-Chronic granulomatous lymphadenitis
 NL-Necrotising lymphadenitis
 SL-Suppurative lymphadenitis
 NSL-Nonspecific lymphadenitis
 RL-Reactive lymphadenitis

References

- [1]. World Health Organization. Global tuberculosis report 2014. Geneva: WHO; 2014.
- [2]. Annual status report, TB India 2015
- [3]. <https://tbcindia.gov.in/showfile.php?lid=3314>. India TB report 2018
- [4]. <https://www.tbfacts.org/tb-statistics-india/>
- [5]. Sharma SK, Mohan A. Extrapulmonary tuberculosis. Indian J Med Res. 2004Oct;120(4):316-53. Review. PubMed PMID: 15520485.
- [6]. Evans CA. GenXpert – a game changer for tuberculosis control? [4] *PLOS MED*. 2011;8:e1001064.
- [7]. Centers for Disease Control and Prevention (CDC). Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. *MMWR Morb Mortal Wkly Rep*. 2009;58:7-10.
- [8]. International standard for tuberculosis care, 3[6] rd edition, 2014 www.who.int/tb/publications/standards-tb-care-2014/
- [9]. Piersimoni C, Scarparo C, Piccoli P, et al. Performance assessment of two [7] commercial amplification assays for direct detection of *Mycobacterium tuberculosis* complex from respiratory and extrapulmonary specimens. *J Clin Microbiol*. 2002;40:4138-42.
- [10]. Saglam L, Akgun M, Aktas E. Usefulness of induced sputum and fiberoptic [8] bronchoscopy specimens in the diagnosis of pulmonary tuberculosis. *J Int Med Res*. 2005;33:260-65.
- [11]. Yassin, MA, Datiko DG, Shargie EB. Ten-year experiences of the tuberculosis control program in the southern region of Ethiopia. *Int J Lung Dis*. 2006;10(10):1166-71.
- [12]. Arora VK, Gupta R. Trends of extrapulmonary tuberculosis under revised national tuberculosis control program: A study from South Delhi. *Ind J Tuberc*. 2006;53:77-83.
- [13]. Rock RB, Sutherland WM, Baker C, Williams DN. Extrapulmonary tuberculosis among Somalis in Minnesota. *Emerging infectious diseases*. 2006 Sep;12(9):1434.

- [14]. Tadesse M, Abebe G, Abdissa K, Aragaw D, Abdella K, Bekele A, et al. GeneXpert MTB/RIF assay for the diagnosis of tuberculous lymphadenitis on concentrated fine needle aspirates in high tuberculosis burden settings. PLoS ONE. 2015;10(9):1-9.
- [15]. Gaur PS, Bhaskar R, Singh S, Saxena P, Agnihotri S. Incidence and clinical profiles of pulmonary and extra-pulmonary tuberculosis patients in North Indian population: a hospital based retrospective study. Inter J Res Development in Pharmacy and Life Science. 2017;6(5):2773-8.
- [16]. Komanapalli SK, Prasad U, Atla B, Vasundhara N, Yendluri D. Role of CB-NAAT in diagnosing extra pulmonary tuberculosis in correlation with FNA in a tertiary care center. Int J Res Med Sci 2018;6:4039-45.
- [17]. Al-Ateah SM, Al-Dowaidi MM, El-Khizzi NA. Evaluation of direct detection of Mycobacterium tuberculosis complex in respiratory and non-respiratory clinical specimens using the Cepheid Gene Xpert® system. Saudi Med J. 2012Oct;33(10):1100-5. PubMed PMID: 23047207
- [18]. Singh KG, Tandon S, Nagdeote ST, Sharma K, Kumar A. Role of CB-NAAT in diagnosing Mycobacterial tuberculosis and rifampicin resistance in tubercular peripheral lymphadenopathy. Int J Med Res Rev. 2017;5(03):242-46.
- [19]. Ligthelm LJ, Nicol MP, Hoek KG, Jacobson R, van Helden PD, Marais BJ, et al. Xpert MTB/RIF for rapid diagnosis of tuberculous lymphadenitis from fine-needle-aspiration biopsy specimens. J Clin Microbiol. 2011;49: 3967-70.

Dr. Gundrajakuppam Lavanya. "Comparison of GeneXpert with ZN Stainin FNA samples of suspectedextrapulmonary tuberculosis." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 18, no. 7, 2019, pp 25-30.