

Fine needle aspiration cytology in the diagnosis of Tuberculous lymphadenitis and utility of Ziehl Neelsen stain benefits and pitfalls

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Abstract

Introduction: In a developing country like India, tuberculous lymphadenitis is one of the most common presentations at OPDs. However, anti-tubercular treatment cannot be administered only on clinical suspicion. Cytomorphology with acid fast staining proves to be a valuable tool in diagnosing these cases along with culture. The study was undertaken to study the utility, limitations of fine needle aspiration cytology and various cytomorphological presentations in reference to Ziehl-Neelsen staining in tuberculous lymphadenitis and correlate the culture findings. **Methods:** The study was conducted for duration of two years with total of 170 cases at a tertiary care centre. The patients with clinically suspected lymphadenopathy were selected. **Results:** The incidence of tuberculous lymphadenitis was 68.8%. Overall AFB positivity was 64.1%. Epithelioid cell granulomas with lymphocytes were the most common cytological picture and cases showing necrosis had highest AFB positivity. Maximum patients presented in second to fourth decade of life. Cervical region was the most common site of involvement with solitary lymphadenopathy as the most common presentation in contrast to matted lymph nodes as reported by others. **Conclusion:** Yet again Fine needle aspiration cytology is a safe, cheap and reliable procedure requiring minimal instrumentation and is highly sensitive to diagnose tuberculous lymphadenitis. The diagnostic index can be further increased by complementing cytomorphology with acid fast staining and culture techniques. However FNAC complimented with techniques like ELISA and PCR would give better dimensions to the current scenario of diagnosis and treatment modalities.

Key words: Lymphadenitis, FNAC, Tubercular Lymphadenitis, Granulomatous, AFB.

Introduction

Ancient man described diseases to be work of an evil spirit, curse brought on man by the wrath of malevolent Gods, or the act of supernatural powers. With evolvement of medical sciences, pioneers Louis Pasteur and Robert Koch shared the insight that diseases were caused by bacteria which evoked specific response in the body. Granulomatous response is one such response. Granulomatous lymphadenitis is a manifestation of several conditions including mycotic, viral and bacterial infections like tuberculosis, leprosy, syphilis, Sarcoidosis, toxoplasmosis and secondary response in lymph nodes draining carcinomas or lymphomas [1]. Tuberculosis is a major health hazard in developing

countries. In India 1000 people per day or one per min account for mortality due to tuberculosis [2,3,4]. Extra pulmonary tuberculosis is on rise world over. It's a protean disease, which can virtually affect all organs [5]. The global increase is believed to be fuelled by HIV related immunosuppression. Peripheral lymph node involvement is the commonest form of extra pulmonary mycobacterial disease and cervical region is the most frequently affected [3,6,7]. Fine Needle Aspiration Cytology (FNAC) provides an inexpensive, quick and safe alternative to histopathology for the diagnosis of tuberculosis. It's a patient friendly technique and provides a good assessment of cytomorphological features. The presence of epithelioid granuloma forms the basis of diagnosis of tuberculosis. Demonstration of AFB in FNAC smears directly or by culture finally nails

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the etiology, even if epithelioid cell granulomas are not seen [2, 8]. The present study was undertaken to evaluate the diagnostic value of FNAC in Tuberculous lymphadenitis and the utility of Ziehl-Neelsen staining, to find the prevalence of tuberculosis in clinically suspected patients of tuberculous lymphadenitis and to analyze the cytomorphological features encountered in aspirates of tuberculous lymphadenitis and correlate them with acid fast bacilli positivity on Ziehl Neelsen stain and with the results of culture.

Materials and Method

The work represents the prospective study undertaken in the department of pathology at a tertiary care centre over a period of 2 yrs. Patient selection was clinically suspected tuberculous lymphadenitis irrespective of age, sex and site of lymphadenopathy. A detailed history of the patient was taken and a through clinical examination was performed.

Results

All the patients of lymphadenopathy with a clinical suspicion of tuberculosis were included in the study. A total of 170 cases have been studied out of which 117 were diagnosed as tubercular lymphadenitis, 41 reactive lesions, 3 metastatic deposits, single case of NHL, two cystic lesions and six cases were inconclusive. (Table 1)

Table-1: Relative frequency of Lymph node lesions.

Lymph Node Lesions	No.	Percentage
Tuberculous lymphadenitis	117	68.8
Reactive lymphadenitis	41	24.1
Metastatic deposits	3	1.7
Non Hodgkin's Lymphoma	1	0.5
Benign Cystic Disease	2	1.1
Inconclusive Aspirates	6	3.5

Amongst the cases of tuberculous lymphadenitis, 46 were male and 71 were females making M: F ratio 1:1.54 and a slight predominance were noted in second to fourth decades of life. Associated clinical features like low grade fever, anorexia, malaise and weight loss were present in 78 patients (66.6%). Ninety seven patients (82.9%) had lymphadenopathy of more than one week. Cervical lymphadenopathy was seen in 66.6%, followed by supraclavicular in 23%, axillary nodes in 16 cases and inguinal in 4.2%. (Table 2) Amongst the cervical lymph node 39 lymph node enlargements were noted in the head and around the external jugular vein. Single enlarged lymph nodes were noted in 58.9% of cases, followed by 27.3% showing multiple and discrete lymph nodes and matted lymph nodes were seen in total of 13.6% of cases.

Table-2: Site of Lymph Nodes.

Site of lymphadenopathy	Percentage of cases
Superficial cervical lymph nodes	38%
Supraclavicular lymph nodes	26%
Axillary lymph nodes	15%
Inguinal lymph nodes	5%
Generalized Lymphadenopathy	16%

The lymph nodes varied in size ranging from less than 1 cm to more than 2 cm. Majority of cases were recorded in the criterion of less than 1 cm (38.4%). A firm consistency of lymph node was observed in 79 cases (67.5%), while 38 cases (32.4%) were soft. The aspirate was admixed with blood in 58 cases (48.7%), while it was cheesy in 23 (19.6%) and purulent in 36 cases (30.7%).

In the current study, following cytomorphological features were observed and the cases of tuberculous lymphadenitis were grouped as follows:

- Group I : Epithelioid cell granulomas without necrosis
- Group II: Epithelioid cell granulomas with necrosis.
- Group III: Only necrosis
- Group IV: Necrosis with poly morphonuclear leukocytosis.

The incidence of Group I was the highest among all being 34.1%, thirty nine cases were included in Group II (33.3%), seven in Group III (5.9%) and 31 in Group IV (26.4%). ZN stain was performed in all the cases. The ZN positivity was 32.5% in Group I, 64.1% in Group II, 71.4% in Group III and 67.7% in Group IV. Overall ZN positivity was 54.7%. (Table 3).

Table-3: Correlation of Cytomorphological features and ZN stain.

Group	Total No of Cases	Total No. of ZN Positive Cases
Group I	40 (34.1)	13 (32.5%)
Group II	39 (33.3%)	25 (64.1%)
Group III	7 (5.9%)	5 (71.4%)
Group IV	31 (26.4%)	21(67.7%)

The ZN positivity was also compared with the nature of aspirate as 44.8% in material mixed with blood, 69.5% in cheesy aspirate and 61.1% in purulent material. Since the demonstration of AFB was diagnostic of tuberculosis, only the ZN negative cases were selected to be inoculated on LJ medium. Out of a total 53 ZN negative cases, culture was attempted in 40 cases only, because of inadequacy of the aspirate and the patient being unwilling for a second attempt at FNAC. Amongst these 40 cases, culture was positive in 10 cases, hence making culture positivity 25%.

Table-4: Culture Positivity.

Groups	ZN Negative Cases	Culture Attempted	No. of Positive Cultures
Group I	27	20	10%
Group II	13	9	55.5%
Group III	2	2	0
Group IV	11	9	33.3%

Among the 117 cases of tuberculous lymphadenitis, 16 were HIV seropositive. Regional lymph node involvement was seen in 12 cases and the other 4 showed generalized lymphadenopathy. Cervical nodes showed maximum presentation with a total of 8 cases, followed by axillary 3 cases and a single case was noted in inguinal region. A confirmative diagnosis of tubercular lymphadenitis was offered in 11 cases and the other five were ZN negative. Three amongst the latter showed epithelioid cell granulomas and the other two showed necrosis and poly morphonuclear inflammatory infiltrate and were inoculated for culture, amongst them one was culture positive.

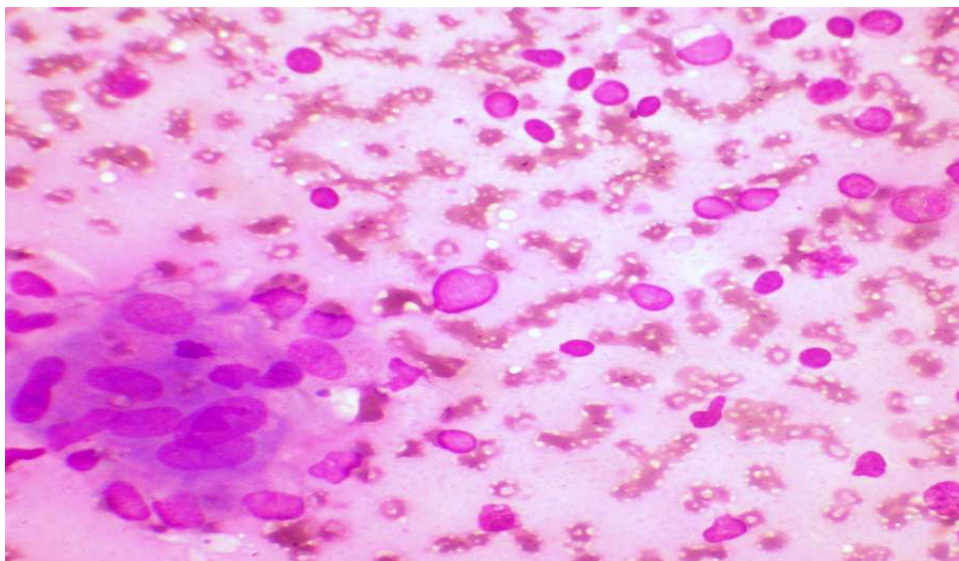
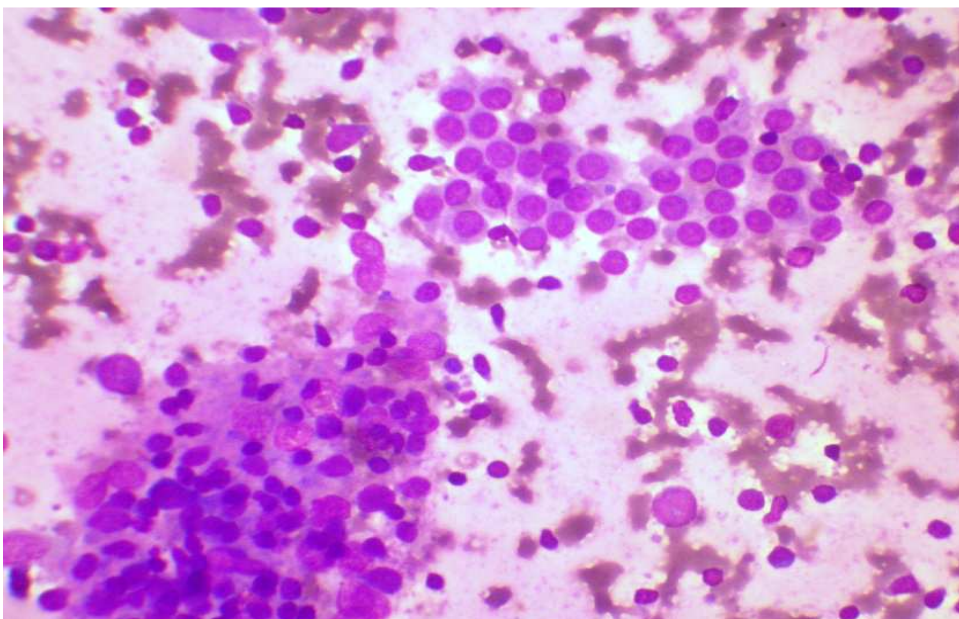
Cytomorphologically, the most common category encountered was Group I & IV, with 6 cases in each (37.5%), followed by group II with 3 cases (18.7%) and Group III with a single case (6.2%). Maximum ZN positivity (100%) was observed in Group II and Group III, followed by Group IV (66.6%) and Group I (50%). There were eleven ZN positive cases in total. (Table 5)

Table- 5: Correlation of cytomorphological Features and ZN stain.

Groups	No. of cases	ZN Positive Cases
Group I	6	50%
Group II	3	100%
Group III	1	100%
Group IV	6	66.6%

The ZN positivity was 100% in cheesy aspirates, followed by aspirates mixed with blood (66.6%) and purulent aspirates (60%). The overall ZN positivity was 68.7%, maximum being in Group. II & III and in cheesy aspirates.

The 5 ZN negative cases were inoculated on LJ medium (3 cases in Group I and 2 cases in Group IV). The culture positivity was 66.6% in Group I (2 out of 3 cases were culture positive) and 50% in group IV (1 out 2 cases was culture positive). The overall culture positivity was 60%.

**Figure-1: Epithelioid cell granuloma with few lymphocytes (Leishman 400x) Group I****Figure-2: Epithelioid cells with necrosis and inflammatory cells (Leishman 400x) Group II**

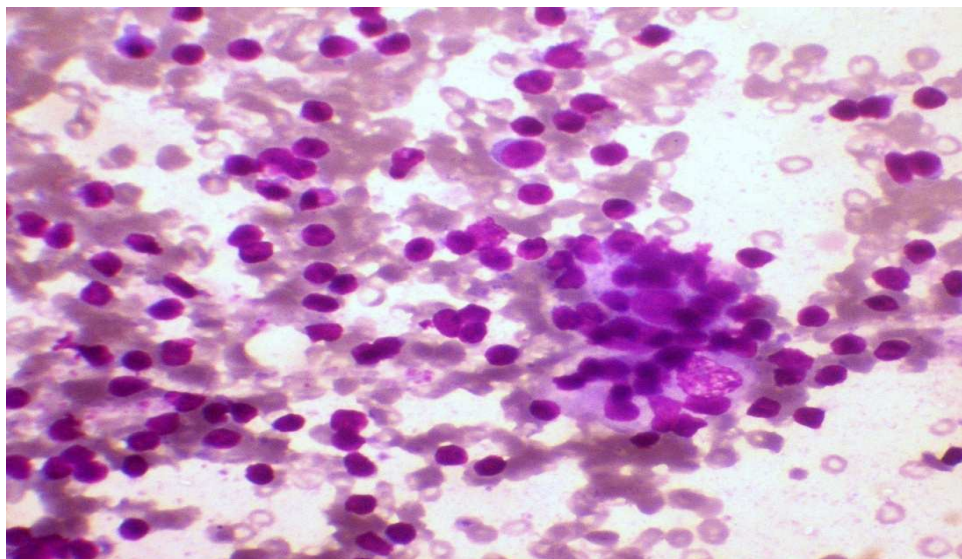


Figure-3: Epithelioid cells with necrosis and inflammatory cells (Leishman 400x) Group II

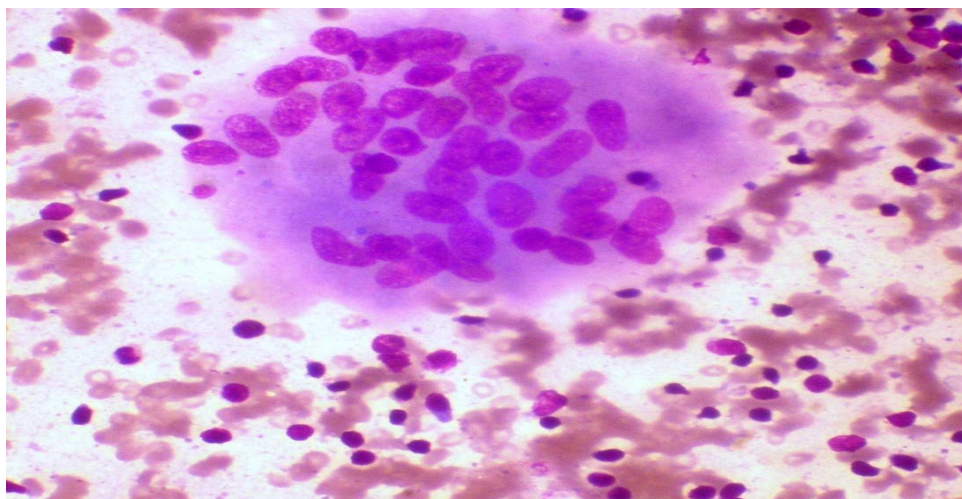


Figure- 4: Epithelioid cells with necrosis and mixed inflammatory cells polymorphs and lymphocytes (Leishman 400x) Group II

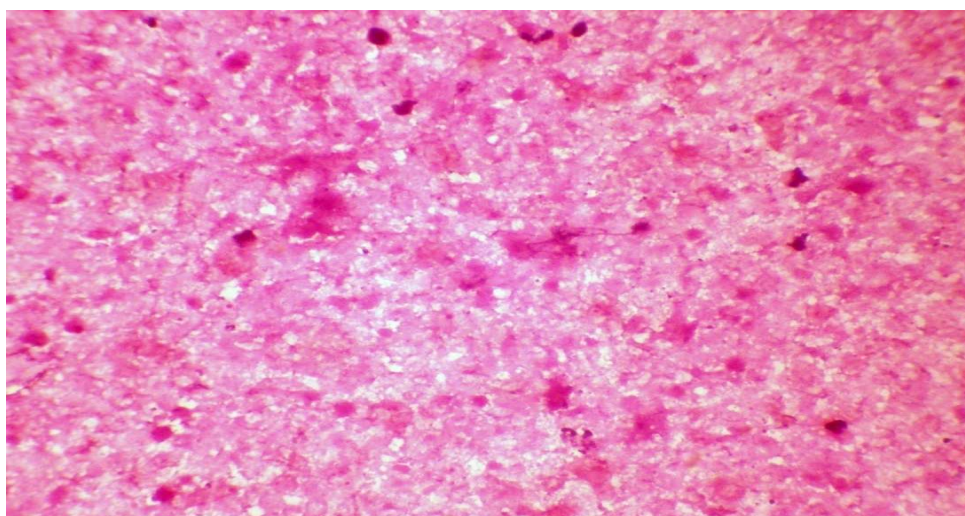


Figure-5: Necrosis (H&E 400 x) Group III

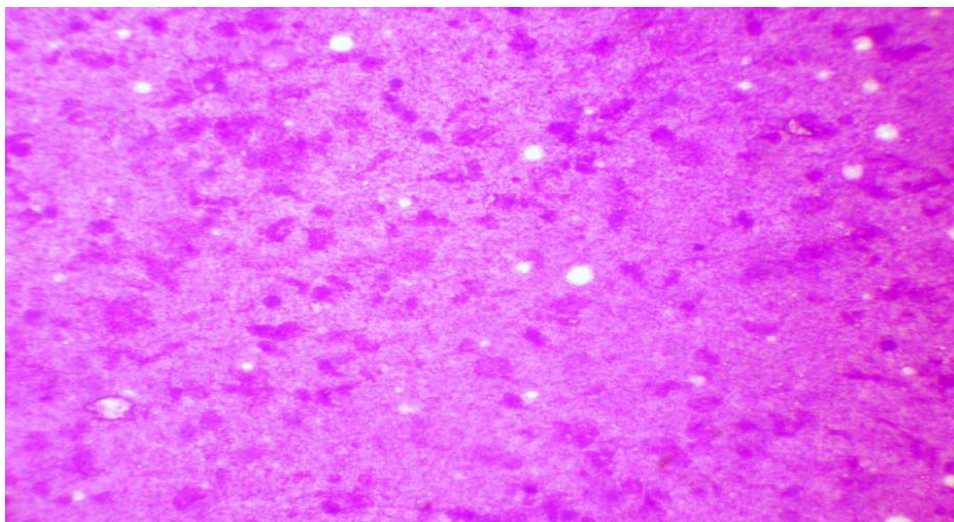


Figure-6: Necrosis (Leishman 400 x) Group III

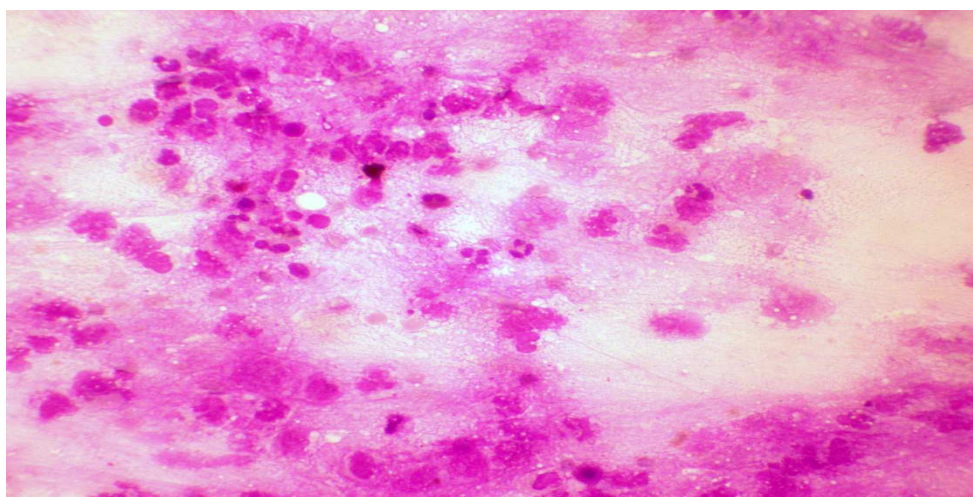


Figure-7: Necrosis and poly morpho nuclear leukocytes (Leishman 400x) Group IV

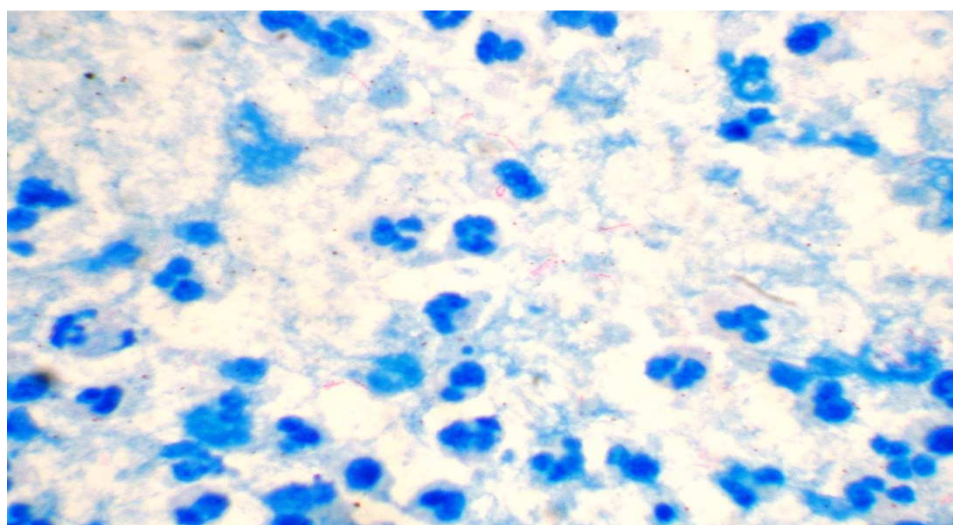


Figure-8: Acid Fast Bacilli (ZN Stain Oil immersion)

Patients were treated with anti tubercular treatment. A clinical follow up of the patients receiving treatment from hospital was carried out. It was found that out of total 117 cases of tuberculous lymphadenitis 47 were taking anti tubercular treatment. Amongst these 28 were ZN positive and 19 were negative for ZN. The material aspirated from 15 out of these 19 cases was inoculated on LJ media out of which 5 cases tested positive.

Discussion

Enlarged symptomatic lymphadenopathy most frequently involving head, neck, axilla and inguinal regions are relatively common clinical findings. The etiology ranges from simple reactive hyperplasia to tuberculosis and malignancies. Diagnosis of mycobacterial cervical lymphadenitis remains a diagnostic challenge for many clinicians despite current advances in diagnostic laboratory techniques. Fine needle aspiration is a simple, rapid and patient friendly diagnostic technique.

A total of 170 cases were aspirated out of which 117 (68.8%) were diagnosed as tubercular lymphadenitis. More than half the patients (56.4%) were in second and third decades and showed a female preponderance (M: F 1:1.5). These findings were in accordance with the studies conducted by Rajshekeran et al and Natraj et al.[9,10].

Associated clinical features such as low grade fever, weight loss, anorexia and malaise were present in 66.6% cases. In the present series, majority of the patients (58.9%) had single enlarged lymph node while in the study conducted by Seth and Donald multiple lymph node involvement was common. [11].

Considering the site superficial cervical lymph nodes were affected in most of the patients. Similar findings were observed by Kusum Verma et al [2], Rajshekeran et al [9], Das et al [12], Gupta et al [13] and Tripathy et al [14]. In contrast to above said findings Dandapat et al [7] and Madhusudan et al [15] observed upper deep cervical nodes to be more commonly involved.

The size of the lymph node ranged from 1 cm to 5 cm in diameter, with 38.4% being less than 1cm and the rest were more than 1 cm in diameter (61.5%). Bedi et al [16] and Ahmed et al [17] have suggested that smaller lymph nodes have less chances of being tuberculous and have experienced that lymph nodes up to 1 cm diameter are indicative of a reactive process. However in the present study lymph node < 1 cm was not accountable as a limiting factor.

A firm consistency was observed in 67.5% cases while the rest (32.4%) were soft. Approximately half (48.7%)

yielded an aspirate mixed with blood, followed by 30.7% cases in which purulent material was obtained and in 19.6% cheesy material was aspirated. The above finding correlated with the clinical stage and immunological status of the patients.

Cytomorphologically the incidence of group I was highest (34.1%), followed by Group II (33.3%). There were 5.9% cases in group III and 26.4% in group IV. The ZN positivity ranged from 32.5% to 71.4% maximum being in Group III. This was followed closely by Group IV (67.7%) and Group II (64.1%). Group I showed the lowest ZN positivity (32.5%). The overall ZN positivity was low (54.7%) and that can be explained as its defined by the clinical stage and immunological status of the patient and a minimum of 5 to 10000 bacilli/ml of the aspirate are required to get a positive result on ZN stain. [18,19] The said pattern of AFB positivity shows an inverse relation with the presence of epithelioid cell granulomas and a direct one with necrotic material, the reason being that liquefaction of a necrotic focus is associated with an enhanced proliferation of AFB. This pattern of ZN positivity was noted with the study conducted by Bibbo et al [20], Das et al [12] Gupta et al [13], Chakraborti et al [21] and Malakar et al [22]. Therefore, cases showing epithelioid cells without necrosis and a negative ZN stain are inconclusive cases. According to Singh et al in India the presence of epithelioid cell granulomas forms the basis of diagnosis of tuberculous lymphadenitis [1,23,24,27].

The demonstration of an AFB is diagnostic of tuberculosis, therefore only ZN negative cases were selected for culture. Out of the 53 ZN negative cases aspirates from 40 cases were inoculated on LJ media. The other 13 cases couldn't be inoculated on culture media because of insufficient material. Ten out of these 40 cases were positive, making the overall culture positivity 25%. In the present study low culture positivity could be attributed to paucity of organisms in the lesion as 10 -1000 bacilli/mm³ of the specimen is required to obtain a positive result on culture [25]. Other variable causes like the course of natural healing, history of previous anti tubercular treatment, unrepresentative lymph node aspirate and presence of

bacteriostatic substances. The low culture positivity in the present study is in accordance with the other studies carried out [2,8,26]. However, Natraj et al in their study reported culture positivity between 50 to 83.3%, regarding the same, culturing pus rather than the tissue may not show growth as bacilli in the pus are already killed by free fatty acids [10,27] Hence resulting in low culture positivity.

Tuberculosis is recognized as the one of the most common opportunistic infection seen in HIV seropositive patients presenting as pulmonary, extra pulmonary and disseminated disease. In the current study out of 117 patients of tuberculous lymphadenitis 16 were HIV positive amongst which 12 cases had presented with regional lymphadenopathy and remaining 4 showed generalized lymphadenopathy. These findings were in contrast to the study conducted by Jayaram et al [28] who found generalized lymphadenopathy in most of the cases. Associated pulmonary tuberculosis was seen in 31% and this was in accordance with study conducted by Rajshekhran et al [9].

Cytomorphologically no particular pattern was found to be specific for tuberculous lymphadenitis in seropositive patients as reported by others [29] although necrotizing lymphadenitis has been considered to be distinctive pattern for diagnosis of tuberculosis in AIDS patients by Ltaljos et al [31,33]. In our study a confident diagnosis of tuberculosis could be given in 11 cases. The overall ZN positivity in seropositive patients was 68.75% as compared to 54.7% in seronegative patients. Culture positivity was also higher in seronegative patients. Similar findings were observed by Nayak et al [29], Arora et al [34], Prasoon et al [30] and Radhika et al [35].

The low rates of positivity on culture and a long wait of 8 weeks before the report can be dispatched questions the actual efficacy and utility of this procedure and hence calls for better and rapid techniques with a higher sensitivity. Flurochrome staining using auramine / rhodamine stains has a popular approach with better sensitivity index. Flurochrome stained smears may be restained with ZN after examination without removing auramine.

On the other perspective despite its usefulness in the diagnosis of tuberculous lymphadenitis, fine needle aspiration cytology (FNAC) faces several limitations, and its sensitivity and specificity are not well

established. Advancements made in the field of molecular and immunological techniques have provided alternative approaches for rapid and reliable diagnosis. These include Polymerase Chain reaction (PCR) and DOT ELISA. In a study conducted by Aljafari et al, the diagnostic accuracy and limitations of FNAC were studied in comparison with conventional microbiological methods and polymerase chain reaction (PCR). Sixty patients with lymphadenopathy and a clinical diagnosis of tuberculous lymphadenitis were subjected to FNA. The aspirate was used for cytological examination, Ziehl-Neelsen staining, mycobacterial culture and PCR. PCR was performed using two sets of oligonucleotide primers for *Mycobacterium tuberculosis* and a single primer for *M.Bovis* species. In this study, the results of FNAC, microbiological methods and PCR correlated with the clinical outcome after follow-up for an average period of 24 months. Twenty-five cases (41.6%) were treated and responded well to anti-tuberculosis therapy, among them 17 were correctly diagnosed by FNAC (68%), eight by microbiological methods (32%) and 24 by PCR (96%).

Where PCR is considered the gold standard, FNAC predicted the correct diagnosis in 62% of cases with a high false negative rate (38%) due to the absence of granuloma/necrosis in smears from cases of early tuberculosis. In the latter group PCR proved to be the most valuable and a diagnostic success of 100% was achieved when FNAC and PCR were combined. In addition, PCR allowed immediate characterization of *M. tuberculosis* in the vast majority (96.2%) of cases in the study population [32].

In addition to being simple and inexpensive ELISA is very sensitive and rapid in diagnosing pulmonary and extra pulmonary tuberculosis. The sensitivity and specificity of ELISA has been found to be 95.4% and 88.5% respectively. In comparison to PCR, ELISA is more sensitive and less specific mainly because of its high specificity index of MOTT antigens detection PCR can demonstrate mycobacterial DNA in lymph nodes even in culture and smear negative cases. Therefore, ELISA is a rapid, easy and cost effective screening test which can be used in AFB negative cases of tuberculous lymphadenitis.

Conclusion

Tuberculous lymphadenitis was one of the most common types of lymphadenitis encountered in our outpatient department. In the present study a total of

170 clinically suspected patients of tuberculous lymphadenitis underwent FNAC and 117 were diagnosed as tuberculous lymphadenitis based on cytomorphological features, ZN staining, culture examination and clinical follow up. The low ZN and culture positivity should not be considered to be a disadvantage of FNAC in diagnosing tuberculous etiology as epithelioid cell granulomas were sufficient to establish the diagnosis and can be useful as first line diagnostic tool in diagnosing tuberculous lymphadenitis coupled with ZN staining and culture examination. However the diagnostic accuracy of FNAC can be enhanced by combination of ELISA, PCR and molecular techniques.

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