FINE NEEDLE ASPIRATES OF ENLARGED LYMPH NODES. ROLE OF ZIEHL NEELSEN STAIN IN THE DIAGNOSIS OF TUBERCULOSIS.

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ABSTRACT

Background: Lymph node tuberculosis (LNTB) is the leading form of extra pulmonary tuberculosis and common in our country. Acid Fast Bacilli (AFB) detection by Ziehl-Neelsen (ZN) method in the fine needle aspirate from these lymph nodes is confirmatory.

Objective: To assess the diagnostic utility of Ziehl-Neelsen staining in lymphadenopathy cases, since it is commonly used method in the diagnosis of TB.

Methods: A total of 150 cases of tuberculous lymphadenitis diagnosed by fine needle aspiration cytology (FNAC) were included in this study. Smears of the aspirates from all these cases were stained with Ziehl-Neelsen stain and examined by the light microscope. Results of Acid Fast Bacilli by Ziehl-Neelsen and cytomorphology by fine needle aspiration cytology were compared.

Results: The classic cytomorphological pattern of tuberculous lymphadenitis (epithelioid granulomas, Langhan's giant cells and caseous necrosis) by Fine Needle Aspiration Cytology was seen in 150 cases. These were divided into three categories among which maximum cases (80.95%) were confirmed in the smears showing caseous material only. By concluding the results routine Ziehl-Neelsen staining confirmed Acid Fast Bacilli in 90 (60 %) cases.

Conclusion: Ziehl-Neelsen staining if coupled with fine needle aspiration cytology of suspected tuberculous lymphadenopathy cases can confirm diagnosis of tuberculosis.

Keywords: Ziehl-Neelsen stain; Tuberculosis (TB); Fine needle aspiration cytology (FNAC); Acid-fast bacilli; lymph node.

INTRODUCTION

Tuberculosis (TB) remains a major health problem of the world[1]. World wide it is the second leading cause of death, killing about 2 million people per year. Most cases of the TB occur in developing countries of the world where there is poverty, lack of health facilities and overcrowding.[2]

Lymphadenopathy is a common presentation of many diseases.[3] Among these diseases TB is the most frequent cause of this condition in developing countries.[1]

To diagnose this condition a simple investigation like FNAC is required. The aspirated material, in this way, besides cytomorphologic examination can also be used for other ancillary tests like ZN staining, culture etc.[3]

As far as Ziehl-Neelsen (ZN) method for acid-fast bacilli (AFB) detection is concerned, it plays an important role in the diagnosis of lymph node TB (LNTB) and also in monitoring its treatment. Main demerit of it is its low sensitivity which ranges from 9 to 46%.[4] Despite this low sensitivity, it remains the most frequently used diagnostic test for TB in the developing countries.[5]

Other sensitive tests like culture and PCR can also be applied for this purpose but due to their limitations they are not used commonly. Culture is time consuming and also requires special safety measures in laboratories. PCR technique cannot be used routinely due to its high cost in developing countries where TB prevalence is high. Serological techniques have also been introduced for this purpose but due to their low specificity and sensitivity cannot be used with full confidence.[4] These specialized tests are used when the results of FNAC and ZN staining are equivocal inspite of strong clinical suspicion.[6]

In short if sensitivity of ZN staining and microscopy is improved, it can be used not only for diagnosis of LNTB but can also be used as a valuable tool for TB control programmes in the world.[4]

One thing about AFB positivity to be noted is that

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they are found mostly in cheesy and purulent aspirates which are usually labeled and disposed of as acute suppurative lymphadenitis.[7] So in such situations ZN staining of the smears must be done. If still there is no AFB positivity, some quantity of the aspirate should be subjected to culture on Lowenstein Jensen medium to improve the diagnostic accuracy of the FNAC and ZN method.[4]

The rationale of this study is to evaluate the diagnostic role of ZN staining when combined with fine needle aspiration cytology in the routine diagnosis of TB in lymphadenopathy cases, frequently reporting to hospitals in our country.

MATERIALS & METHODS

This observational study was conducted at the laboratory of Khalifa Gul Nawaz teaching hospital Bannu from 1st October 2013 to 30th September 2014. The patients of lymphadenopathy after initial clinical examination by the clinicians were referred to the laboratory for Fine Needle Aspiration Cytology for cytological evaluation of their enlarged lymph nodes. After taking informed consent enlarged lymph nodes of the patients were aspirated using 23 gauge needle attached to 10 ml disposable syringe taking strict aseptic measures. During each pass the needle was moved to and fro several times while maintaining the negative pressure. The aspirate in each case was used to make two smears, one for cytomorphological examination after fixation in 95% alcohol and staining with H and E stain and the other for AFB detection after air drying and staining with ZN stain. If there was granulomatous inflammation in the lymph node aspirate on cytomorphological examination, then the other slide was stained with ZN stain for AFB otherwise discarded. This ZN stained smear was examined for AFB under oil immersion (1000X) using light microscopy. If AFB were seen on direct microscopy, the case was confirmed to be due to tuberculosis.

All the cases showing AFB after ZN staining were isolated and counted. The data was interpreted by using Microsoft Excel. Diagnostic efficacy of ZN staining was determined in the form of percentages.

RESULTS

General characteristics of the patients: During the study period of one year a total of 150 cases were diagnosed by FNAC to have chronic granulomatous lymphadenitis. There were no complications of fine needle aspiration except pain at the site of aspiration.

Out of 150 patients of chronic granulomatous lymphadenitis, 80 (53.33%) were females and 70 (46.67%) were males ranging in age from 02 years to 70 years with a mean of 28.5 years (Table 1). Out of these, 115 (76.67%) cases belonged to poor families regarding their socio economic condition (Table 2). Profession wise most of the patients were housewives and students comprising 55 (36.67%) and 48 (32%) cases respectively.

Table 1: Age and sex wise distribution of tuberculous lymphadenopathy

Age group	Male	Female	Total
1-10	07	10	17
11-20	13	09	22
21-30	19	26	45
31-40	14	19	33
41-50	09	09	18
51-60	05	06	11
61-70	03	01	04
Total	70	80	150

Table 2: Distribution of patients according to economic condition

Economic condition	Frequency	Percentage
Poor	115	76.67
Mediocre	20	13.33
Good	15	10
Total	150	100

Table 3: Confirmation of cases by ZN staining (n=150)

AFB confirmation status	Number & % age of cases
Confirmed	90 (60%)
Not confirmed	60 (40%)
Total	150 (100%)

The most frequently involved region by lymphadenopathy was cervical; where left cervical area was involved in 88 (58.67%) cases while right cervical area in 47 (31.33%) cases. The next frequently involved sites were axillary and supraclavicular regions comprising of 7 (4.67%) and 5 (3.33%) cases respectively. There were also 2 (1.33%) cases of bilateral cervical and 01 (0.67%) case of inguinal lymphadenopathy. Regarding the duration of lymphadenopathy, 101 (67.33) patients reported within 6 months while 49 (32.67%) patients reported after 6 months to hospital for complaint of their lymphadenopathy.

Cytomorphologic features of the aspirates and its categorization: The cytomorphologic diagnosis was divided into three main categories, the first having

Table 4: AFB positivity by ZN method in different cytomorphologic categories

Cytomorphologic category	Number of cases in each category	Number of positive cases in each category	Percentage
Caseous material without granulomas	42	34	80.95
Granulomas with inflammatory cells	29	21	72.41
Granulomas with hemorrhagic background	79	35	44.30
Total	150	90	60

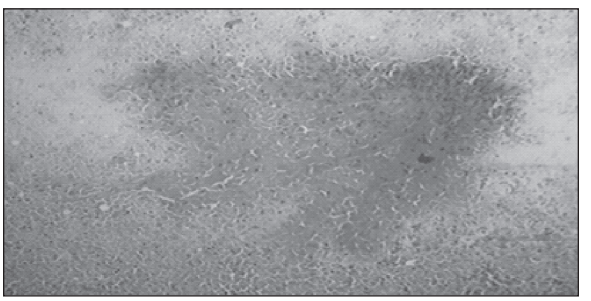

Figure 1: A photomicrograph of a smear prepared from FNA of a lymph node showing an eosinophilic acellular granular caseous necrotic material. (H & E Stain; X100).

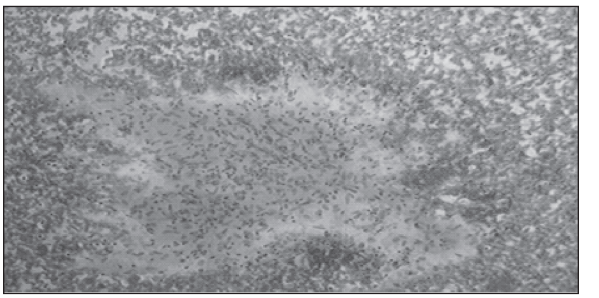

Figure 3: A photomicrograph of a smear prepared from FNA of a lymph node showing Granuloma in a hemorrhagic background (H & E Stain; X100)

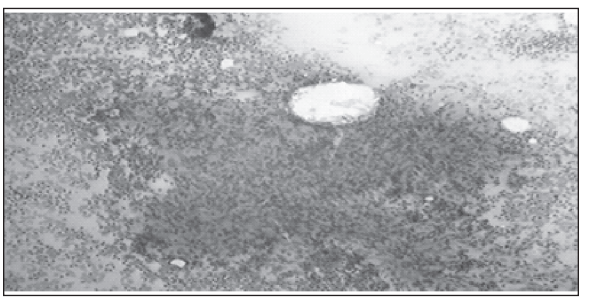

Figure 2: A photomicrograph of a smear prepared from FNA of a lymph node showing Granuloma in the centre and scattered lymphocytes and neutrophils (inflammatory cells) in the background (H & E Stain; X100)

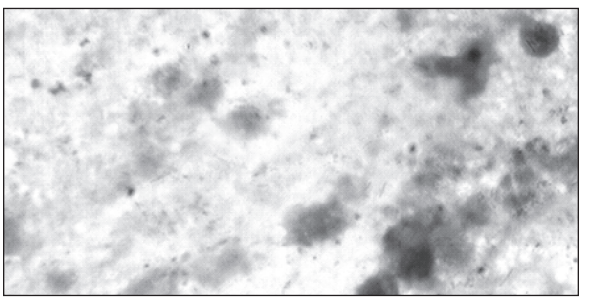

Figure 4: Photomicrograph of Acid Fast Bacilli seen as pink straight and slightly curved rods. (Z.N Stain; X1000)

caseous material only (figure 1), the second showing epithelioid granulomas with inflammatory cells (figure 2) and the third showing granulomas with hemorrhagic background (figure 3)

In the first category there were 42 (28 %) cases. The second category contained 29 (19.33 %) cases while there were 79 (52.67 %) cases in the third category.

Results of ZN staining: Smears of all the 150 cases were stained with ZN stain. Patients whose smears showed AFB after ZN staining (figure 4) were confirmed to have tuberculous lymphadenopathy. Thus out of these 150 cases, AFB were seen in 90 (60%) cases by direct microscopy (Table 3)

Regarding different cytomorphological categories, out of 42 cases having caseous necrotic material without granulomas AFB were detected in 34 (80.95%) cases by ZN method. In the second category of 29 cases having granulomas with a background of inflammatory cells, AFB was found in 21 (72.41%) cases. In the third category of granulomas with hemorrhagic background only 35 (44.30) out of 79 cases were confirmed for presence of AFB by ZN staining method (Table 4). Thus AFB positivity by ZN method was 80.95%, 72.41 % and 44.30% in the first, second and third cytomorphologic categories respectively. Combining results of all the three categories, AFB positivity came to be 60%.

DISCUSSION

Lymphadenitis is the most common extra-pulmonary presentation of extra pulmonary tuberculosis.[8] Diagnosis of the lymph node TB can be suspected from its signs and symptoms. However, to start anti-tuberculous drugs a morphological diagnosis by FNAC is necessary. FNAC is a non invasive, simple, cheap and sensitive technique in the diagnosis of TB in tuberculous lymphadenopathy cases.[9]

If cytological diagnosis by FNAC is confirmed by ZN stain for AFB detection then it becomes an authentic and valuable diagnosis of tuberculous lymphadenitis and treatment can be initiated without any doubt.[4]

Age wise, tuberculous lymphadenopathy cases range from an early to advanced age. In our study, the youngest patient was two-year-old and the oldest was 70 years old well correlating with a study conducted by Ahmad et al where the age of the youngest patient was two-years while 95 years of the oldest one.[6] Majority (75%) of the cases in our study were in the age range of 20-40 years of age. Other studies by Ergete et al[10] and Purohit et al [11] have also showed similar age distribution.

Male to female ratio in our study was 1:1.4 showing a mild female predominance. Similar ratio was noted by Ergete et al[10] and Purohit et al[11] while male predominance was noted by Ahmad et al[6] and Rajsekaran et al.[12] Reason of this high number of females to be suffering from tuberculosis is that the females in developing countries have weak immune system as they usually do more work, consume less and low quality food and bear the high nutritional and physical burden during repeated pregnancies and lactation.[13, 14, 15]

Cervical region was involved in 137 (91.33%) cases in our study. This finding well correlated with that found by Bezabih et al[16] and Sharma et al.[17] They observed cervical involvement in 74.2% and 88.2 % of cases respectively.

In our study, maximum positivity of AFB (80.95 %) was observed in cheesy aspirates well supported by another study conducted by Nidhi et al[9] where they found 85.5% AFB positivity in the smears showing necrosis. The reason is that AFB concentration is more in the aspirates showing purulent or necrotic material both by gross or microscopic examination.[18]

The overall positivity of the smears for AFB in our study was 60% which correlates closely with the results of other studies by Nidhi et al (71.02%)[9] and Ahmad SS et al (46.4%)[7].Other studies conducted by Lakhey M et al[19], Mistry Y et al[18], Aftab R et al[5] and Chandrasekher B et al[4] showed quite opposite results i.e.31.74%, 229%, 31% and 12.5% respectively. The reason may be that there were more cheesy and purulent aspirates in our study as well as conducted by Nidhi P et al[9] and Ahmad SS et al[7] while less number of such aspirates in the other mentioned studies.

CONCLUSION

ZN staining is confirmatory test for TB in the aspirates from lymphadenopathy cases. Therefore fine needle aspirates of the lymph nodes showing caseous or granulomatous inflammations, if subjected to ZN stain for AFB detection, can make the diagnosis of TB accurate to the level that lengthy, expensive and toxic Anti TB treatment can be started with full confidence. Most importantly if both FNAC and ZN staining are accompanied in all suspicious Tuberculous cases, we can save many patients of lymphadenopathy from undue surgical biopsy.

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