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# 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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**Keywords**: Tuberculosis, TB, FNAC, Tuberculous lymphadenitis, TBLN, GeneXpert.

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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.

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## 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. (See table 1).

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**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 |

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| | | | |
|---|---|---|---|
| | 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 | 69.5 |

**(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. (See 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. (See 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 |

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| | | | |
|---|---|---|---|
| | HIV | 1 | 1.0 |
| | None | 96 | 91.4 |
| **Disease history** | History of contact | 21 | 20.2 |
| | History of pulmonary TB | 2 | 1.9 |
| | History of extrapulmonary TB | 2 | 1.9 |
| | None | 80 | 76.2 |
| **History of treatment** | 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 |

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| | | | |
|---|---|---|---|
| | 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 (See table 3). Sinuses were present in 10.5% of patients, primarily in the 19-30 age group. (See table 2).

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

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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%) (See table 4).

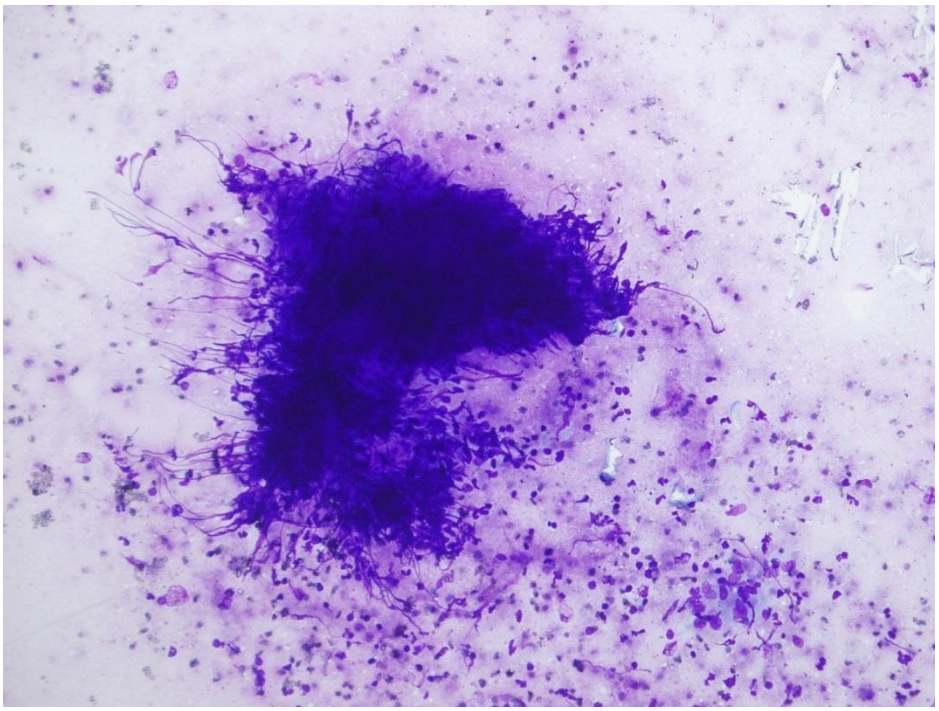

Figure 1: a photomicrograph of a granuloma in a background of necrosis (necrotizing granulomatous reaction). (Diff-Quick® stain X100)

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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 | |
| **Consistency** | Blood | 39(92.9%) | 2(4.8%) | 1(2.4%) | .005 | |
| | 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%) | | |
| | Firm + Cystic | 1(50.0%) | 1(50.0%) | 0(0.0%) | | |
| **GeneXpert** | Negative | 53(67.1%) | 5(29.4%) | 4(44.4%) | .011 | 62.87 |
| | Positive | 26(32.9%) | 12(70.6%) | 5(55.6%) | | |
| **Culture** | Negative | 50(63.3%) | 4(23.5%) | 3(33.3%) | .005 | 64.81 |
| | Positive | 29(36.7%) | 13(76.5%) | 6(66.7%) | | |
| **ZN stain** | Negative | 75(94.9%) | 17(100.0%) | 8(88.9%) | .435 | 51.22 |
| | Positive | 4(5.1%) | 0(0.0%) | 1(11.1%) | | |
| **Auramine-Rhodamine stain** | Negative | 75(94.9%) | 16(94.1%) | 8(88.9%) | .760 | 51.47 |
| | Positive | 4(5.1%) | 1(5.9%) | 1(11.1%) | | |

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%) | .000 | 80.0% | 89.06% |
| | positive | 7(16.3%) | 36(83.7%) | | | |
| **ZN stain** | negative | 55 (55.0%) | 45 (45.0%) | .511 | 51.61% | 96.61% |
| | positive | 2 (40.0% | 3 (60.0%) | | | |
| **Auramine-Rhodamine stain** | negative | 57 (57.6%) | 42 (42.4)% | .006 | 53.33% | 100% |
| | positive | 0 (0.0%) | 6 (100.0%) | | | |

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GeneXpert yielded positive results in 41.0% of cases (See 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 (See table 5). Purulent aspirate and Necrotizing suppurative background/inflammatory process pattern were significantly correlated with positive GeneXpert results (See tables 5, 6). Furthermore, a strong statistical significance was observed between positive GeneXpert results and culture positivity P-value= 0.000 (See 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 (See 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 (See tables 5, 6 and 7).

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.

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Table 5: 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 (n= 105)

| Characteristic | Variable | Consistency | | | | P- value |
|---|---|---|---|---|---|---|
| | | firm | cystic | soft | firm+ cystic | |
| **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%) | |
| | multiple lymph nodes in single group | 11(73.3%) | 1(6.7%) | 1(6.7%) | 2(13.3%) | .034 |
| | 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%) | |
| | 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%) | .997 |
| | 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%) | |
| | normal weight (18.5-24.9) | 39(44.3%) | 7(58.3%) | 2(66.7%) | 1(50.0%) | .985 |
| | 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%) | |

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## 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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