

## *Original Article*

# Cytomorphological Spectrums in Tuberculous Lymphadenitis: Understanding the Stages of Disease

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## **Abstract**

**Introduction:** Tuberculous lymphadenitis is the most common form of extrapulmonary tuberculosis and one of the main causes of lymphadenopathy. Fine Needle Aspiration Cytology (FNAC) has played a substantial role in diagnosis of tuberculous lymphadenopathy and it has become a first-line diagnostic technique. The aim of this study was to describe and understand the spectrum of cytomorphological changes seen in tuberculous lymphadenitis corresponding to stages of disease and to associate the cytomorphological changes with Acid Fast Bacilli (AFB) positivity.

**Methods:** This is a retrospective cytomorphological study of cytologically diagnosed tuberculous lymphadenitis. The recorded details of clinical presentation and site of the lymph nodes were noted. The slides were stained with Papanicolaou, Giemsa and AFB.

**Result:** There were a total of 203 cases with age ranging from 3- 75 years. Four cytomorphological patterns observed were Necrotising Granulomatous Lymphadenitis (NGL; 45.32%), Granulomatous Lymphadenitis (GL, 18.22%), Necrotising Suppurative Lymphadenitis (NSL; 21.18%) and Necrotising Lymphadenitis (NL; 15.27%). Necrotising patterns were observed in immunocompromised individuals like HIV infected patients, in patients with previous history of tuberculosis and in patients with chronic renal disease. Strong AFB positivity was observed in necrotising patterns. Lymph nodes of head and neck region were the most common site of involvement with cervical being the commonest.

**Conclusion:** Necrotizing patterns are observed in the later stages of disease or in the immunocompromised patients. Strong positivity for AFB is observed in the smears with necrotizing patterns and less in the granulomatous pattern.

**Keywords:** Cytomorphology, Fine Needle Aspiration Cytology, Granulomatous Lymphadenitis, Necrotising Suppurative Lymphadenitis, Necrotising Granulomatous Lymphadenitis, Tuberculous Lymphadenitis.

## **Introduction**

Tuberculosis is still a major problem even in the twenty first century despite of awareness of the disease and the available treatment. It is still a leading cause of death among infectious diseases.<sup>1</sup> It is estimated that 1.7 billion individuals are infected worldwide, with 8 to 10 million new cases and 3 million deaths per year.<sup>2</sup> The strong resurgence of tuberculosis has been caused by the epidemics of drug abuse and

human immunodeficiency virus (HIV) infection.<sup>1</sup> Although its incidence has steadily decreased in developed countries, it remains a major health problem in developing nations, like our country Nepal.

Tuberculosis is one of the foremost public health problems in Nepal, causing a significant burden of morbidity and mortality. About 45% of the total population is infected with TB, out of which 60% are adults (aged 15-64). Every year, 44,000 people develop active TB, of whom 20,000 have infectious tuberculosis. TB causes an estimated 8,000- 11,000 death per year.<sup>3</sup>

Tuberculous Lymphadenitis is the most common form of extrapulmonary

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tuberculosis<sup>1,4,5</sup> and one of the most common causes of lymphadenopathy.<sup>5</sup> FNAC has played a substantial role in diagnosis of Tuberculous Lymphadenopathy and it has become a first-line diagnostic technique.<sup>6-8</sup> It is simpler, safer, more rapid and economical procedure than core-needle or excisional biopsy.<sup>5-7,9</sup> Spectrum of morphological changes seen in tuberculous lymphadenitis denotes the stages of the disease by itself and also the immune status of the patient.<sup>10-12</sup> Hence, understanding the different morphologies observed in the smear of tuberculous lymphadenitis helps in early diagnosis and treatment of the disease. The study was conducted with an aim to describe the spectrum of cytomorphological changes seen in fine needle aspirate smears of Tuberculous Lymphadenitis corresponding to stages of diseases and to associate it with AFB positivity. Site distribution of various lymph nodes in tuberculous lymphadenitis was also observed.

### **Methodology**

A retrospective cytomorphological study of cytologically diagnosed tuberculous lymphadenitis was conducted in the Department of Pathology, BPKIHS, during 1<sup>st</sup> Jan- 31<sup>st</sup> Dec 2010.

Among fine needle aspiration performed in the Department of Pathology from enlarged lymph nodes, only the cases of tuberculous lymphadenitis were included in the study. The recorded details of clinical presentation and site of the lymph nodes were noted.

FNAC were performed using a 23G needle and a 10ml syringe under strict safety conditions in the Department of Pathology.

Immediate smears were made from the aspirate material. Two slides were immediately fixed in 95% ethanol and later Papanicolaou stained. Three slides were air-dried and later fixed for

May-Grünwald Giemsa for morphology and Ziehl – Neelson for acid fast bacilli.

Tissue biopsies were not included in the study. No molecular techniques were used in the FNA material. Culture of the tissue for microbiological examination of the aspirated material was not performed.

**Statistical analysis:** Descriptive statistics was calculated. In inferential statistics, association was found out at  $p= 0.05$  where  $CI= 95\%$  using  $\chi^2$  test.

### **Result**

Among fine needle aspiration done on enlarged lymph nodes from 1<sup>st</sup> January to 31<sup>st</sup> December 2010, in the Department of Pathology, BPKIHS, 203 cases were diagnosed as tuberculous lymphadenitis.

The age of the patients in the study ranged from 3 to 75 years. The most common clinical presentation was swelling at the site of enlarged lymph nodes. Evening rise fever associated with cough was seen in 19 cases. Patients with enlarged mesenteric nodes had complaint of abdominal pain with distension and loss of appetite. There were no other specific complaints. Out of 203 cases, 24 had past history of tuberculous lymphadenitis and 3 had pulmonary tuberculosis. Four cases were associated with HIV infection and 5 cases had chronic kidney disease.

The most common site of tubercular lymphadenopathy was head and neck region (164). In the head and neck region, cervical was the most common site (126). This was followed by submandibular (20), supraclavicular (16) and submental (2) nodes. After head and neck region, other sites were axillary (15), multiple (10), mesenteric (9) and inguinal region (5) (Table 1).

**Table No. 1:** Sites of involvement of various lymph nodes and their frequency.

| S. No. | Sites              | Number of cases | %     |
|--------|--------------------|-----------------|-------|
| 1      | Head and neck      |                 |       |
|        | a. Cervical        | 126             | 62.06 |
|        | b. Submandibular   | 20              | 9.85  |
|        | c. Submental       | 2               | 0.98  |
|        | d. Supraclavicular | 16              | 7.88  |
| 2      | Axillary           | 15              | 7.38  |
| 4      | Inguinal           | 5               | 2.46  |
| 5      | Mesenteric         | 9               | 4.43  |
| 6      | Multiple           | 10              | 4.92  |
|        | Total              | 203             | 100   |

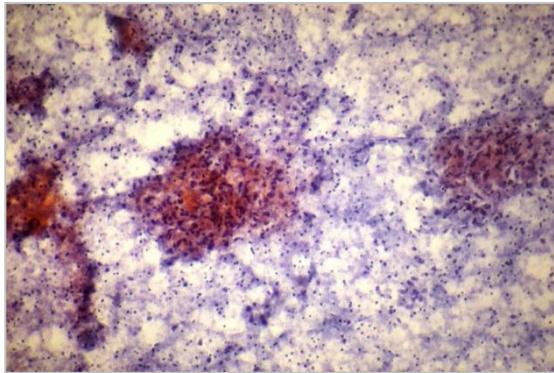
**Table No. 2:** Cytomorphological patterns of Tuberculous Lymphadenitis with corresponding AFB positivity.

| S. No | Cytomorphology                          | No. of cases | %     | AFB positivity | %     | P value |
|-------|---|--------------|-------|----------------|-------|---------|
| 1     | Necrotizing Granulomatous Lymphadenitis | 92           | 45.32 | 36             | 39.13 | <0.001  |
| 2     | Granulomatous Lymphadenitis             | 37           | 18.22 | 2              | 5.4   | <0.001  |
| 3     | Necrotizing Lymphadenitis               | 31           | 15.27 | 30             | 96.77 | <0.001  |
| 4     | Necrotizing Suppurative Lymphadenitis   | 43           | 21.18 | 41             | 95.34 | <0.001  |
|       | Total                                   | 203          | 100   |                |       |         |

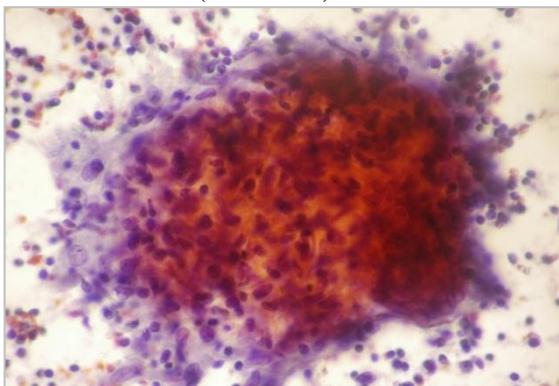
**Table No. 3:** Comparison of cytomorphological patterns in tuberculous lymphadenitis of various studies.

| S. No | Study          | Various cytomorphologies |        |        |        |
|-------|----------------|--------------------------|--------|--------|--------|
|       |                | NGL                      | GL     | NL     | NSL    |
| 1     | Laishram et al | 50.90%                   | 29.25% | 13.30% | 6.52%  |
| 2     | Nayak et al    | 47.70%                   | 23.80% | 5%     | 10%    |
| 3     | Tarun et al    | 33.33%                   | 51.50% |        | 15.50% |
| 4     | Present study  | 45.32%                   | 18.22% | 15.27% | 21.18% |

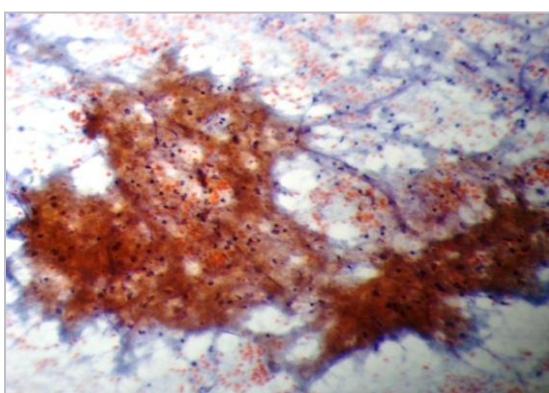
**Figure 1:** Presence of epithelioid cell granuloma and caseous necrosis in NGL (PAP stain).



**Figure 2:** GL showing presence of epithelioid cell granuloma and lymphocytes without caseous necrosis (PAP stain).



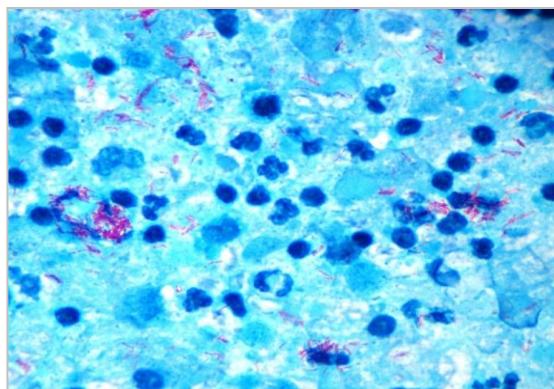
**Figure 3:** PAP stained smear showing neutrophils and their nuclear dust along with macrophages, lymphocytes and occasional degenerated epithelioid histiocytes against a background of abundant caseous necrosis in NSL. Granulomas are absent in the smears.



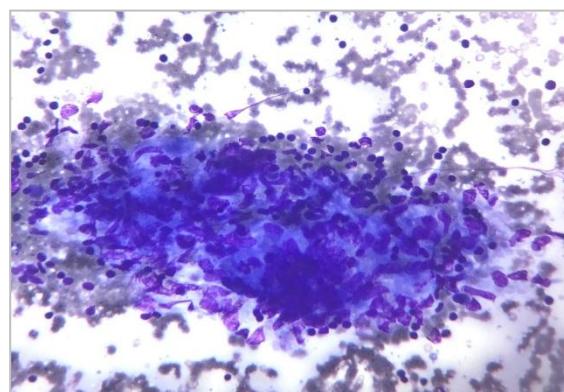
**Figure 4:** PAP stained smear revealing abundant caseous necrosis and occasional lymphocytes in NL.



**Figure 5:** Strong AFB positivity in a smear revealing necrotising suppurative pattern (Ziehl-Neelson stain).



**Figure 6:** Giemsa stain smear revealing a well formed granuloma with scant caseous necrosis.



The overall morphological features observed in the smears were caseous necrosis, epithelioid cell granulomas, langhans giant cells, histiocyte like giant cells, neutrophils and their nuclear dust, macrophages, few plasma cells and lymphocytes. Occasional presences of granulation tissue were seen.

After thorough examination of the smears, four cytomorphological patterns were observed and categorized accordingly (Table 2).

1. Necrotising Granulomatous Lymphadenitis (NGL): 92 cases (45.32%). These cases showed presence of caseous necrosis along with fair number of epithelioid cell granulomas with or without presence of Langhans giant cells. There was also presence of few histiocytic giant cells. Figure 1
2. Granulomatous Lymphadenitis (GL): 37 cases (18.22%). There was presence of epithelioid cell granulomas with or without giant cells. Caseous necrosis was absent. Figure 2
3. Necrotising Suppurative Lymphadenitis (NSL): 43 cases (21.18%). These cases showed presence of numerous neutrophils and their nuclear dust along with lymphocytes and macrophages against a background of abundant caseous necrosis. Histiocytic type giant cells were also seen in few cases. Epithelioid cell granulomas were absent. Occasional scattered epithelioid cells were observed. Figure 3
4. Necrotising Lymphadenitis (NL): 31 cases (15.27%). Abundant caseous necrosis was seen in these cases with occasional degenerating epithelioid cells. Epithelioid cell granulomas were absent. Figure 4

In the NGL pattern, AFB was positive in 36 cases (39.13%) out of 92 cases. The patterns revealing predominantly caseous necrosis like NL and NSL showed more frequency of AFB positivity. The patterns with necrotizing suppuration showed 95.34% AFB positivity and those of necrotizing lymphadenitis showed 96.77% AFB positivity. Out of 37 cases of GL,

only 2 cases (5.4%) showed AFB positivity. There was a statistically significant association of AFB positivity with the cytomorphological patterns.

There were 24 cases with recent history of tubercular lymphadenitis, 23 of which showed necrotising pattern and one case (4.16%) showed granulomatous pattern. In the necrotising patterns, NGL was seen in 9 (37.5%), NSL in 8 (33.33%) and NL in 6 (25%). 15 out of 24 of these cases showing necrotising pattern showed AFB positivity.

In this study, there were 3 cases with previous history of pulmonary tuberculosis and all the cases showed necrotising patterns in the smears with one case each of NGL, NL and NSL. AFB positivity was seen in all the three cases.

Association of tuberculous lymphadenitis with HIV infection was seen in 4 out of 203 total cases. This group also showed necrotising pattern in the smears with 2 cases of NGL and one case each of NSL and NL. AFB positivity was seen in 3 out of 4 cases.

There were 5 cases of tuberculous lymphadenitis in patients with chronic renal disease. Smears in this group also showed necrotising patterns with 3 cases of necrotising granulomatous pattern, one case of necrotising suppuration and one case with caseous necrosis. AFB positivity was seen in 3 out of 5 cases.

## Discussion

Despite decades of TB control efforts; the case numbers are still increasing today. The rising global incidence rates of TB, fueled by HIV, and the colossal gap between the notified and the estimated number of new TB cases has recently prompted global action.<sup>13</sup> Prompt diagnosis and effective treatment leads to reduced morbidity and mortality.<sup>14</sup>

Progress in controlling TB is critically constrained by the inadequacy of available diagnostic tools.<sup>13</sup> Since recently, fine needle aspiration cytology has been used extensively in the diagnosis of tuberculous lymphadenitis and has become a first line diagnostic technique.<sup>6,8</sup> It

is simple, safe, cost-effective and conclusive.<sup>9,10,14</sup> Diagnosis of tuberculous lymphadenitis can be established by demonstrating acid-fast bacilli (AFB) in FNA smears with Zeil-Neelsen stain or auramin-rhodamine stain, culture of the organism, or by polymerase chain reaction (PCR).<sup>14</sup> But in developing countries like Nepal, demonstration of bacilli in FNA smears by Zeil-Neelsen stain is most widely used due to certain factors like time, cost etc. involved in other methods.

There were 203 cases of cytologically diagnosed tuberculous lymphadenitis in our study. Age of the patients ranged from 3 to 75 years. The commonest clinical mode of presentation was swelling at the site of enlarged nodes.

The most common location of clinically apparent tubercular lymphadenopathy is the cervical region (scrofula).<sup>7,15</sup> The other sites are supraclavicular, mediastinal and axillary chains in order of decreasing frequency.<sup>4</sup> In our study, the most common site of lymphadenopathy was head and neck region, with cervical region being the commonest. This was followed by axillary (7.38%), mesenteric and multiple (4.43% each), inguinal (2.46%) and supratrochlear lymph node (0.49%). In a study conducted by Laishram RS et al,<sup>10</sup> cervical lymph nodes (50.49%) were most commonly involved, followed by axillary (24.13%), multiple (14.87%), inguinal (9.66%), supraclavicular (0.82%). Similar result was seen in a study conducted by Bezabih M et al<sup>16</sup> revealing cervical region as the most common site (74.2%). Post cervical (50%) followed by submandibular groups (47.8%) were commonly involved in tubercular lymphadenopathy in a study of fine needle aspiration cytology in childhood TB lymphadenitis by Balaji J et al.<sup>9</sup>

*Mycobacterium tuberculosis* is an intracellular parasite. Engulfed by the alveolar macrophages, the mycobacteria multiply within the cells and are transported to new locations. When macrophages laden with bacilli encounter CD4+ T cells, the T cells become activated and produce a variety of lytic enzymes that kill mycobacteria but also cause tissue necrosis.<sup>1</sup>

The macrophages transform into uni- or multinucleated epithelioid macrophages. Thus, the smears of tuberculous lymphadenitis show epithelioid granulomas, caseation necrosis, lymphocytes, and occasional Langhans-type giant cells.<sup>4</sup> Orell et al emphasized the criteria for diagnosis of tuberculous lymphadenitis suggested by the presence of lymphocytes, epithelioid cells, Langhans giant cells, neutrophils, caseous necrotic background (eosinophilic granular material without recognizable cellular elements) and hemorrhage.<sup>5,17,18</sup> We had similar cytomorphological findings in our study.

The broad range of lesions that can be observed in tuberculosis is the result of continuous interaction between bacterial virulence and individual hypersensitivity and immunity to infection.<sup>10,12</sup> Many authors have described different cytomorphological patterns in the smears of tuberculous lymph nodes (Table 3). Laishram RS et al<sup>10</sup> have described four cytomorphological patterns of smears which are similar to our findings. Nayak S et al,<sup>19-20</sup> have described similar four patterns in their two studies of Fine Needle Aspiration Cytology of Tuberculous Lymphadenitis; one in HIV patients only and the other study in patients with both HIV positive and negative. Hemalatha A et al in their study of cytomorphological patterns of tubercular lymphadenitis revisited have also described four similar patterns.<sup>25</sup> Many authors have described three patterns by combining necrotizing pattern with or without suppuration into one group.<sup>16,21,26,27</sup> Balaji J et al<sup>9</sup> have described five cytomorphological patterns: necrosis with granuloma, epithelioid cell granuloma, caseation necrosis, focal granuloma with inflammatory exudates and inflammatory exudates only with occasional langhan's giant cell. Chakrabarti AK et al<sup>12</sup> have described five histologic types in their study of morphologic classification of tuberculous lesions. Sridhar CB et al<sup>22</sup> have described four patterns in a comparative cytological study of lymph node Tuberculosis in HIV infected individuals and in patients with diabetes in a developing country.

The major immune responses of the host to tuberculous infection are macrophage activation, specific T-cell-mediated reactivity, and granuloma formation.<sup>1</sup> Granulomatous inflammation develops under the regulating influence of cytokines produced by local mononuclear phagocytes, T cells, dendritic cell etc.<sup>23</sup> With the development of specific immunity and the accumulation of large numbers of activated macrophages at the site of the primary lesion, granulomatous lesions are formed.<sup>24</sup> The tuberculous granuloma is classically described as having a necrotic center and concentric areas of epithelioid cells, Langhans giant cells, and lymphocytes.<sup>1</sup> Initially, the tissue-damaging response can limit mycobacterial growth within macrophages. It not only destroys macrophages but also produces early solid necrosis in the center of the tubercle.<sup>24</sup> Though development of granulomas is a major morphological feature of tuberculosis; they can be present in a number of other diseases including sarcoidosis, viral or bacterial adenitis, fungal infections, toxoplasmosis, cat scratch disease, collagen vascular diseases, carcinoma, lymphoma or sarcoma and the diseases of reticuloendothelial system.<sup>5</sup> In developing countries where tuberculosis is common and other granulomatous diseases are rare, presence of granulomas in FNA smears are highly suggestive of tuberculosis.<sup>14</sup>

We had 18.22% of GL with 5.4% AFB positivity. The low AFB positivity in GL denotes the fact that the presence of lymphocytes and epithelioid cells limit the proliferation of bacilli.<sup>1,12,22</sup>

The cytomorphological patterns, to some extent, denote the immune status of the individuals. The presence of acid-fast bacilli in smears is directly proportional to the necrosis and inversely to the granulomas.<sup>4,18,22</sup> The bacilli are bright red, slender, and beaded by the Ziehl–Neelsen acid-fast stain.<sup>4</sup> Presence of caseous necrosis and suppuration are more commonly seen in immunocompromised patients with higher and heavy AFB positivity. In our study, the predominance of caseous necrosis was seen in

patients with past history of tuberculosis, immunocompromised individuals and in patients with chronic illness. It was associated with a higher and heavy AFB positivity (Figure 5). Nayak S et al<sup>19</sup> in their study of FNAC in Lymphadenopathy of HIV-positive patients have shown the predominant cytomorphological patterns consisting of caseous necrosis; necrotizing lymphadenitis, necrotizing granulomatous and necrotizing suppurative lymphadenitis and they were associated with higher AFB positivity. Nayak et al<sup>20</sup> in their other study of FNAC in Tuberculous lymphadenitis of patients with and without HIV infection have also shown a higher incidence of necrotizing pattern of smears in HIV infected patients. In a study conducted by Mahana S et al, the highest smear and culture positivity was noted in cases with only necrosis.<sup>28</sup> Similarly various studies have shown higher positivity rate of AFB TB in predominant necrosis pattern which are concurrent with our study.<sup>25-28</sup>

### **Conclusion**

Four types of tuberculous lymphadenitis patterns were observed in our study: GL, NGL, NSL and NL. Necrotizing patterns were observed in patients having recent history of tuberculous lymphadenitis with completion of antitubercular treatment but having persistence of lymphadenopathy, in patients co-infected with HIV and in patients with chronic renal disease. As the presence of AFB in smears was directly proportional to the necrosis and inversely to the granulomas, higher and heavy positivity for AFB was observed in the smears with necrotizing patterns and lesser in the granulomatous pattern.

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