Comparison of Culture, GeneXpert and Two Staining Techniques with Fine Needle Aspiration in Diagnosis of Tuberculous Lymphadenitis, Khartoum, Sudan: A Prospective Cross-Sectional Study

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Abstract:

Introduction: Investigations of tuberculous lymphadenitis are challenging for clinicians. Using multimodality patterns may yield a better outcome. The objective is to compare the various laboratory methods; GeneXpert, culture, ZN stain and Auramine-Rhodamine stain in diagnosing tuberculous lymphadenitis. **Method:** This study is a prospective cross-sectional laboratory-based study. The study was conducted in Khartoum, Sudan. Specimens obtained from lymph nodes were examined cytologically, culture, GeneXpert, Auramine-Rhodamine stain and ZN stain laboratory methods were applied for all specimens, and all cases were initially diagnosed as tuberculous lymphadenitis by FNAC. Patients' sociodemographic data anthropometric measurements, clinical examination and diagnostic tools were also gathered. SPSS version 23 was used for data analysis.

Results: a total number of 105 cases were included, 53.3% were males. The mean age was 27.29 ± 15.94. The most affected age group was 19 – 30 (33.3%). The most affected lymph node group was the cervical group 83 (79.0%); furthermore, 81(77.1%) patients had a single lymph node affected while 24(22.9%) cases had multiple lymph nodes. The main cytomorphological pattern identified was necrotizing granulomatous lymphadenitis 79(75.2%). GeneXpert Positive cases were (41.0%), ZN staining (4.8%), Auramine-rhodamine stain (5.7%) and culture showed growth among (45.7%) of the cases. Sensitivity against FNAC was as follow: culture 64.81%, GeneXpert 62.87%, ZN stain 51.22% and Auramine-Rhodamine stain 51.47%. On the other hand, sensitivity and specificity against culture was calculated as follow: FNAC 100%, GeneXpert 80.0%, ZN stain 51.61% and Auramine-Rhodamine stain 53.33%. Specificity, as based on culture, was as follows FNAC 54.28%, GeneXpert 89.06%, ZN stain 96.61% and Auramine-Rhodamine stain 100%.

Conclusion: combining various diagnostic methods for caseating necrotizing cytomorphology cases yields less than 50% positivity. FNAC is recommended as the first-line test for suspected tuberculous lymphadenitis due to its higher sensitivity, rapidity, practicality, and cost-effectiveness. In inconclusive cases or those with a suppurative background, especially suspected TBLN, culture and GeneXpert are advised.

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Introduction

Tuberculosis is the nightmare of developing countries; this endemic disease affects millions each year and considered fatal. In the Eastern Mediterranean region nine countries contribute 95% of the TB burden in the region. These are Pakistan, Afghanistan, Sudan, Morocco, Somalia, Iraq, Egypt, Islamic Republic of Iran and Yemen [1].

Sudan is one of the endemic countries of tuberculosis and tuberculous lymphadenitis with prevalence of 158 cases per 100,000 of the population in 2016 with an incidence rate of 88 per 100,000 populations [1]. The use of diagnostic tools done by culture or by polymerase chain reaction (PCR) is not practical in clinical set-up in Sudan due to unavailability, time consumption for the culture and the high cost of PCR, for these reasons studies on sensitivity and specificity of FNAC in diagnosing tuberculous lymphadenitis are needed since this method is highly sensitive, available, easy, rapid and cheap [1-3].

Extra pulmonary TB disease is increasing, mainly, causing lymphadenopathy which in many cases the only presenting symptom made the diagnosing very challenging due to the variable presentations of infected patients. Currently most tuberculous lymphadenitis is caused by M. Tuberculosis and atypical mycobacteria, particularly M. scrofulosum and M. avium complex (MAC). The process is indolent and slowly progressive and is not accompanied by systemic symptoms [2,3,4].

Definitive diagnosis of TBLN done by culture or by polymerase chain reaction (PCR) showing M. Tuberculosis in involved LN. However, culture needs up to 8 weeks to show the results. Specimen for histopathology and acid-fast bacilli (AFB) smear can establish the diagnosis, especially in the endemic area with TB. Fine needle aspiration cytology (FNAC) is very useful as first step due to its availability, less invasive and more beneficial in an area where HIV and TB endemic, however, scarce information about the role of these techniques in the diagnosis of TLN has been published, and results are very different depending on the study [5-7].

TBLN is very common in Sudan and the most common diagnostic tool to start with is FNAC, especially when patients lack any pulmonary symptoms. The main cytomorphological pattern seen for diagnosis is smears revealing granulomas with necrosis, granulomas without necrosis, or sometimes necrosis only. In immunocompromised patients, there may be only loose aggregates of histiocytes rather than true granulomas. Special stains for bacteria, acid-fast bacilli, and fungi are important whenever granulomas, necrosis, and/or a neutrophilic infiltrate is present. Importantly, organisms are best seen with special stains in necrotic material. [7-12]. The objective of this study is to compare the various laboratory methods; GeneXpert, culture, ZN stain and Auramine-Rhodamine stain in diagnosing tuberculous lymphadenitis.

Materials and methods

This research constitutes an observational cross-sectional prospective study conducted at Almobarak laboratory, a private facility situated in Khartoum, overseen by a consultant cytopathologist and three other pathologists. Serving as both a training center for pathology registrars and a primary laboratory for Fine-Needle Aspiration Cytology (FNAC) procedures, the laboratory annually receives approximately 4000 to 4500 patients, including around 120 diagnosed with tuberculous lymphadenitis. Patients hail from diverse Sudanese states and rural areas, and the laboratory is renowned for its expertise in FNAC procedures within Khartoum. Apart from lymphadenopathy cases, the laboratory also handles gynecological specimens and various body fluids, such as pleural fluid, ascetic fluid, and bronchoalveolar lavage fluid (BAL), sent by clinicians. The study encompasses 105 patients diagnosed with tuberculous lymphadenitis through FNAC, and data collection occurred between March 2021 and December 2021.

Data Collection:

Data collection involved a standardized, pretested questionnaire administered through interviews, covering sociodemographic details, anthropometric measurements, clinical examinations, and diagnostic tools. Clinical

procedures included Fine-Needle Aspiration, with a 22-gauge needle inserted into the targeted lymph node for cell aspiration via negative pressure. The aspirated material underwent microscopic slide staining for diagnosis, and the remaining sample was utilized for additional laboratory tests.

Laboratory tests:

All the following tests were done in National Tuberculosis Reference Laboratory in Stack Medical Research Laboratories. GeneXpert PCR, Xpert MTB/RIF Assay performed on the GeneXpert Instrument systems, is a qualitative, nested real- time polymerase chain reaction in vitro diagnostic test for the detection of Mycobacterium tuberculosis complex DNA in raw sputum or aspirated body fluids and cells. Performed for all cases.

Culture: this is considered the gold standard test for diagnosing tuberculosis, but it needs a long time to get the result. The aspirated sample is placed in a media convenient for the bacteria to grow and is checked between interval periods until the 8 weeks are passed then the resulted growth is documented. Culture was done for all cases. Ziehl–Nelsen (ZN) stain. ZN staining is a bacteriological stain used to identify acid- fast organisms, mainly Mycobacteria. This stain will be used on the prepared slides (from fine needle aspiration) to visualize the Mycobacterium. One slide with ZN stain done for all cases.

Auramine-Rhodamine stain: also known as Truant auramine stain is a histological and cytological technique used to visualize acid- fast bacilli using fluorescence microscopy. This stain will be used on microscopic slides smeared by the aspirated material and results will be documented. One slide with Auramine-Rhodamine stain was done for all cases.

Data Analysis:

Statistical analysis employed the Statistical Package for the Social Sciences (SPSS) version 23, specialized in organizing and analyzing scientific data. Socio-demographic, physical, and clinical examination data, along with laboratory results, were analyzed for sensitivity and specificity using FNAC and culture as the gold standard. The Chi-square test assessed the significance of differences between values, with significance determined by a P-value <0.05. Assistance from a specialized statistician was sought for data analysis.

Ethical issues:

Ethical approval from research committee at Federal Ministry of Health in Khartoum was obtained. Patients consent was obtained and no harm was imposed on patients. Permission from medical and laboratory directors was also obtained.

Results:

The sample size included in the study was 105 cases and all were diagnosed as tuberculous lymphadenitis by cytomorphology.

Sociodemographic data:

In terms of sociodemographic data, a slight male predominance of 53.3% was observed, with ages ranging from 10 months to 63 years. The (19-30) age group was most affected, comprising 33.3% of cases. Gender-based analysis revealed the highest incidence in the (19-30) age group for both females (36.7%) and males (30.4%), though the results were statistically insignificant (P-value=0.710). Body Mass Index (BMI) calculations indicated that 46.7% of patients were within the normal weight range. Among the 94 Sudanese patients, 75.2% resided in Khartoum, and 43.8% were married, with 37.1% having children. Educational levels varied, with 36.2% having basic primary education and 23.8% having secondary school education. Employment status included 26.7% self-employed and 25.7% students (Table 1).

Table 1 **Sociodemographic characteristics of patients with tuberculous lymphadenitis diagnosed at Almobarak Laboratory in 2021 (n= 105)**

Characteristic	Variable	n	%
Gender	Male	56	53.3
	Female	49	46.7
Age Group	Under 5 Years	7	6.7
	5-10 Years	8	7.6
	11-18 Years	17	16.2
	19-30 Years	35	33.3
	31-45 Years	24	22.9
	46-60 Years	12	11.4
	Over 60 Years	2	1.9
BMI category	Underweight below 18.5	42	40
	Normal weight 18.5-24.9	49	46.7
	Overweight 25-29.9	7	6.7
	Obese 30+	7	6.7
Nationality	Sudanese	94	89.5
	Sudanese (south)	7	6.7
	Eritrean	4	3.8
Residency	Khartoum	79	75.2
	Central states (Gazira, White Nile)	19	18.1
	Western states (Kordofan, Darfur)	4	3.8
	Eastern states (Red Sea, Kassala, Gedarif)	1	1.0
	Northern states (River Nile, Northern)	1	1.0
	Southern states	1	1.0
Marital status	Married	46	43.8
	Single	30	28.6
	Divorced	2	1.9
	Not Applicable (child)	27	25.7
Having Children	Yes	39	37.1
	No	13	12.4
	Not Applicable (child or single)	53	50.5
Educational level	Not Applicable (below school age)	11	10.5
	Illiterate	13	12.4
	Basic Primary (less than 10 years)	38	36.2
	Secondary school	25	23.8
	College and above	18	17.1
Occupation	Not Applicable (Child)	12	11.4
	Employee	9	8.6
	Housewife	20	19.0
	Jobless	9	8.6

	Self-employee	28	26.7
	Student	27	25.7
Habits	Smoking	5	4.8
	Snuffing	7	6.7
	None	93	88.6
Keeping pets	Yes	32	30.5
	None	73	

(Some data in table 1 appeared in our previous publication: Mirghani, Hagir; Elmadhoun, Wadie1; Ahmed, Mohamed H.; Ahmed, Musaab; Almobarak, Ahmed O. The Utility of Polymerase Chain Reaction GeneXpert Test in the Diagnosis of Tuberculous Lymphadenitis. Journal of Microscopy and Ultrastructure ():10.4103/jmau.jmau_123_22, June 20, 2024. | DOI: 10.4103/jmau.jmau_123_22-Wolters Kluwer Medknow Publications gave permission to use such data under license number (5838900637951).

In terms of social habits, the majority did not engage in any particular habits (88.57%), while 6.7% used snuff and 4.8% were smokers. Most patients did not keep pets at home (69.5%). Symptom presentation was common, with 47.6% experiencing weight loss and 38.1% having fever. Age-wise correlation revealed a higher percentage of symptomatic patients in the 19-30 age group (38.4%), with statistical significance (P-value=0.042). The majority of patients did not have comorbidities, with hypertension (2.9%) being the most prevalent (Table 2).

Furthermore, 20.0% of patients had a history of contact, 1.9% had a history of pulmonary TB, 1.9% had a history of extra-pulmonary TB, and 2.9% had a history of treatment, with only one patient completing the treatment (Table 2).

*Table 2 **The frequency distribution of the clinical, cytomorphological and diagnostic parameters among patients with tuberculous lymphadenitis diagnosed at Almobarak Laboratory in 2021 (n= 105)***

			Frequency	%
Presenting symptoms	Fatigability	Yes	36	34.3
		No	69	65.7
	Fever	Yes	40	38.1
		No	65	61.9
	Cough	Yes	20	19.0
		No	85	81.0
	Loss of appetite	Yes	32	30.5
		No	73	96.5
	Loss of weight	Yes	50	47.6
		No	55	52.4
	Night sweats	Yes	16	15.2
		No	89	84.8
	Chills	Yes	4	3.8
		No	101	96.2
	Chest pain	Yes	6	5.7
		No	99	94.3
Risk factors	Diabetes mellitus		1	1.0
	Hypertension		3	2.9
	Kidney disease		2	1.9
	Diabetes+ Hypertension		2	1.9
	HIV		1	1.0

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Disease history	None	96	91.4
	History of contact	21	20.2
	History of pulmonary TB	2	1.9
	History of extrapulmonary TB	2	1.9
History of treatment	None	80	76.2
	Yes	3	2.9
	No	102	97.1
Lymph node size grouped	1-3 cm	50	47.6
	3-5 cm	36	34.3
	Above 5	19	18.1
LN cervical or else	Cervical	83	79.0
	Other site	22	21.0
LN site	Cervical	75	71.4
	Supraclavicular	6	5.7
	Axillary	3	2.9
	Submandibular	12	11.4
	Cervical+ Supraclavicular	4	3.8
	Cervical+ Submandibular	3	2.9
	Supraclavicular+ Inguinal	1	1.0
	Cervical+ Inguinal	1	1.0
Number of LN involved	Single Lymph node in single group	81	77.1
	Multiple lymph nodes in single group	15	14.3
	Multiple lymph nodes in multiple groups	9	8.6
Consistency	Firm	88	83.8
	Cystic	12	11.4
	Soft	3	2.9
	Firm+ Cystic	2	1.9
Nature of aspirate	Purulent	63	60.0
	Blood	42	40.0
Presence of sinuses	Yes	11	10.5
	No	94	89.5
Discharge from sinuses	Yes	6	5.7
	No	99	94.3
Cytomorphological parameters and other diagnostic tools			
Cellularity	Cellular	77	73.3
	Hypocellular	28	26.7
Background	Extensive necrosis	69	65.7
	Focal necrosis	36	34.3
Predominant cells	Lymphocytes	66	62.9
	Neutrophils	39	37.1
Epithelioid cells	Aggregate	79	75.2
	Scattered	26	24.8

Giant cells	Present	32	30.5
	Not Present	73	69.5
Cytological category	Necrotizing granulomatous lymphadenitis	79	75.2
	Necrotizing Suppurative background/ inflammatory process	17	16.2
	Necrotizing lymphadenitis	9	8.6
GeneXpert	Negative	62	59.0
	Positive	43	41.0
ZN stain	Negative	100	95.2
	Positive	5	4.8
Auramine-Rhodamine stain	Negative	99	94.3
	Positive	6	5.7
Culture	Negative	57	54.3
	Positive	48	45.7

Lymph nodes clinical examination

Clinical examination of enlarged lymph nodes revealed a predominance of cervical involvement (79.0%). Within the 19-30 age group, the highest percentages of cervical, supraclavicular, axillary, and submandibular lymph nodes were observed. Most lymph nodes were within the 1-3 cm range (47.6%), with the highest percentages within the 19-30 age group. The majority of cases (77.1%) involved a single lymph node in one group, with firm consistency observed in 83.8% of cases. Additionally, 12 (11.4%) cases exhibited cystic consistency. Lymph node aspirate nature varied, with 60.0% being purulent and 40.0% bloody. Females predominantly exhibited purulent aspirate (71.4%). Purulent aspirate was significantly associated with sinuses and a history of contact (Table 3). Sinuses were present in 10.5% of patients, primarily in the 19-30 age group. (Table 3).

*Table 3 **The correlation between the nature of aspirate with laboratory and clinical findings among patients with tuberculous lymphadenitis diagnosed at Almobarak Laboratory in 2021 (n= 105)***

Characteristic	Variable	Nature of aspirate		
		purulent	blood	P- value
GeneXpert	Negative	29(46.8%)	33(53.2%)	.001
	Positive	34(79.1%)	9(20.9%)	
ZN stain	Negative	59(59.0%)	41(41.0%)	.350
	Positive	4(80.0%)	1(20.0%)	
Culture	Negative	23(40.4%)	34(59.6%)	

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	Positive	40(83.3%)	8(16.7%)	.000
Auramine- Rhodamine stain	Negative	59(59.6%)	40(40.4%)	
	Positive	4(66.7%)	2(33.3%)	.731
Gender	Male	28(50.0%)	28(50.0%)	
	Female	35(71.4%)	14(28.6%)	.025
Disease history	History of contact	7(33.3%)	14(66.7%)	
	History of pulmonary TB	1(50.0%)	1(50.0%)	
	History of extrapulmonary TB	1(50.0%)	1(50.0%)	.041
	None	54(67.5%)	26(32.5%)	
Lymph node size grouped	1-3cm	24(48.0%)	26(52.0%)	
	3-5cm	25(69.4%)	11(30.6%)	.054
	above 5cm	14(73.7%)	5(26.3%)	
Presence of sinuses	Yes	6(54.5%)	5(45.5%)	
	No	57(60.6%)	37(39.4%)	.696
Discharge from sinus	Yes	4(66.7%)	2(33.3%)	
	No	59(59.6%)	40(40.4%)	.731
Consistency	Firm	49(55.7%)	39(44.3%)	
	Cystic	12(100.0%)	0(0.0%)	
	Soft	1(33.3%)	2 (66.7%)	.022
	Firm+ Cystic	1(50.0%)	1(50.0%)	

Diagnostic techniques:

All diagnoses were established through FNAC, identifying three primary cytomorphological patterns: Necrotizing granulomatous lymphadenitis (75.2%) (Image1), Necrotizing suppurative background/inflammatory process (16.2%), and Necrotizing lymphadenitis (9.5%) (Table 2). The specificity of FNAC against culture was 54.28%. Age group analysis revealed no statistically significant association with cytomorphology patterns. However, there was a significant correlation between the nature of aspirate and cytomorphology, with purulent aspirate predominantly associated with Necrotizing granulomatous lymphadenitis (92.9%). The consistency of lymph nodes also demonstrated a statistically significant association with cytomorphology patterns, such as firm consistency in Necrotizing granulomatous lymphadenitis (78.4%) (Table 4).
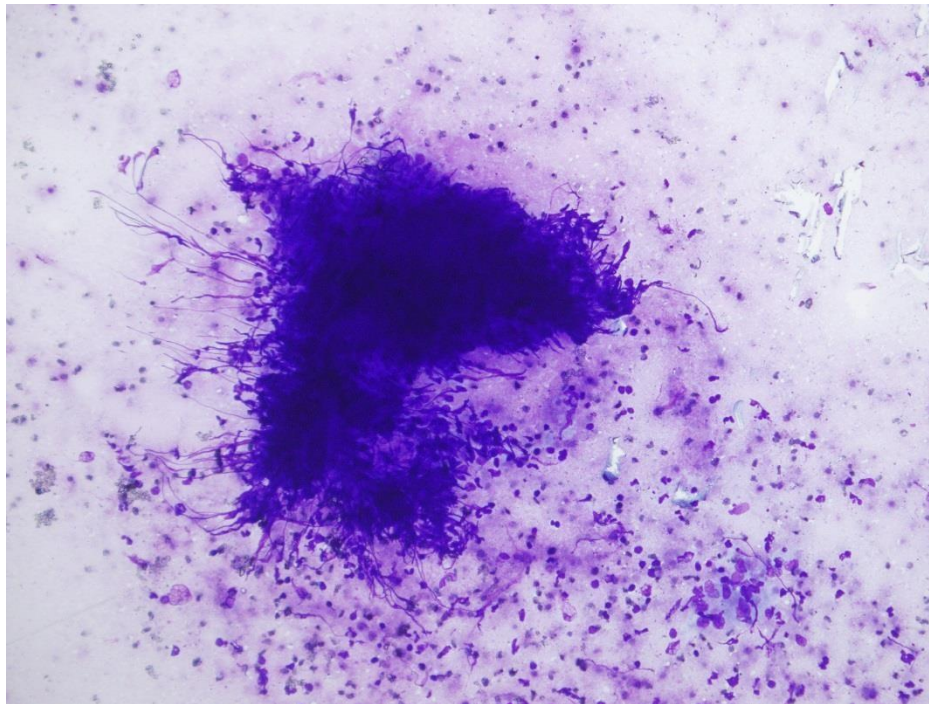
Figure 1 ***a photomicrograph of a granuloma in a background of necrosis (necrotizing granulomatous reaction). (Diff-Quick® stain X100)***

Table 4 ***The correlation between the cytological category and (nature of aspirate, consistency, GeneXpert, culture, ZN stain, Auramine-Rhodamine stain) of patients with tuberculous lymphadenitis diagnosed at Almobarak Laboratory in 2021 (n= 105)***

		Cytological category				
		Necrotizing granulomatous lymphadenitis	Necrotizing Suppurative background/inflammatory process	Necrotizing lymphadenitis	P- value	Sensitivity %
Nature of aspirate	Purulent	40(63.5%)	15(23.8%)	8(12.7%)	.003	
	Blood	39(92.9%)	2(4.8%)	1(2.4%)		
Consistency	Firm	69(78.4%)	12(13.6%)	7(8.0%)		
	Cystic	8(66.7%)	4(33.3%)	0(0.0%)		
	Soft	1(33.3%)	0(0.0%)	2(66.7%)	.005	
	Firm + Cystic	1(50.0%)	1(50.0%)	0(0.0%)		
GeneXpert	Negative	53(67.1%)	5(29.4%)	4(44.4%)		62.87

Culture	Positive	26(32.9%)	12(70.6%)	5(55.6%)	.011	
	Negative	50(63.3%)	4(23.5%)	3(33.3%)		64.81
ZN stain	Positive	29(36.7%)	13(76.5%)	6(66.7%)	.005	
	Negative	75(94.9%)	17(100.0%)	8(88.9%)		51.22
Auramine-Rhodamine stain	Positive	4(5.1%)	0(0.0%)	1(11.1%)	.435	
	Negative	75(94.9%)	16(94.1%)	8(88.9%)		51.47
	Positive	4(5.1%)	1(5.9%)	1(11.1%)	.760	

*Table 5 **The correlation between culture and (GeneXpert, ZN stain and Auramine- Rhodamine stain) of patients with tuberculous lymphadenitis diagnosed at Almobarak Laboratory in 2021 (n= 105)***

		Culture				
		negative	**positive**	**P-value**	**sensitivity**	**specificity**
GeneXpert	negative	50(80.6%)	12(19.4%)		80.0%	89.06%
	positive	7(16.3%)	36(83.7%)	.000		
ZN stain	negative	55 (55.0%)	45 (45.0%)		51.61%	96.61%
	positive	2 (40.0%	3 (60.0%)	.511		
Auramine-Rhodamine stain	negative	57 (57.6%)	42 (42.4)%		53.33%	100%
	positive	0 (0.0%)	6 (100.0%)	.006		

GeneXpert yielded positive results in 41.0% of cases (Table 2), with a sensitivity against FNAC at 62.87%. Sensitivity and specificity against culture were 80.0% and 89.06%, respectively. Age group analysis showed no significant correlation with positive GeneXpert results. However, a statistically significant association was found between positive GeneXpert results and cystic lymph nodes (Table 5). Purulent aspirate and Necrotizing suppurative background/inflammatory process pattern were significantly correlated with positive GeneXpert results (Tables 5, 6). Furthermore, a strong statistical significance was observed between positive GeneXpert results and culture positivity P-value= 0.000 (Table 4).

ZN stain showed a sensitivity against FNAC of 51.22%, with age group and consistency having statistically insignificant associations. Purulent aspirate, Necrotizing lymphadenitis pattern, and culture positivity were not significantly associated with ZN stain results. Auramine-rhodamine stain demonstrated a sensitivity against FNAC of 51.47%, with a statistically insignificant correlation with age group. However, positive Auramine-rhodamine results were significantly associated with firm consistency. Purulent aspirate and Necrotizing lymphadenitis pattern were not significantly associated with positive Auramine-rhodamine results. Positive Auramine-rhodamine results were strongly associated with both culture positivity (Table 4) and positive GeneXpert results p-value=.002.

Culture displayed growth in 45.7% of cases, with a sensitivity against FNAC of 64.81%. Age group analysis and consistency showed statistically insignificant associations. However, culture positivity was significantly associated with cystic lymph nodes, purulent aspirate, and Necrotizing suppurative background/inflammatory process pattern (Tables 4, 5 and 6).

All patients exhibited a positive response to anti-tuberculous treatment, demonstrated by lymph

node size regression and symptom alleviation within 3 to 4 weeks of treatment initiation.

Table 6 **The correlation between consistency and (GeneXpert, ZN stain, Auramine-Rhodamine stain, culture, number of lymph nodes involved, lymph node site, BMI category) of patients with tuberculous lymphadenitis diagnosed at Almobarak Laboratory in 2021**

Characteristic

		firm	cystic	soft	firm+ cystic	P- value
GeneXpert	Negative	59(95.2%)	1(1.6%)	1(1.6%)	1(1.6%)	.001
	Positive	29(67.4%)	11(25.6%)	2(4.7%)	1(2.3%)	
ZN stain	Negative	86(86.0%)	10(10.0%)	2(2.0%)	2(2.0%)	.015
	Positive	2(40.0%)	2(40.0%)	1(20.0%)	0(0.0%)	
Auramine-Rhodamine stain	Negative	85(85.9%)	10(10.1%)	2(2.0%)	2(2.0%)	.048
	Positive	3(50.0%)	2(33.3%)	1(16.7%)	0(0.0%)	
Culture	Negative	53(93.0%)	2(3.5%)	1(1.8%)	1(1.8%)	.034
	Positive	35(72.9%)	10(20.8%)	2(4.2%)	1(2.1%)	
Number of lymph nodes involved	single lymph node in single group	69(85.2%)	10(12.3%)	2(2.5%)	0(0.0%)	.034
	multiple lymph nodes in single group	11(73.3%)	1(6.7%)	1(6.7%)	2(13.3%)	
	multiple lymph nodes in multiple groups	8(88.9%)	1(11.1%)	0(0.0%)	0(0.0%)	
Lymph node site	Cervical	63(84.0%)	8(10.7%)	2(2.7%)	2(2.7%)	.997
	Supraclavicular	4(66.7%)	2(33.3%)	0(0.0%)	0(0.0%)	
	Axillary	3(100.0%)	0(0.0%)	0(0.0%)	0(0.0%)	
	Submandibular	10(83.3%)	1(8.3%)	1(8.3%)	0(0.0%)	
	cervical+ supraclavicular	3(75.0%)	1(25.0%)	0(0.0%)	0(0.0%)	
	cervical+ submandibular	3(100.0%)	0(0.0%)	0(0.0%)	0(0.0%)	
	supraclavicular+ inguinal	1(100.0%)	0(0.0%)	0(0.0%)	0(0.0%)	
	cervical+ inguinal	1(100.0%)	0(0.0%)	0(0.0%)	0(0.0%)	
BMI category	underweight (below 18.5)	37(42.0%)	3(25.0%)	1(33.3%)	1(50.0%)	.985
	normal weight (18.5-24.9)	39(44.3%)	7(58.3%)	2(66.7%)	1(50.0%)	
	overweight (25-29.9)	6(6.8%)	1(8.3%)	0(0.0%)	0(0.0%)	
	Obese (30+)	6(6.8%)	1(8.3%)	0(0.0%)	0(0.0%)	

Discussion:

The age distribution in this study revealed a significant impact in the third decade, with statistical significance noted in association with the presence of symptoms (P-value= 0.042) and lymph node site (P-value= 0.003). Comparable investigations by Aamer Ikram et.al. [6], in Sri Lanka [10], and others [15], [19], [20], [21], [22], [26], [30], [37], and [39] also explored age groups in relation to the prevalence of tuberculous lymphadenitis. Discrepancies in age-related findings across studies, such as those by Yeshi Metaferia et.al. [13] and in the UK [14], may be attributed to variations in sample composition. Our study's average BMI closely aligns with a study conducted in India [31].

Gender distribution displayed a slight male predominance, consistent with findings in studies by Brijesh Thakur et.al. [7], Yeshi Metaferia et.al. [13], and Emile Musoni et.al. [15]. Conversely, several studies reported a female predominance, as observed in Spain [5], Pakistan [6], and studies in India [21], [22], [24], and [26]. Variances in gender patterns may stem from sampling biases rather than inherent differences.

Geographically, the distribution was primarily in Khartoum, echoing findings in a study highlighting urban predominance [21]. However, a study reported approximately half of the participants residing in rural areas [13]. Discrepancies in geographic distribution across studies underscore the influence of data collection locations.

In this study, most patients were married, with basic primary education, followed by secondary school education, aligning with a study by Mengistu Fantahun I.D et. al. [20]. Varied occupational distributions were noted, with self-employment and student statuses prevailing. These occupational patterns may be attributed to local cultural, traditional, and economic disparities.

Habits such as smoking and snuffing were uncommon in our study, resembling findings in Ethiopia [13], while a study in India reported a significant percentage of smokers [21]. These disparities reflect diverse social habits influenced by distinct lifestyle choices.

The majority of patients presented with symptoms, with weight loss being the most common, followed by fever. Comparable findings were reported in studies where systemic symptoms predominated [30] or more than half of participants were symptomatic [37]. In contrast, certain studies noted fewer constitutional symptoms at presentation [14], with fever and cough being the most common associated symptoms [19].

Comorbidities were infrequent, with hypertension being the most prevalent, followed by diabetes with hypertension, kidney disease, and a single case of HIV positivity. This aligns with a study by Wubshet Assefa et.al. [29], while others reported diabetes as the commonest comorbidity [10] or a substantial percentage of diabetes mellitus among subjects [20].

A notable finding in this study was a significant history of contact, consistent with results from studies in India [24] and by Tamanna-E-Nur et.al. [19]. However, other studies reported lesser contact history [29], suggesting social stigma and denial may contribute to varied reporting.

The distribution and characteristics of affected lymph nodes revealed a prevalence of cervical involvement, consistent with over 10 studies in literature. The majority of cases involved a single lymph node in one group, and firm consistency predominated. Our results were consistent with previous studies that also described unilateral lymphadenopathy and predominantly solid consistency.

Regarding lymph node size, most were between 1-3 cm, similar to a study in Bangladesh [38], while other studies reported larger lymph nodes [34]. Purulent aspirate was common, correlating with positive results in GeneXpert and culture. These findings align with studies noting various aspirate appearances, including grossly purulent material, caseous material, and blood-mixed material.

Sinuses were present in 10.5% of cases in our study, primarily discharging. Comparable findings were noted in studies where approximately one-fourth of participants had sinus tract formation [29]. Varying reports of abscesses and/or draining sinuses highlight differences in disease stages and FNAC techniques.

In this study, all diagnosed cases of tuberculous lymphadenitis demonstrated regression of lymph node size and symptom alleviation after 3 to 4

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weeks of treatment, emphasizing the efficacy of anti-tuberculous treatment.

Cytomorphologically, three main patterns were identified, with necrotizing granulomatous lymphadenitis being the most common. FNAC specificity against culture was low, and significant associations were found with the nature of aspirate and consistency. Similar studies reported varying patterns and FNAC specificity, with some highlighting the high sensitivity but low specificity of cytomorphology.

ZN stain exhibited limited positivity, consistent with the paucibacillary nature of aspirate. Auramine-rhodamine stain showed slightly higher sensitivity than ZN stain. Both stains demonstrated sensitivities against FNAC and specificities against culture. Comparable studies reported variable positivity rates and sensitivities for ZN and Auramine-rhodamine stains.

Culture positivity rates in our study were higher compared to literature, likely due to the inclusion of exclusively FNAC-positive cases. GeneXpert showed significant positivity, with associations observed with cytomorphology, GeneXpert, and Auramine-Rhodamine stain. The findings align with studies emphasizing the sensitivity and specificity of GeneXpert, although positivity rates may vary.

Conclusion:

In conclusion, our study provides comprehensive insights into the demographic, clinical, and diagnostic aspects of tuberculous lymphadenitis. The findings contribute to the existing body of knowledge, emphasizing the importance of FNAC, bacterial stains, and molecular techniques for accurate diagnosis and subsequent monitoring of treatment response. Positive cases for GeneXpert and culture collectively constitute less than half of the total cases, suggesting a robust correlation. Both Auramine-Rhodamine and ZN stains display low sensitivity but high specificity. The necrotizing suppurative background/inflammatory process pattern exhibits the highest positivity rates in both GeneXpert and culture. Importantly, negative results from GeneXpert, culture, ZN stain, and Auramine-Rhodamine stain, either individually or combined, do not exclude the diagnosis of tuberculous lymphadenitis.

Recommendations:

Building upon these findings, several recommendations emerge:

FNAC should be the primary diagnostic tool for suspected cases of tuberculous lymphadenitis due to its superior sensitivity, rapidity, practicality, and cost-effectiveness. Cases with a suppurative background/abscess in cytomorphology, inconclusive for TBLN, warrant further investigation through culture and GeneXpert. While cytomorphological techniques demonstrate high sensitivity in the presence of pathognomonic features, inconclusive cases may require confirmation through complementary tests. ZN staining and Auramine-Rhodamine stain, with their notably low sensitivity, should be judiciously employed in specific diagnostic scenarios for tuberculous lymphadenitis.

Limitations of the Study:

The study's scope was confined to individuals diagnosed with tuberculous lymphadenitis through FNAC, potentially leading to the oversight of cases that lack the characteristic morphological features of tuberculous lymphadenitis. The prevailing political instability in the country, coupled with the challenges imposed by the COVID-19 pandemic, introduced various impediments, ultimately diminishing the overall sample size. Significant inter-observer disparities among pathologists played a crucial role in the diagnostic process, underscoring the imperative need for the presence of highly experienced cytopathologists to ensure diagnostic accuracy.

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