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Original Article

Comparison of different diagnostic modalities for isolation of *Mycobacterium Tuberculosis* among suspected tuberculous lymphadenitis patients

Comparação de diferentes modalidades de diagnóstico para isolamento de Mycobacterium tuberculosis entre pacientes com suspeita de linfadenite tuberculosa

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Abstract

Tuberculosis is a communicable disease with high morbidity and mortality rates in developing countries. The study's primary objective is to compare conventional methods such as acid-fast bacillus (AFB) culture and microscopy with rapid diagnostic methods. The secondary objective is to compare histopathological and microbiological findings in suspected patients with tubercular lymphadenitis. A total of 111 samples (August 2018 to September 2019) of lymph nodes were processed for AFB microscopy, AFB cultures, drug-susceptibility testing (DST), histopathology, and Xpert *Mycobacterium Tuberculosis* (MTB)/resistance to Rifampin (RIF) assays. Out of 111 lymph node samples, 6 (5.4%) were positive for AFB smear microscopy, 84 (75.6%) were positive for AFB culture, 80 (70.7%) were positive on Gene Xpert, and 102 (91.8%) were indicative of tuberculosis for histopathology studies. Mycobacteria growth indicator tube (MGIT) culture positivity was 84 (75.6%) higher than solid Lowenstein-Jensen (LJ) culture 74 (66.6%). Positive cultures underwent phenotypic DST. Two cases were Multidrug-resistant (MDR) on DST, while three cases were Rifampicin resistant on Gene Xpert. The sensitivity of Genexpert was (62%) against the conventional AFB culture method. The poor performance of conventional lymphadenitis diagnostic methods requires early and accurate diagnostic methodology. Xpert MTB/RIF test can help in the treatment of multidrug-resistant TB cases. Nonetheless, rapid and conventional methods should be used for complete isolation of Mycobacterium tuberculosis.

Keywords: DST: Drug Susceptibility Testing, MGIT: Mycobacterium Growth Indicator Tube, LJ: Lowenstein-Jensen, EPTB: Extra-pulmonary Tuberculosis, ZN: Ziehl-Neelsen.

Resumo

A tuberculose é uma doença transmissível com altas taxas de morbimortalidade nos países em desenvolvimento. O objetivo principal do estudo é comparar métodos convencionais, como cultura de bacilo álcool-ácido resistente (BAAR) e microscopia, com métodos de diagnóstico rápido. O objetivo secundário é comparar os achados histopatológicos e microbiológicos em pacientes com suspeita de linfadenite tubercular. Um total de 111 amostras (agosto de 2018 a setembro de 2019) de gânglios linfáticos foi processado para microscopia de AFB, culturas de AFB, teste de susceptibilidade a drogas (DST), histopatologia e Xpert Mycobacterium tuberculosis (MTB)/ensaios de resistência à rifampicina (RIF). Das 111 amostras de linfonodos, 6 (5,4%) foram positivas para baciloscopia de AFB, 84 (75,6%) foram positivas para cultura de AFB, 80 (70,7%) foram positivas para o GeneXpert e 102 (91,8%) foram indicativas de tuberculose para estudos histopatológicos. A positividade da cultura do tubo indicador de crescimento de micobactérias (MGIT) foi 84 (75,6%), maior que a cultura sólida de Lowenstein-Jensen (LJ), 74 (66,6%). As culturas positivas foram submetidas a DST fenotípico. Dois casos eram multirresistentes (MDR) ao DST, enquanto três casos eram resistentes à rifampicina no GeneXpert. A sensibilidade do GeneXpert foi 62% contra o método convencional de cultura AFB. O fraco desempenho dos métodos convencionais de diagnóstico de

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linfadenite requer metodologia de diagnóstico precoce e precisa. O teste Xpert MTB/RIF pode ajudar no tratamento de casos de tuberculose multirresistente. No entanto, métodos rápidos e convencionais devem ser usados para o isolamento completo do Mycobacterium tuberculosis.

Palavras-chave: DST: Teste de susceptibilidade a drogas, MGIT: Tubo indicador de crescimento de Mycobacterium, LJ: Lowenstein-Jensen, EPTB: Tuberculose extrapulmonar, ZN: Ziehl-Neelsen.

1. Introduction

Tuberculosis (TB) is a global health problem with high morbidity and mortality. Tuberculosis mainly disturbs the lungs and is known as pulmonary TB. Extra-Pulmonary tuberculosis (EPTB) defines the isolated onset of tuberculosis in body sites other than the lungs and is observed in about 15%-20% of all tuberculosis cases. Tuberculous lymphadenopathy is the best-known type of EPTB, which includes 35% of all EPTB cases. Cervical lymph nodes are the most typical tuberculous lymphadenopathy site in 60-90% of cases (Roy et al., 2016).

The frequency of tuberculosis in underdeveloped countries is a snowball, and this is believed to coexist with poor hygiene environments and a higher incidence of acquired immunodeficiency syndrome. In 2017, approximately 10 million persons (range 9-11.1 million) were infected globally with TB, out of which 3.2 million are women, 5.8 million are men, and 1.0 million are children. Approximately 3 million people die from tuberculosis every year (Zumla et al., 2015).

Massive progress in the fight against TB is achieved in the last 18 years, mainly by including TB in Millennium Development Goals and funded by Global Fund. In 2016, TB was also included in the United Nations Sustainable Development Goals to end the TB epidemic by 2030. Tuberculosis cases are decreasing across the globe in all WHO regions and other countries, but this pace is not enough to achieve the (2020) goals of the End TB Strategy. In the year 2020, It is necessary to reduce TB's incidence rate to 4%-5% and the death ratio of TB to 10% annually (Friis, 2018).

Pakistan is a developing country with low health standards. According to the Health and Quality of life (HAQ) index, Pakistan is ranked 154th out of 195 countries globally (Khalil et al., 2018). The incidence of new tuberculosis cases in 2015 was 0.51 million each year and approximately 15,000 (4.2%) of which were multidrug resistance (MDR) cases. This prevalence rate accounts for 61% of the Eastern Mediterranean Region's tuberculosis burden. Pakistan has the 5th highest prevalence of tuberculosis cases globally and the 4th highest prevalence of MDR Tuberculosis cases (WHO, 2015a).

Early and accurate diagnosis is a priority for Tuberculosis control, both for patient treatment and for public health intervention to reduce further transmission to the community. Numbers of diagnostic methods are available in the market to detect TB infection. Acid-fast bacillus (AFB) smear and culture were widely used to detect infection in suspected tuberculous lymphadenitis patients. There is a low sensitivity of conventional AFB smears, but the sensitivity of culture for detecting *Mycobacterium Tuberculosis* (MTB) is high. Recently, molecular and non- molecular-based tests are available for early and accurate diagnosis of EPTB with or without detection of drug resistance (Ryu, 2015; Lopes et al., 2021). Xpert® MTB/resistance to **R**ifampin **(**RIF) assay is a molecular test for the rapid diagnosis of tuberculosis and is currently endorsed by WHO. It has also been suggested for use in children with suspected Tuberculosis infection and to diagnose specific forms of extra-pulmonary tuberculosis. It has greater accuracy than smear microscopy (Kasa Tom et al., 2018).

Currently, AFB culture is considered the gold standard, but it requires advanced laboratory measurements and can generate 6-8 weeks (Lewinsohn et al., 2017). For the extra-pulmonary tuberculosis (EPTB) diagnosis, histological evidence plays a vital role. To define a positive test in histology, evidence of granuloma, caseous necrosis, and AFB presence on Ziehl-Neelsen (ZN)-stained histopathological slides are mainly used. The diagnostic accuracy may further increase when biopsy, histopathological, and Xpert® MTB/RIF assay results are combined with culture results (Lewinsohn et al., 2017).

Morbidity and mortality associated with tuberculous lymphadenitis can be potentially reduced by accurate diagnosis and early treatment. EPTB diagnosis is puzzling for many reasons: the lack of sufficient sample amounts or volumes, the specimen's paucibacillary nature, the sample distribution for several diagnostic tests, and non-uniform distribution micro-organisms. Smear microscopy and culture offer sub-optimal sensitivity because of paucibacillary nature. Smear microscopy is positive in < 10%of patients, while cultures for mycobacteria were positive in 39-80% of cases (Mahadevia and Brandwein-Gensler, 2009).

Though the differential diagnosis of tuberculous lymphadenitis is wide-ranging and laboratory identification is of supreme importance to guide and monitor proper treatment. Granulomatous lymphadenitis and caseation necrosis on histopathological examination may occur in other diseases (sarcoidosis, fungal infections, carcinoma, and other inflammatory conditions), so it may not be beneficial. In light of the above, relying on a single diagnostic method with less sensitivity is not a good practice. Therefore, conventional methods and histopathological examination combined with molecular techniques should be done to cover the concern of the low sensitivity of individual tests and further help diagnose tuberculous lymphadenitis. Therefore, it is essential to conduct a study on precise and efficient diagnostic and therapeutic modalities (Cohen and Scheimberg, 2015).

In this study, we compared different diagnostics methods used to detect TB, including Xpert® MTB/RIF assay, LJ culture, ZN staining, fluorescence staining, mycobacterial growth indicator tube (MGIT) 960, and histopathological examination of patients having tuberculous lymphadenopathy. Our main goal was to

estimate the diagnostic potential of different methods to detect TB infection.

2. Material and Methods

2.1. Sample selection

This study was conducted from August 2018 to September 2019 at the Department of Microbiology and Histopathology of a Tertiary care Hospital in Lahore, Pakistan. The inclusion criteria were patients with age of >14 years, clinically suspected of tuberculous lymphadenitis, single or multiple nodes (cervical node, inguinal nodes & axillary node) with associated symptoms like fever, weight loss, and anorexia. Criteria for exclusion were patients taking anti-tuberculosis care or non-consenting people.

2.2. Study techniques

Informed consent was taken from all the patients to include in the study, and their clinical and demographic data were collected. Initially, basic tests performed for assessment were performed, including complete blood count, erythrocyte sedimentation rate (ESR), and chest radiography. Consequently, excisional biopsy was performed using standard aseptic technique, and the specimens were immediately divided into two parts. One portion of the tissue sample was sent to the microbiology department, while the second portion was sent to the histopathology department.

AFB on microscopy or MTB on culture or Xpert MTB/RIF assay was characterized as confirmed tubercular lymphadenitis. On the other hand, the condition was described as presumptive tubercular lymphadenitis if histopathological findings indicate tuberculosis, but microbiological findings did not indicate tuberculosis. Anti-Tuberculosis Treatment (ATT) was started in patients diagnosed with tuberculous lymphadenitis. Newly diagnosed cases were given a four-drug regimen (Rifampicin, Pyrazinamide, Isoniazid, and Ethambutol) in the intensive phase for two months. While in the continuation phase, a weight-based dose of Isoniazid and Rifampicin was given four months following the intensive phase. Re-treatment cases were managed in an intensive phase with five drugs Rifampicin, Isoniazid, Pyrazinamide and Ethambutol, and Streptomycin for eight months while in the continuation phase was treated with three drugs (Isoniazid, Rifampicin, Ethambutol). Second-line ATT was prescribed to treat drug-resistant cases identified on Xpert MTB/RIF assay or culture. Treatment outcomes or medication side effects were tracked every month.

Xpert MTB/RIF: it is an assay comprises nucleic acid amplification test which detects the DNA of Mycobacterium Tuberculosis and RIF, i.e., the mutation in rpoB gene) within 2 hours. Besides, the standard culture method can take 2 to 6 weeks for mycobacterium growth, and if the drug resistance testing is required, it could take 03 more weeks (CDC, 2013).

2.3. Laboratory techniques

Samples were processed for AFB microscopy (ZN and Auramine stains), mycobacterial culture (Solid and Liquid), histopathological and Xpert MTB/RIF assay testing (Figure 1). TB's microbiological criteria were positivity by at least one of the following: Auramine microscopy/ZN microscopy, Xpert MTB/RIF assay, Lowenstein-Jensen (LJ) culture/MGIT culture. The specimens were processed according to modified Petroff's

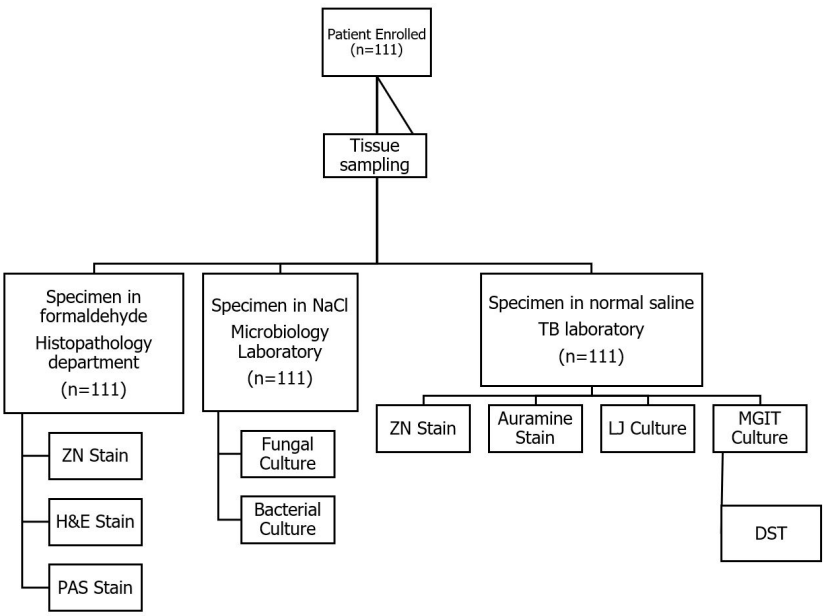

Figure 1. Flowchart of tests performed in different departments. DST = Drug susceptibility testing; LJ = Lowenstein Jensen; H&E = Hematoxylin and eosin stain; ZN = Ziehl-Neelson stain; PAS = Periodic acid Schiff; n = number; MGIT = Mycobacterium growth indicator tube; TB = Tuberclosis.

method (4% NaOH solution) for AFB smear and culture. Each sample was decontaminated using the traditional (NALC–NaOH) technique. Then sediment was neutralized with PBS; subsequently, samples were centrifuged and re-suspended in 1-2 mL PBS and slides were made for ZN and Auramine stain. The sediment was subjected to culture on an automated liquid medium (BACTEC MGIT 960) and solid medium (Lowenstein-Jensen). The LJ medium slopes were incubated at 37 °C in the incubator to grow *Mycobacterium Tuberculosis* while MGIT medium tubes were incubated in BACTEC MGIT 960 machine. The culture readings were observed regularly, and the tubes were discarded if there was no growth at the end of the 8th week. Strains obtained by liquid culture underwent phenotypic Drug Susceptibility Testing (DST) by the proportion method.

For Xpert MTB/RIF assay, homogenized tissue was mixed with the manufacturer's buffer at a 2:1 ratio and placed at room temperature for 10 minutes. After 5 minutes, 1.8 ml of the fluid was moved to the genexpert cartridge. Subsequently, after 02 hours, results were detected, and when MTB was found positive, RIF resistance was identified. Besides, for histopathology, formalin-fixed paraffin-embedded tissue blocks were used. In the morphological examination, the following histological patterns were considered as suggestive of tuberculous lymphadenitis: histology evidence of granuloma, caseous necrosis, and AFB presence on ZN-stained histopathological slides.

2.4. Ethical approval

Ethical Review Committee approved the study of The University of Haripur. Informed written consent was taken from all patients enrolled in the study.

2.5. Statistical analysis

Statistical analysis was done by using IBM SPSS Statistics version 21.0, the Chi-square test, receiver operating characteristic (ROC) curve, specificity, sensitivity, negative predictive value (NPV), positive predictive value (PPV) was calculated to evaluate the diagnostic accuracy of different modalities.

3. Results

In this study, 150 patients were enrolled, out of which 130 (86.6%) were with significant investigations. Among these 130 patients, 111 (85.38%) were diagnosed with tuberculous lymphadenitis, 12 (9.2%) with other malignancy, and 7 (5.3%) with reactive lymphoid hyperplasia (Figure 2). The clinical, demographic, and laboratory characteristics of these patients are shown in Table 1. Tuberculous lymphadenitis usually affects young ages (median age was 23 years), whereas reactive lymphadenitis affects older ages (median age 45). Gender-wise prevalence revealed females' predominance in tuberculous lymphadenitis, whereas males in reactive lymphadenitis. Cervical lymphadenopathy (90%) was the most common presentation, while multiple nodal involvements were seen (10%). Similarly, unilateral and bilateral involvements were seen in (90%) and (10%) of cases, respectively. Overall fever, anorexia, night sweats, and weight loss were frequently observed in these patients (Table 1).

The sensitivity of AFB smear, Gene Xpert, and culture was 5.9%, 71.6%, and 72%, respectively, when these findings were compared with histopathological results suggestive of tuberculosis. The specificity of AFB smear, Gene Xpert, and culture was 100%, 78.8%, and 89%, respectively, compared

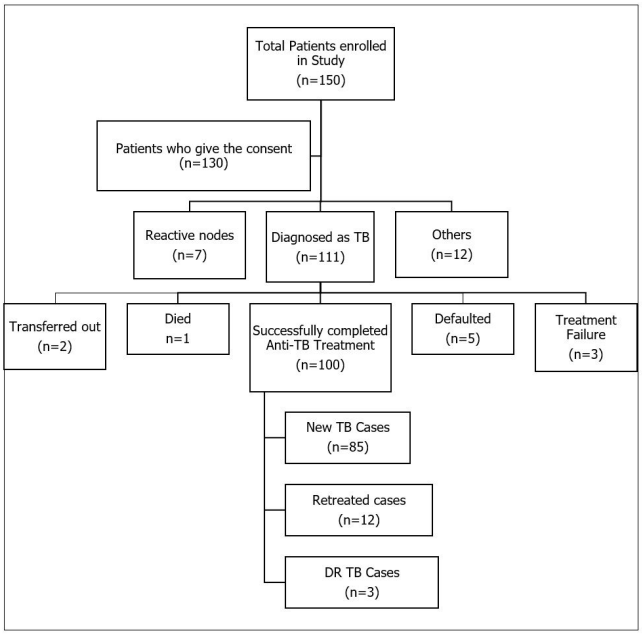

Figure 2. Enrolled patient's outcome. TB = Tuberculosis; DR = drug resistance.

with histopathological findings (Table 2). The ROC analysis was done to calculate the area under the curve (Table 3; Figure 3). Comparative analysis of two culturing media, Liquid culture (MGIT), showed more positivity than solid culture (LJ), as shown in Table 4. Similarly, the comparison of microbiological and histopathological findings is shown in Table 5; Figure 4. The sensitivity of smear and Gene Xpert was found to be 4.1% and 64.9%, respectively, when these findings were compared with culture suggestive of Tuberculosis (Table 6). In 102 (91.9%) cases, the presumptive

Table 1. Demographical data, clinical findings and laboratory data of patients enrolled.

	Parameters	Count (%)
Age	Years, median	23 (15-40 years)
Gender	Female, n (%)	56 (51.5)
	Male, n (%)	55 (49.5)
	Weight loss, n (%)	60(54)
	Fever, n (%)	72 (65)
Lymph node site, n (%)	Cervical	90 (81)
	Cervical and Auxiliary	11 (9.9)
	Cervical and inguinal	10 (9)
Type of node	Unilateral, n (%)	90 (81)
	Bilateral, n (%)	21 (19)
ESR	mm/h, median	35 (20-65)
Abnormal chest X-ray	Parenchymal	27 (24.3)
	Mediastinal	34 (30.6)
	Hilar	12 (10.8)
	Effusion	2 (1.8)

ESR = erythrocyte sedimentation rate.

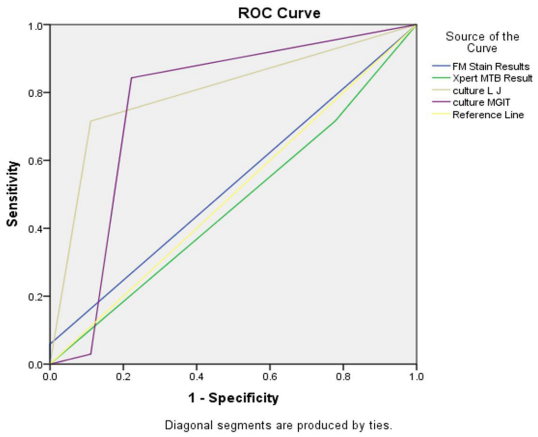

Figure 3. ROC curve.

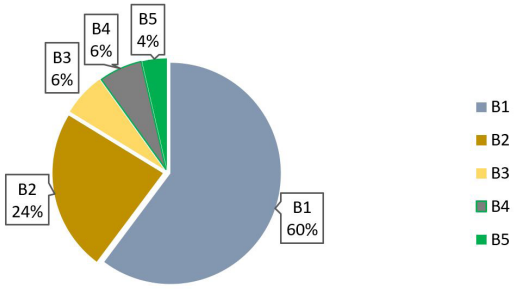

Figure 4. Histopathological results in the study cohort. B1 = necrotizing granulomatous inflammation; B2 = chronic caseating granulomatous lymphadenitis; B3 = Reactive lymphoid hyperplasia; B4 = cold abscess; B5 = others (Atypical lymphoid infiltrate, repeat biopsy, Nonspecific lymphadenitis, Necrotizing histiocytic lymphadenitis).

Table 2. Diagnostic accuracy of AFB smear, Mycobacterial culture, and GeneXpert against suggestive histopathology as gold standard.

		Histopathological Results		Total n (%)	p-value	Sensitivity	PPV	Overall diagnostic Accuracy (95% CI)
		Not Tuberculous lymphadenitis	Tuberculous lymphadenitis			Specificity	NPV	
		n (%)	n (%)					
AFB Smear	-ve	9	6	15	0.454	5.9	100	94.5
	+ve	0	96	96		100		
	Total	9	102	111			8.6	
Gene Xpert	MTB not detected	2	29	31	0.691	71.6	91.2	67.5
	MTB detected	7	73	80				
	Total	9	102	111		22.2	6.5	
AFB Culture	Negative	8	29	37	0.000	71.6	98.6	73
	Positive	1	73	74				
	Total	9	102	111		89	21.6	

AFB = acid-fast bacillus; PPV = Positive predictive value; NPV = Negative predictive value; MTB = Mycobacterium tuberculosis; CI = Confidence interval, diagnostic accuracy was calculated by ROC curve; p value <0.05 was significant; -ve = Negative; +ve = Positive.

Table 3. Area Under the Curve.

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
FM Stain Results	.529	.096	.771	.341	.717
Xpert MTB Result	.469	.098	.758	.277	.661
culture L J	.802	.068	.003	.668	.936
culture MGIT	.767	.108	.008	.555	.978

The test result variable(s): FM Stain Results, Xpert MTB Result, culture L J, culture MGIT has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased. [a]Under the nonparametric assumption; [b]Null hypothesis: true area = 0.5. FM = fluorescence microscopy ;LJ = Lowenstein-Jensen medium; MGIT = Mycobacteria Growth Indicator Tube.

Table 4. Comparison of solid and liquid culture results.

		Culture LJ		Total
		Negative	Positive	
Culture MGIT	Contamination	1	3	4
	Negative	9	14	23
	Positive	27	57	84
Total		37	74	111

This table shows that liquid culture (MGIT) has more positivity than solid culture (LJ).

Table 5. Comparison of histopathological findings with microbiological findings.

Description	B1	B2	B3	B4	B5
AFB Culture +ve	44(39.6%)	22(19.81)	1(0.9%)	6(5.4%)	1(0.9%)
Gene Xpert: MTB detected	50(45%)	17(15.31)	5(4.5%)	5(4.5%)	1(0.9%)
AFB smear +ve	2(1.8%)	2(1.8%)	1(0.9%)	1(0.9%)	0(0%

B1 = necrotizing granulomatous inflammation; B2 = chronic caseating granulomatous lymphadenitis; B3 = Reactive lymphoid hyperplasia; B4 = cold abscess; B5 = others; -ve = Negative; +ve = Positive.

diagnosis of tuberculous lymphadenitis was based on histopathological findings. Positive microbiological findings with genexpert, AFB culture, and smear were observed in 80 (72.1%), 84 (75.6%), and 6 (5.4%) cases, respectively, as shown in Table 6.

Drug susceptibility testing profile for Mono-Resistant (INH), Mono-Resistant (OFX), Mono-Resistant (PZA), Mono-Resistant (RIF), Mono-Resistant (STREP), and Pan-Sensitive (INH) are shown in Table 7. A comparison of DST results with Gene Xpert Rif resistance is shown in Table 8. A total of 100 (90.01%) patients treated with anti-Tuberculosis treatment presented either complete resolution or reduced glands' size after treatment completion (Figure 1). The response was consistent in both male and female patients. Notably, adverse effects, such as bone spread and joint pain, have been seen in 51% of enrolled patients, as illustrated in Figure 2.

4. Discussion

In Pakistan, tuberculous lymphadenitis is endemic; its diagnosis is difficult due to non-specific clinical findings, an extensive range of differential diagnoses, and absent microbiological evidence. As the number of tuberculosis cases decreases globally, extra-pulmonary tuberculosis cases increase (Salvador et al., 2015). In many healthcare units in Pakistan, culture, biopsy, and histopathology are not available due to resource limitations. In the present study, inflamed lymph nodes' common reason was tuberculous lymphadenitis, followed by reactive lymphoid hyperplasia and other malignancy; similar findings have been published in other studies (Fatima et al., 2011; Sarfaraz et al., 2018). Patients diagnosed with tuberculous lymphadenitis usually offered with slowly progressive swellings, cervical involvement most commonly, unilateral distribution; these features are regularly described in the literature (Sarfaraz et al., 2018; Purohit et al., 2009). The age of patients in recent studies ranges 30-40 years(Fontanilla et al., 2011; Sarfaraz et al., 2018), while in the present study, the median age observed is 23 years. Women to men ratio was 1.1:1, which is nearly close to other studies, in which a 1.4:1 ratio was reported (Biadglegne et al., 2013; Imtiaz et al., 2012; Sarfaraz et al., 2018). The basis for female dominance was not clear. However, hormonal basis, genetic susceptibility to disease, less access to healthcare units, and nutritional status might play a role in their dominasnce over males (Ribeiro et al., 2016). In this study, ESR, fever, number, and size of the nodes, location of nodes have no differentiating value between tuberculous lymphadenitis and reactive lymphoid hyperplasia, which is contrary to the literature (Khalil et al., 2013), but this is agreed with a study of Karachi (Sarfaraz et al., 2018). The chest radiography of tuberculous lymphadenitis patients showed 55% abnormalities, a useful ancillary test, while literature accounts for 10-40% abnormalities (Fontanilla et al., 2011; Khalil et al., 2013; Sarfaraz et al., 2018).

In this present study, conventional methods were compared with rapid methods for diagnosing tuberculous lymphadenitis. Recent global tuberculosis goals are based on proper diagnosis and adequate tuberculosis treatment. In developing countries, rapid diagnosis of TB is necessary for successful treatment and reduced transmission of the disease to others. The major drawback of Ziehl-Neelsen (ZN) staining is its low sensitivity due to specimens' paucibacillary nature. In the present study AFB, smear positivity rate is 5.4%, which is reported in the range

Table 6. Diagnostic accuracy of Gene Xpert and AFB smear against Mycobacterial culture taking as gold standard.

		AFB Culture		Total	p-value	Sensitivity	PPV	Overall Diagnostic Accuracy (95% CI)
		Negative	Positive			Specificity	NPV	
AFB Smear	Smear –ve	34	71	105	0.374	4.1	50	33
	Smear +ve	3	3	6				
	Total	37	74	111		91.9	32.4	
Gene Xpert	MTB not detected	5	26	31	0.017	64.9	60	48
	MTB detected	32	48	80		13.5	16.1	
	Total	37	74	111				

AFB = acid-fast bacillus; NPV = Negative predictive value; MTB = Mycobacterium tuberculosis; CI = Confidence interval; PPV = Positive predictive value; -ve = Negative; +ve = Positive.

Table 7. Drug sensitivity testing results.

Drug Susceptibility Testing Profile	Frequency	Percent
Mono_R (INH)	3	2.7
Mono_R (OFX)	1	.9
Mono_R (PZA)	7	6.3
Mono_R (RIF)	1	.9
Mono_R (STREP)	2	1.8
NA	22	19.8
Pan-S (N)	75	67.6
Total	111	100.0

This table showing results of drug susceptibility testing performed on 84 cases. INH = Isoniazid; OFX = Ofloxacin; PZA = Pyrazinamide; RIF = Rifampicin; STREP = Streptomycin; Mono-R = Mono-resistant; NA = Not applied; Pan-S = Pan Sensitive (sensitive to all drugs).

Table 8. Comparison of DST Profile with Gene Xpert Rifampicin Resistance.

		Xpert Rifampicin Resistance				Total
		NA	RRD	RRID	RRND	
DST Profile	Mono_R (INH)	1	1	0	1	3
	Mono_R (OFX)	1	0	0	0	1
	Mono_R (PZA)	3	0	0	4	7
	Mono_R (RIF)	0	1	0	0	1
	Mono_R (STREP)	0	0	0	2	2
	NA	0	0	0	22	22
	Pan-S	28	1	1	45	75
Total		33	3	1	74	111

Three cases of Rifampicin resistance were diagnosed on Gene Xpert but out of these 3 cases only one case was resistant on DST. NA = Not applied; RRD = Rifampicin resistance detected; RRID = Rifampicin resistance indeterminate; RRND = Rifampicin resistance not detected; INH = Isoniazid; OFX = Ofloxacin; PZA = Pyrazinamide; RIF = Rifampicin; STREP = Streptomycin; Mono-R = Mono-resistant; Pan-S = Pan Sensitive (sensitive to all drugs).

of 0-40% (Derese et al., 2012; Biadglegne et al., 2013; Sarfaraz et al., 2018). Culture-based methods are currently considered as the gold standard, but these required advanced laboratory measurements and can generate results in 6-8 weeks (Vadwai et al., 2011). In the present, 66.6% of the samples were positive for *Mycobacterium Tuberculosis*, which is consistent with the literature (Vadwai et al., 2011; Ghariani et al., 2015; Sarfaraz et al., 2018). In this study, liquid culture (MGIT) gave results earlier than solid media (LJ) results, which is also reported in many other studies (Nagpal et al., 2016; Roy et al., 2016).

However, Gene Xpert may be the leading diagnostic tool among all molecular techniques for diagnosing tuberculosis, but it has drawbacks. Rifampicin resistance detection is an important marker for MDR-TB cases. However, some strains are only resistant to Rifampicin that may not permit proper MDR therapy, so that patients misdiagnosed as MDR-TB cases, which leads to overtreatment, is a significant drawback of gene Xpert. Other disadvantages are stable power supply requirements, maintenance of proper temperature, and calibration of the machine every year (Sarfaraz et al., 2018; Nagpal et al., 2016). Since 2010, over 80 peer reviews for diagnostic efficacy of Gene Xpert for other different types of tuberculosis have been reported, with promising results published (WHO, 2015b). In this study, the optimal sensitivity of Gene Xpert for tuberculous lymphadenitis is 28.4% only when it is compared with histopathology, as the reference standard, which agrees with a study (Sarfaraz et al., 2018; Ramos et al., 2015). In a previous study, the sensitivity of Gene Xpert against mycobacterial culture varied from 50% to 100% (Denkinger et al., 2014), while the present study showed 62.2% sensitivity of gene Xpert against AFB culture taking as the reference standard, which agrees with the literature (Salvador et al., 2015; Vadwai et al., 2011). Nevertheless, the specificity of Gene Xpert against histopathology is about 79% in our study, which was lower than most reported ranged 86-99% (Denkinger et al.,

2014; Penz et al., 2015; Ligthelm et al., 2011), while it is almost same to a study (Sarfaraz et al., 2018). A total of 32 culture-negative specimens were positive on Gene Xpert, probably true positives due to scanty organism present in lesions (Sarfaraz et al., 2018; Biadglegne et al., 2013). Gene Xpert is a surrogate marker in MDR cases to detect Rifampicin resistance (rpo B gene mutation). The PPV of GeneXpert of Rifampicin susceptibility ranges from 70% to 90%, and NPV is over 99% (Organization). In the present study, three Rifampicin resistance cases were reported on Gene Xpert, which agrees with a study in China (Che et al., 2017).

The histopathological patterns seen in this study are necrotizing granulomatous inflammation, chronic caseating granulomatous lymphadenitis, reactive lymphoid hyperplasia, a cold abscess. These findings have been reported in the literature to diagnose tuberculous lymphadenitis (Handa et al., 2012; Sarfaraz et al., 2018). Chronic granulomatous inflammation is suggestive of tuberculosis in endemic countries. However, it is not pathognomonic, and it can also be perceived in non-tuberculous mycobacterium diseases, leprosy, fungal infections, cat scratch disease, lymphoma, tularemia, syphilis, sarcoidosis, and Kikuchi's disease (Shin et al., 2014; Fontanilla et al., 2011; Sarfaraz et al., 2018). In reactive lymphoid hyperplasia findings, we do not rule out tuberculous lymphadenitis; in the present study, reactive lymphoid hyperplasia has bacteriological findings (Gene Xpert positive were 5, AFB culture positive was 1, AFB smear-positive was 1) (Sarfaraz et al., 2018). Similarly, in a previous study, 38% of patients with reactive lymphoid hyperplasia were *Mycobacterium Tuberculosis* complex PCR positive (Derese et al., 2012; Mittal et al., 2011).

5. Conclusions

As the sensitivity of conventional methods for diagnosing tubercular lymphadenitis is limited, there is no single procedure for an accurate diagnosis of tubercular lymphadenitis. Gene Xpert, AFB smear, and histopathology must be done for their diagnosis according to the feasibility of setting the society to prevent inappropriate diagnosis and treatment of patients. Furthermore, this study discussed the diagnostic accuracy of different modalities for isolation of *Mycobacterium tuberculosis* among suspected tuberculous lymphadenitis patients. However, further research studies are required to enhance the specificity, sensitivity, and reproducibility of tests to set up a cost-effective diagnostic road maps.

List of Abbreviations

AFB: acid-fast bacillus; DST: drug-susceptibility testing; EPTB: Extra-pulmonary tuberculosis; ESR: Erythrocytes Sedimentation Rate; LJ: Lowenstein-Jensen; MDR: multidrug resistance; MGIT: Mycobacteria growth indicator tube; MTB: Mycobacterium Tuberculosis; RIF: resistance to Rifampin; ROC: receiver operating characteristic; ZN: Ziehl-Neelsen.

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