Molecular Diagnostics and Precision Medicine in Tubercular Lymph Nodes

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Dedicated to

The book is dedicated to individuals afflicted with tuberculosis who are still seeking a precise diagnosis and, consequently, a cure.

I extend my heartfelt gratitude to all whose support and contributions have made completing this book possible.

First and foremost, I am deeply thankful to my supervisor, ***Dr. Arvind Bhake****, professor in the Department of Pathology at Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha, whose guidance and expertise were invaluable throughout the writing process. His insights and encouragement inspired me to reach new heights in this work.*

I would also like to express my respectful obeisance to my ***grandparent****s for their blessings, which will always remain with me. I express my appreciation to my wife,* ***Dr. Prerna****, for her continued support and encouragement. She has always given her unwavering support and understanding during the ups and downs of this creative journey. It is a great pleasure to thank my parents, brothers, uncles, and aunts for their support and prayers. I am blessed to have a wonderful father,* ***Mr. Y. K. Gupta****, who introduced me to the world of research; my mother,* ***Mrs. Shakuntla Gupta****, who continuously prayed for my success; and my brother,* ***Mr. Vishal Gupta****, for their unstinted support, cooperation, and understanding, and unfathomable love.*

Dr. Vivek Gupta

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PROLOGUE

Tubercular lymphadenitis (TBLN) accounts for 20–40% of cases of extrapulmonary tuberculosis; the common presenting symptoms of tuberculosis, like fever, cough, weight loss, fatigue, and night sweats, are not always associated with TBLN. Often, patients present only with enlarged lymph nodes to the clinician, who then prescribes an initial two weeks of antibiotic treatment. If the enlarged lymph node does not regress even after treatment, TBLN is suspected.

This book evaluates the molecular diagnostic intervention of such lymph nodes and how to treat them. In subsequent chapters, its emphasis uses real-time polymerase chain reaction (PCR) for the Mycobacterium tuberculosis complex, targeting the multicopy insertion sequence for diagnosing tubercular lymph node cases. It correlates with the cytological or histological diagnosis of tuberculosis.

It circumvents the detection of TBLN-caused non-tubercular mycobacterium and drug-resistant Mycobacterium tuberculosis, as the molecular target used for MTB detection was specific.

The findings show that real-time PCR diagnoses have the distinct advantage of detecting TBLN at an early stage. It proves to enable the highly sensitive, specific, and accurate diagnosis of TBLN in pediatric populations, where clinicians often miss the diagnosis.

This book has a broad scope of laboratory research so as to reach out to clinicians in addition to describing sensitive and specific diagnostic tools for evaluating TBLN. The book enables not only the early-case detection of TBLN but also emphasizes the patients, to inform them about the treatment protocol of TB. This book, by its relevant results, presents, in a real sense, a blend of efficient molecular diagnostics and a public health service. It catches the audiences through its uniqueness of taking molecular diagnostics to accurately tailor a treatment for lymph node tuberculosis.

Research that ends empirically in the laboratory and fails to be translated for clinical applications is more academic. However, the present book is an exception as it has the following unique and engaging components. The scientific knowledge generated by this book will attract all laboratory research personnel (basic medical sciences), para-clinical, clinical, and super-specialties in medical sciences. It will also entice undergraduate, post-graduate, and doctoral students studying tuberculosis.

Biotechnology plays a vital role in probe-making, and the information presented here would make technicians wiser as to what is specifically needed for the comprehensive blending of nucleic acid sequences of Mycobacterium tuberculosis to widen the scope of translatory research for the highest benefit of the community. Thus, the book also engages biotechnology personnel working in probe-making.

The book also engages tuberculosis patients, teaching them about a disease that is rampant in many countries. Its focus on the diagnostic dilemmas of tubercular lymph nodes and the application of molecular diagnostics modalities (RT-PCR) in such cases offers them guidelines for precise treatment.

The book addresses the following valuable aspects:

- The early diagnosis of tubercular lymph nodes
- The challenges of diagnosing tubercular lymph nodes in children and how to overcome these challenges
- The translatory component of the research work
- A bibliographic analysis
- The scope for future research in this area

This work offers a new perspective by describing misdiagnosed and undiagnosed tubercular lymph node cases and providing a viewpoint on how to bring such cases to early and precise treatment. It describes how the numerical catchment of patients suffering from tubercular lymph nodes increases. It also gives a new perception of evaluating children with tubercular lymph nodes, thus helping to manage such cases.

PREFACE

Lymphadenopathy represents the most common extrapulmonary tuberculosis manifestation. Tubercular lymphadenitis (TBLN) may manifest without the typical tuberculosis symptoms, such as fever, cough, weight loss, fatigue, and night sweats. Patients may initially seek medical attention due to enlarged lymph nodes, leading clinicians to prescribe an initial two-week course of antibiotics. If the lymph nodes remain enlarged despite treatment, TBLN is suspected, prompting further investigations such as cytology or histopathology.

Granuloma, with or without multinucleated giant cells and caseation necrosis, provides cytological/histopathological evidence of TBLN. The development of these findings is influenced by the patient's immune response and immune status. When we cannot see granulomas, we may miss the diagnosis of TBLN cases. Additionally, lymph nodes often display reactive changes during the evolution of granuloma formation, which complicates the diagnosis, particularly in pediatric cases with no apparent symptoms or immunological markers.

This book evaluates the molecular diagnostic intervention of such lymph nodes and their accurate treatment. Subsequent chapters emphasize real-time PCR for the Mycobacterium tuberculosis complex and discuss various clinical and laboratory scenarios for diagnosing TBLN. The findings suggest that a PCR-based diagnosis has a distinct advantage in detecting TBLN cases at an early stage and proves particularly effective in diagnosing TBLN in the pediatric population, where doctors often miss the diagnosis.

It circumvents the detection of TBLN caused by non-tubercular mycobacterium and drug-resistant Mycobacterium tuberculosis, as the molecular target used for MTB detection was specific.

The material outlined in this book holds substantial potential for laboratory research. It offers valuable insights to clinicians seeking sensitive and specific diagnostic tools for TBLN that eventually allow for the early-case detection of TBLN and bring the cases to the TB treatment protocol. It presents an efficient molecular diagnostic, blending in public health service, in a real sense, through its relevant results.

The scientific knowledge generated by this book will attract all laboratory research personnel (basic medical sciences), para-clinical, clinical, and super-specialties in medical sciences. It will also entice

undergraduate, post-graduate, and doctoral students studying tuberculosis, and engage the biotechnology personnel working in probe-making for tuberculosis. Finally, the book also benefits patients by making them aware of the rampant tuberculosis in many countries.

SECTION A

CHAPTER 1

EXTRAPULMONARY TUBERCULOSIS AND TUBERCULAR LYMPH NODES

Overview

This chapter overviews the burden of extrapulmonary tuberculosis and tubercular lymph nodes. It also lays the foundation for their investigation and management in subsequent chapters, which would lead to precision medicine in clinically suspected and unsuspected tubercular lymph nodes.

Introduction

Tuberculosis (TB) is one of the greatest killers worldwide. It has threatened the human race since time immemorial, due not only as a medical problem but also to its impact as a social and economic tragedy. The WHO End TB Strategy serves as a blueprint for countries to reduce TB incidence by 80%, reduce TB deaths by 90%, and eliminate catastrophic costs for TB-affected households by 2030. The Strategy is not a "one-size-fits-all" approach, and its success depends on its adaptation in diverse country settings. Thus, it has become imperative to imperatively study TB from a new perspective to help WHO achieve its goal.

TB global data

In 2021, the World Health Organization recorded 10 million new cases of TB and attributed 1.6 million deaths to TB in the same year. Six million men, 3.4 million women, and 1.2 million children were affected. TB is present in all countries in every part of the world and in all age groups. In 2021, the most significant number of new TB cases occurred in WHO's South-East Asian Region (46%), followed by the African Region (23%) and the Western Pacific (18%). Around 87% of new TB cases occurred in the 30 high TB burden countries, with more than two-thirds of the global total in Bangladesh, China, the Democratic Republic of the Congo, India, Indonesia, Nigeria, Pakistan, and the Philippines.

Over 95% of TB deaths occur in low- and middle-income countries, and it is among the top five causes of death among women aged 15 to 44 years. In 2014, about 80% of reported TB cases occurred in 22 countries. The six countries with the most significant number of incident cases in 2014 were India, Indonesia, Nigeria, Pakistan, the People's Republic of China, and South Africa. India accounts for 23% of global tuberculosis incidence, with an estimated 2.2 million cases reported in 2014.

Extrapulmonary tuberculosis

In addition to the lungs, TB affects many extrapulmonary sites; this is known as extrapulmonary TB (EPTB). The Global TB Report 2020 found that EPTB constituted 16% of notified TB cases worldwide, ranging from 8% in the Western Pacific Region to 24% in the Eastern Mediterranean Region. There has also been a rise in EPTB cases in high-income countries, particularly those in the European Union.

Because of its nonspecific symptoms and signs, along with a lack of constitutional features, EPTB is often not suspected early in the course of illness and its diagnosis is often delayed.

EPTB affects the lymph nodes, pleural, meningeal, pericardial, skeletal, gastrointestinal, genitourinary, and miliary TB. Tubercular lymphadenitis (TBLN) accounts for 20–40% of cases of EPTB. Cervical adenopathy is the most common, but inguinal, axillary, mesenteric, mediastinal, and intramammary involvement are also present.

Symptoms of TBLN

The frequency of symptoms in patients with tubercular lymphadenopathy is variable. In one of the studies by Gupta and Bhake, 129 cases involved the lymph nodes, with patients aging from 1 year to 74 years (mean 30.49 ± 16.69). The female-to-male ratio was 1:1.18. The cervical lymph nodes were the most involved among all lymph nodes. A total of 23 cases were in the pediatric age group (≤14 years), and 107 patients were in the adult age group (>14 years). As regards cytopathology, 48 patients were diagnosed with TBLN, 63 patients with reactive lymphoid hyperplasia, one patient with suppurative lymphadenitis, and 17 cases with malignancy.

Of the 48 cases diagnosed as TBLN, a history of exposure through family members was present in three patients, and three patients had undergone a partial or complete treatment of tuberculosis. Fever was the most common presenting symptom, involving 19 (39.3%) patients, followed by weight loss in six (12.5%) patients, and cough or loss of appetite in three

(6.3%) patients. Six (12.5%) patients had more than two symptoms, and 4 (8%) patients had more than three symptoms, whereas 19 (40%) patients had no symptoms suggestive of tuberculosis (Fig. 1).

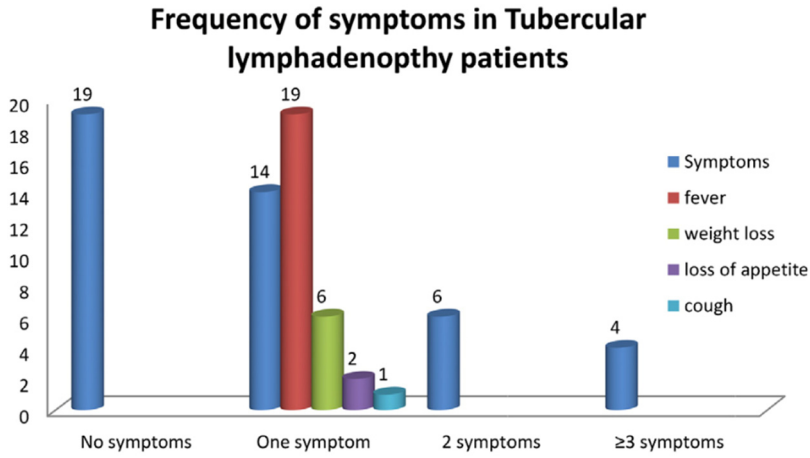

Figure 1. Frequency of symptom in tubercular lymphadenopathy patients
(Used with permission of *Elsevier Science & Technology Journals,* Diagnosis of clinically suspected and unsuspected tubercular lymphadenopathy by cytology, culture, and smear microscopy, Gupta V, Bhake A, *Indian Journal of Tuberculosis;* 2017: vol 64, 314–317; permission conveyed through Copyright Clearance Center, Inc.)

Diagnosing TBLN is problematic as patients' presentation ranges from asymptomatic to nonspecific; clinicians can be perplexed about whether to start the anti-tubercular treatment, which significantly impacts controlling the EPTB.

Further, despite the data on global case numbers, research on extrapulmonary TB is limited, possibly because it is difficult to investigate with the available resources and knowledge. EPTB, if not investigated thoroughly with proper evidence, is difficult to manage.

The subsequent chapters aim to provide a better understanding of extrapulmonary tuberculosis cases, with specific reference to tubercular lymph nodes, to facilitate the precise treatment based on evidence and, thereby, the better management of tuberculosis.

Scope

We should study the management of tuberculosis from a new perspective, one based on the facts for the number of pulmonary and extrapulmonary tuberculosis cases. The new scientific perspective would help control tuberculosis.

Summary

The chapter summarizes:

- The data on the global burden of tuberculosis
- The significant number of extrapulmonary tuberculosis cases
- The spectrum of TBLN symptoms

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CHAPTER 2

CELL-MEDIATED IMMUNITY AND TUBERCULAR LYMPH NODES

Overview

Cell-mediated immunity (CMI), or cell-mediated immune response or cellular immunity, is a vital component of the immune system that plays a central role in defending the body against intracellular pathogens and abnormal cells. Cell-mediated immunity plays a pivotal role in the body's defense against intracellular pathogens, including the bacterium responsible for causing tuberculosis (TB) and Mycobacterium tuberculosis. It is closely associated with the formation of tubercular lymphadenitis (TBLN). Multiple factors influence CMI and lead to TBLN formation. This chapter helps readers understand CMI in TBLN and its impact on TBLN diagnosis.

Introduction

CMI, an adaptive immunity, is a central component of the body's defense mechanism against intracellular pathogens, such as Mycobacterium tuberculosis, the bacterium responsible for tuberculosis (TB). When Mycobacterium tuberculosis infects the body, it primarily resides within macrophages and other immune cells. Cell-mediated immunity is particularly critical in controlling and combating TB infection, and is closely associated with the formation and function of tuberculous lymph nodes. Various factors influence CMI and, thereby, TBLN granuloma formation. These factors have diagnostic outcomes; therefore, knowing the CMI in TBLN and how various factors influence it is necessary.

CMI-TBLN

The key steps involved in CMI and TBLN granuloma formation are as follows:

Step 1: Antigen Presentation

- TB infection begins with the inhalation of Mycobacterium tuberculosis into the lungs.
- Antigen-presenting cells (APCs), such as dendritic cells and macrophages, encounter the bacteria.
- APCs engulf and process the bacteria, breaking them down into antigen fragments.

Step 2: Activation of Helper T Cells

- APCs present the antigen fragments on their cell surfaces using major histocompatibility complex (MHC) molecules.
- CD4+ helper T cells recognize these antigen-MHC complexes and become activated.
- Activating helper T cells is a critical step in initiating the immune response.

Step 3: Cytokine Release

- Activated helper T cells release cytokines, particularly interferon-gamma (IFN-γ).
- IFN-γ plays a central role in enhancing the bactericidal activity of macrophages.

Step 4: Macrophage Activation

- INF-γ activates macrophages infected with Mycobacterium tuberculosis.
- Activated macrophages become more effective at killing intracellular bacteria.

Step 5: Recruitment of Immune Cells

- The immune response leads to the recruitment of additional immune cells, including more macrophages and T cells, to the site of infection.

Step 6: Granuloma Formation

- Granulomas are organized structures composed of immune cells, including macrophages, T cells, and fibroblasts.
- These structures encapsulate infected cells, containing the bacteria and preventing their spread to other tissues.
- Granuloma formation is a hallmark of TB infection and reflects the host's attempt to control the pathogen.

Step 7: Tissue Healing and Scarring

- Over time, the granuloma may change, leading to tissue healing and scarring.
- Bacteria do not become entirely eradicated. Instead, they become dormant within the granuloma.

Step 8: Immune Memory

- Some activated T cells, particularly memory T cells, persist within the body.
- Memory T cells "remember" the TB antigens, providing long-term immunity.
- If the infection reactivates, memory T cells mount a rapid immune response.

Tubercular lymph node granulomas often form in response to TB infection and can be found in the lymph nodes draining the infected area. These granulomas are complex structures that help contain the infection and can contribute to tissue damage and scarring over time. The interplay between the immune response and the bacterium's persistence can lead to the various clinical manifestations of TB.

Factors influencing cell-mediated immunity in tuberculosis

The clinical manifestations of cell-mediated immunity, such as tuberculous lymphadenitis, are believed to represent a localized manifestation of a systemic disease. The CMI and granuloma formation in TBLN, in addition to the bacterial factors, are influenced by various host factors, including age, gender, nutritional status, genetic predisposition, family history of contact with tuberculosis, and the patient's immune competence. Consequently, this results in a wide range of clinical and morphological presentations. These observations likely stem from various factors, including biological, hormonal, social, environmental, and behavioral distinctions between men and women.

A fundamental biological divergence exists in the immune systems of men and women, which may contribute to these differences. Researchers suggest hormonal influences on immunity as an underlying cause for the distinct disease pattern observed in women.

Women in developing countries often experience a lower socioeconomic status and nutritional deficiencies, which can impact their CMI response to the disease. Additionally, it is proposed that women are more conscious of

their appearance and may seek healthcare services earlier than men, who may neglect their condition until it reaches an advanced stage.

The formation and organization of granulomas can vary depending on the effectiveness of the cell-mediated immune response. Here is how the degree of organization is related to the immune response:

Effective Cell-Mediated Immune Response:

- In cases where the cell-mediated immune response is strong and effective, granulomas tend to be well organized.
- These granulomas exhibit minimal necrosis—the death of cells or tissue in the area.
- Individuals with well-organized granulomas observe few, if any, clinical symptoms.
- The immune system successfully contains the infection, and the disease remains relatively controlled.

Poor Cell-Mediated Immune Response:

- When the cell-mediated immune response is weak or inadequate, granulomas may be less organized.
- These less-organized granulomas often show scattered clusters of epithelioid macrophages—immune cells in defense against pathogens.
- Massive necrosis within the granulomas may indicate extensive tissue damage and cell death.
- Individuals with the formation of poorly organized granulomas may experience frequent and severe clinical manifestations, including disease symptoms.

In summary, the organization and characteristics of granulomas in the context of tuberculosis and other infectious diseases reflect the host's immune response. A robust cell-mediated immune response tends to result in well-organized granulomas with minimal tissue damage and fewer clinical symptoms. In contrast, a weakened immune response can lead to disorganized granulomas with significant tissue damage and more pronounced clinical manifestations of the disease.

CMI from childhood to adulthood

The immune system undergoes a gradual maturation process during infancy. Newborns receive crucial protection against many infectious diseases from their mothers through the passive transfer of IgG antibodies, which occurs both transplacentally during pregnancy and through breastfeeding. As this

maternal immunity gradually wanes, young children become more susceptible to infections, although their immune systems are developing and becoming more robust.

Over time, the ability of the immune system to provide protection increases, and young adults experience fewer infections. The accumulation of immunological memory is a dynamic aspect of the adaptive immune response. This immunological memory persists into old age but may gradually diminish over time.

CMI declines with age

As individuals age, their immune system undergoes significant changes and deterioration, known as immune senescence. This aging-related immune remodeling has substantial implications for health and longevity. One notable consequence of the immune senescence is that it renders older adults more susceptible to tuberculosis.

CMI factors affecting and clinical implications

The multiple factors that affect the CMI and adaptive immunity in TBLN leading to granuloma formation carry a clinical significance, and we should consider them while evaluating TBLN. A female patient may not have evident signs or symptoms or cytomorphological features of tubercular lymph nodes due to low CMI and yet may have tuberculosis. Similarly, due to low or no CMI, a pediatric patient or an elderly patient with TBLN may not be diagnosed correctly.

Scope

This chapter describes the CMI and granuloma formation in TBLN and also the factors affecting them. It also outlines the effect of age on CMI. These elements must be taken into account when evaluating cases of TBLN.

Summary

The chapter describes

- The steps involved in CMI and granuloma formation for TBLN
- The factors affecting CMI
- The importance of aging on CMI

- The clinical implications of CMI on the diagnosis of TBLN

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CHAPTER 3

CLINICAL AND CELLULAR FEATURES IN TUBERCULAR LYMPHADENITIS

Overview

It is challenging to diagnose tuberculosis lymphadenitis clinically; in resource-poor countries, laboratories are often unavailable. This chapter describes the symptoms, clinical characteristics, and results of cytological analysis in peripheral tuberculous lymphadenitis patients. It further recommends that constitutional symptoms and clinical and cytological features help diagnose peripheral tubercular lymphadenitis and open new frontiers to enhance research that studies the effect of the cytological features of these cases.

Introduction

The incidence of extrapulmonary tuberculosis (EPTB) has been on the rise over the last few years. Peripheral tuberculous lymphadenopathy is the most common form of extrapulmonary tuberculosis. It accounts for 25–60% of all EPTB cases in regions where mycobacterial infection is prevalent, and commonly presents in lymph nodes draining the head and neck. In India, 43% of tuberculous lymphadenitis (TBLN) cases are diagnosed on clinical grounds alone without laboratory confirmation, as these facilities are often unavailable. The conventional methods of diagnosis for tuberculosis, like examination of sputum for acid-fast bacilli and a chest X-ray, are relatively accurate in detecting the active pulmonary component of the disease. However, they do not help detect the extrapulmonary tubercular components.

Fine-needle aspiration cytology (FNAC) is usually the first line of investigation in diagnosing tuberculous lymphadenitis and has a high diagnostic yield (97%); however, laboratory facilities are not available at all centers in developing countries. We were interested in reviewing the clinical parameters of TBLN lymphadenitis and the morphological changes

observed in cytology. This chapter describes the symptoms, clinical characteristics, and results of cytology analysis in tuberculous lymphadenitis patients to assess their diagnostic value in patients.

Fine-needle aspirates

Gupta and Bhake performed FNAC using a 23-gauze needle under aseptic conditions, and the material was aspirated using a 20 ml disposable syringe attached to the Franzen handle. Their study described the aspirate as sticky, purulent, hemorrhagic, and cheesy white. The aspirate from each case was taken for cytological examination and smeared on three slides: two air-dried and one wet-fixed. The study protocol stained smears with May-Grünwald-Giemsa, Ziehl-Neelsen (ZN) stain on air-dried smears, and Papanicolaou stain on alcohol-fixed smears. The study carried out cytomorphological typing on smears stained with Papanicolaou and grouped them into three categories:

- Type 1— epithelioid granuloma with caseous necrosis. In addition to epithelioid cells, the smear contained clumps of amorphous debris or caseous necrotic material. It may be associated with lymphocytes, Langhans giant cells, and neutrophils.
- Type 2 — epithelioid granuloma without caseous necrosis has groups of epithelioid cells and a variable number of lymphoid cells. A foreign body or Langhans giant cells may or may not be present.
- Type 3 — a tubercular abscess with no epithelioid granuloma presents necrotic materials with a marked degeneration and variable polymorphonuclear infiltration.

Diagnostics results of fine-needle aspirates

The authors (Gupta and Bhake) studied 156 patients with peripheral lymphadenopathy suspected of being of tubercular origin and performed FNAC. The study diagnosed 70 cases of reactive lymphadenitis, 69 cases of TBLN, and 5 cases of other conditions. The remaining 12 cases were inconclusive. Among the 69 cases, the female-to-male ratio was 1.3:1. The ratio supports the findings of studies that more women than men have tuberculous lymphadenitis. The mean age of females was 29.9 +- 15.18 years, with 75% of the patients aged between 18 and 42 years. The mean age of males was 30.5 +-12.57, with 75% of the patients aged between 19 and 39 years. The mean age of females and males was not statistically significant.

The most common site for FNAC among the lymph nodes was cervical (70.3%), followed by supraclavicular (11%), submandibular (10%), axillary (7.4%), and inguinal (1.3%). Tuberculous lymphadenitis is one of the most common manifestations of extrapulmonary tuberculosis. Cervical lymphadenitis, caused by Mycobacterium tuberculosis, is generally considered to originate in the lymphatic spread of organisms from a primary pulmonary focus. However, in a minority of cases, it can originate from a primary focus in the mouth, tonsils, oropharynx, or head and neck tissues.

Table 3a summarizes the clinical characteristics of 69 patients with tubercular lymphadenopathy. Of the lymph nodes diagnosed, 62 (89.9%) were of size ≥2x2 cm, and most of them presented as multiple, either discrete or matted (59, 85.5%), and solid (55, 79.7%), lymph nodes. Constitutional symptoms were present in 41 (59.4%) patients. The triad of size, multiplicity, and matting helps reach the diagnosis of tuberculous lymphadenitis. The presence of constitutional symptoms helps raise the suspicion of tuberculous lymphadenitis, especially in resource-poor countries like India, where diagnosis and treatment are usually based on clinical suspicion alone. Thus, there is a need to improve the clinical criteria used to diagnose tuberculous lymphadenitis.

Table 3a. Clinical characteristics of 69 patients with tuberculous lymphadenitis

Lymph node characteristics	No. of instances
Size[a] <2x2 cm	07 (10.1%)
2–4 cm	48 (69.5%)
>4 4 cm	14 (20.4%)
Characters	
Single	10 (14.5%)
Multiple	
(Discreet)	28 (40.6%)
(Matted)	31 (44.9%)
Solid	55 (79.7%)
Fluctuant	14 (20.2%)
Constitutional symptoms	
Any symptom	41 (59.4%)
Fever	29 (42.0%)
Cough	23 (33.3%)
Night sweat	33 (47.8%)
Fatigue	37 (53.6%)
Weight loss[b]	17 (24.6%)

1. a =Transverse diameter of the largest cervical mass
2. b >10% of body weight in 3 months

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The diagnosis of tuberculosis is often difficult given that symptoms and signs might be nonspecific, the collection of bacteriologic specimens is problematic, and the bacteriologic yields are low. However, clinical signs are usually apparent in patients with peripheral tuberculous lymphadenitis, and fine-needle aspiration (FNAC) intervention provides excellent bacteriologic yields. Although the authors have demonstrated the diagnostic value of FNAC in resource-limited settings, it remains underutilized as a routine diagnostic modality in most endemic areas. They also categorized cytomorphological findings in 69 cases as well-formed epithelioid cell granulomas, giant cells, and caseous necrosis (34 cases), epithelioid cell granulomas but no caseous necrosis (18 cases), and caseous necrosis but no granulomas (17 cases). Forty-five cases were ZN-positive with maximum positivity, with 26/34 (76.5%) smears showing both granuloma and necrosis, followed by smears having necrosis (68.9%) and only three cases (16.7%) of granuloma only. Table 3b shows the cytological features and ZN staining for acid-fast bacilli (AFB).

Table 3b. Cytological findings and Ziehl-Neelsen staining for AFB-positive cases

Cytological findings	No. of cases	AFB-positive cases
Type 1	34	26
Type 2	18	03
Type 3	17	16
Total	69	45

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Cytomorphological features identified in purulent aspirate (26) cases were epithelioid cell granulomas, with caseation necrosis in 12/26 cases and

necrosis only seen in 14/26 cases. Eighteen out of 26 cases were AFB-positive. Cytomorphological features in hemorrhagic aspirate (29) cases were epithelioid cell granuloma, with caseation necrosis in 18/29 cases and epithelioid cell granuloma only in 10/29 cases. Cheesy aspirate was seen in 14 cases only. Table 3c shows the characteristics of aspirate and cytomorphological patterns.

Table 3c. Nature of aspirate and cytomorphological spectrum

Nature of aspirate	Type 1	Type 2	Type 3	Total
Purulent	12	00	14	26
Hemorrhagic	18	10	01	29
Cheesy	04	08	02	14
Total	34	18	17	69

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The aspirate demonstrated a tendency to correlate with cytomorphological patterns and had the p-value <0.0001 (highly significant). Cheesy aspirates are most commonly associated with Type 2 cytomorphological patterns. The type 1 pattern was most commonly associated with hemorrhagic aspirate, and type 3 was associated with purulent aspirate.

A total of 70 patients were diagnosed with reactive lymphadenitis on FNAC. The female:male ratio was 4:5, and the mean age of presentation for females was 22.8 ±13.4 years, and for males, 29 ± 18.6 years. Out of 70 patients with reactive lymphadenitis, 65 (93%) presented as single lymph nodes with a size <2 cm, and the remaining 5 (7%) had multiple lymph nodes of 1 cm. The reactive lymphadenitis in 60 patients (86%) was not associated with any symptoms, and the remaining 10 (14%) cases had only fever as a presenting symptom.

The types of aspirates that were associated with the diagnosis of reactive lymphadenitis were as follows: particulate and sticky in 60 (85.7%) cases, hemorrhagic in 8 (11.5%) cases, and purulent and cheesy in 1 case (1.4%) each. Aspirate showed a highly significant correlation with