ROLE OF FINE NEEDLE ASPIRATION CYTOLOGY AND ZIEHL NEELSEN STAINING IN DIAGNOSIS OF PATIENTS WITH SUSPECTED TUBERCULOUS LYMPHADENITIS

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ABSTRACT

Objective: To determine the role of Fine Needle Aspiration Cytology (FNAC) and Ziehl Neelsen staining in diagnosis of patients with suspected tuberculous lymphadenitis

Methods: This cross-sectional study was carried out in the department of Pathology, Khyber Medical College Peshawar. A total of 125 patients clinically suspected of having tuberculosis with well palpable and enlarged cervical lymph node were included. Fine needle aspiration cytology was performed according to standard protocols. Cytological smears were examined under microscope using hematoxylin and eosin stain (H and E) for the presence of granuloma, necrosis, Langhans giant cells, plasma cells, lymphocytes, macrophages, and neutrophils. Smears stained with ZN stain were examined under oil immersion objective for presence of Acid Fast Bacilli (AFB).

Results: Among 125 cases there were 33% male and 67% female patients with an age range of 1-55 years. Based on cytomorphological findings, out of total 125 lymph node aspirates, 89% (111/125) cases revealed cytomorphological features suggestive of tuberculous lymphadenitis. Out of 111 cases showing cytological picture of tuberculous lymphadenitis, most common cytomorphological pattern in our study was epithelioid granulomas with caseous necrosis in 69% (77/111) of the cases, followed by necrosis only without epithelioid granulomas 19% (21/111) cases and epithelioid granuloma without necrosis in 12% (13/111) of the cases. Microscopic examination using Ziehl Neelsen stain revealed AFB positivity in 65% cases (81/125) with high frequency (54%) among cases with caseous necrosis as compared to cases without necrosis (11%).

Conclusion: Based on our study findings we conclude that in patients presenting with cervical lymphadenopathy especially in developing countries where tuberculosis is endemic and where advanced diagnostic facilities are not available, combination of fine needle aspiration cytology and Ziehl Neelsen staining is an alternate, non-invasive and cost-effective out-patient technique for initial diagnosis of tuberculous patients.

Keywords: FNAC, Tuberculous lymphadenitis, Zeihl Neelsen Stain

INTRODUCTION

Tuberculosis (TB), a chronic communicable disease continues to be an important public health issue affecting approximately one third of the world's population especially in the developing countries[1]. In 2014, tuberculosis was responsible for the death of nearly 1.5 million people, representing a global mortality impact larger than any other infectious disease[2]. Although lungs (pulmonary TB) are the main site of infection by *Mycobacterium* TB, extrapulmonary infection commonly involves lymph nodes, spine, or brain[3]. Lymphadenopathy is one of the most common presentation of extrapulmonary tuberculosis[4]. Precise identification of TB is imperative as satisfactory results can be achieved with anti-tuberculous chemotherapy without surgical removal of the involved lymph nodes[5]. Recently, FNAC of the cervical lymph nodes has provided an alternative, non-invasive and reliable technique to collect material for both bacteriological and cytomorphologic examination[6]. An important consistent indicator of cervical mycobacterial infection is conventional Ziehl Neelsen (ZN) stain of a smear from the aspirated specimen[7]. A characteristic feature of tuberculous infection is cytomorphological appearance of granulomatous inflammation[8]. Culture is important for diagnostic confirmation, however; it takes 1 to 4 weeks to obtain a positive result, and its sensitivity is also comparatively low in paucibacillary conditions[9]. Cytologically granulomatous inflammation is clustering of epithelioid cells in a lymphoid background[10]. Epithelioid cells are cells of the mononuclear phagocyte system having elongated nuclei, fine granular chromatin with small nucleoli and are mainly associated with intense immunological activity[11]. Multinucleated and Langhans type of giant cells as well as caseous necrosis may or may not be present. Granulomatous inflammation is not only a feature of tuberculosis but also a variety of other bacterial, fungal and protozoal infections and other granulomatous diseases[12]. TB is highly prevalent in developing countries and maximum number of

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cases presenting as granulomatous inflammation on cyotological or histological examination are presumed to be cases of TB and treated accordingly[13]. FNA has been used as the first line diagnostic step in making a diagnosis of tuberculous lymphadenitis. Definitive diagnosis of tuberculosis by FNA rests on cytomorphological demonstration of epithelioid granulomas with or without caseous necrosis along with AFB staining. In tuberculous lymphadenitis, FNAC smears sometimes reveal only caseous necrosis without epithelioid cell granuloma which can pose a diagnostic problem. FNAC is an effective out-patient procedure, has high accuracy; with a sensitivity of over 90% and a specificity of 100%[14]. ZN positive rate from the aspirated material falls within the reported range of (12–75%)[15]. ZN stain in smears from aspirates diagnosed to be tuberculous in etiology needs to be evaluated in the laboratory to confirm the cytomorphologic diagnosis of TB.

This cross-sectional study presents an analysis of the findings from 125 cases of suspected tuberculous patients in Peshawar. The purpose of this study was to evaluate the role of FNAC and ZN stain for AFB in the diagnosis of tuberculous lymphadenitis and to determine correlation of the cytomorphologic diagnosis and subsequent demonstration of AFB in the smears.

MATERIAL AND METHODS

This cross-sectional study was carried out at Khyber Medical College Peshawar from August 2012 to July 2013. A non-probability consecutive sampling technique was used to select the study participants. A total of 125 clinically suspected patients of tuberculosis with cervical lymphadenopathy were included after taking their consent. Baseline parameters including Full Blood Count (FBC), Erythrocyte Sedimentation Rate (ESR), Mantoux test, and chest radiograph were performed. Demographic data, relevant clinical history, and physical examination findings of each patient were recorded on a predesigned questionnaire. Both males and females with well palpable and enlarged cervical lymph nodes were included. Those patients were excluded who had very small or non-palpable lymph nodes or were known cases of malignant, allergic, or skin disorders. FNA was performed according to standard protocols using 20-22-gauge needle with a 10ml attached syringe. For aspiration, needle was moved many times throughout the lesion. Initially four smears were prepared from aspirated material and air dried. The pattern was described as caseous for yellow-white aspirate, pus for greenish yellow, and blood mixed for hemorrhagic material. Two smears were used for cytomorphological evaluation using hematoxylin and eosin stain (H and E), which were fixed directly in absolute ether and alcohol. Cytological smears were examined under microscope for the presence of granuloma, necrosis, Langhans giant cells, plasma cells, lymphocytes, macrophages, and neutrophils. Smears stained with ZN stain were examined under oil immersion objective for AFB. Presence of sheets of epithelioid cells with lymphocytes and plasma cells with or without multinucleated giant cells were diagnosed as granulomatous lymphadenitis, and eosinophilic granular material containing inflammatory cells and necrotic cell debris was defined as caseous necrosis. The remaining two smears were examined for AFB positivity using ZN stain. ZN stained smears were examined for AFB under oil immersion (100 X) using light microscopy which appeared as pinkish/red, thin curved rod-shaped bacterium measuring 0.5 to 3 μm against a bluish background. SPSS version 21 was used for data analysis and results were presented as tables and graphs.

RESULTS

A total of 125 patients suspected of having tuberculosis were included in the study. There were 33% male and 67% female patients with an age range of 1-55 years. Table 1 shows age and gender wise distribution of study participants. Chest X-ray showed evidence of active pulmonary lesions or mediastinal lymphadenopathy in 21% cases, while 79% of cases had normal chest radiograph findings. Sixty one percent of the patients were in the age range of 16-40 years, out of which 71% were female and 75% were male patients. Out of 125 cases, ninety-two cases (74%) had a positive Mantoux test. Posterior triangle nodes were affected in 43% of cases, followed by anterior triangle and supraclavicular in 24% and 11% of cases respectively. The pattern of aspirate was purulent or caseous in 74% of the cases, while it was hemorrhagic and clear in 24% and 1% of cases respectively. Based on cytomorphological findings, out of total 125 lymph node aspirates, 89% (111/125) cases revealed cytomorphological features suggestive of tuberculous lymphadenitis, while 11% (14/125) were found negative for granulomatous lesions. Out of 125 cases showing cytological picture of tuberculous lymphadenitis most common cytomorphological pattern in our study was epithelioid granulomas with caseous necrosis in 69% (77/111) of the cases, followed by necrosis only without epithelioid granulomas 19% (21/111) cases and epithelioid granuloma without necrosis in 12% (13/111) of the cases (Table 2). Microscopic examination using

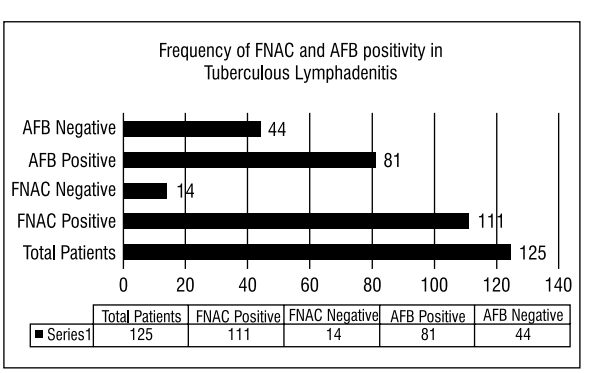

Figure 1. Shows comparison of FNAC and Zeihl Neelsen stain for AFB positivity in patients (n=125) suspected of having Tuberculous lymphadenitis

Table 1: Distribution of patients according to age and gender (n=125)

Age in Years	Female (n=41)	Male (n=84)
1-15 years	9 (22%)	16 (19%)
16-40 years	29 (71%)	63 (75%)
40-55 years	3 (7%)	5 (6%)

Table 2: Distribution of cases according to cytomorphological pattern on FNAC and AFB positivity on Ziehl Neelsen staining

Features	Number	Percentage	AFB Positivity
Epithelioid granulomas with caseous necrosis	61	69%	28 (23%)
Epithelioid granulomas without caseous necrosis	17	19%	14 (11%)
Caseous necrosis only without epithelioid granuloma	11	12%	39 (31%)

ZN stain revealed AFB positivity in 65% cases (81/125), while 35% cases (44/125) were found AFB negative. Frequency of AFB positivity was high in patients with cytological pattern of caseous necrosis (54%) with and without epithelioid granulomas as compared to cases without necrosis (11%). Comparison of FNAC and ZN stain for AFB positivity is demonstrated in Figure 1.

DISCUSSION

TB is a widespread bacterial infectious disease, commonly caused by *Mycobacterium* TB. It is estimated to have a prevalence of almost 8 million individuals each year, and an annual mortality rate of 3 million due to complications of the disease[16]. In the recent years frequency of mycobacterial lymphadenitis has increased due to increase in the incidence of mycobacterial infection worldwide. TB lymphadenitis is the most common clinical presentation of extrapulmonary TB accounting for approximately 15 to 20 per cent of all cases of TB. For the diagnosis of TB lymphadenitis, a high level of suspicion is required. A detailed history, general physical and radiological examination, Mantoux test, demonstration of AFB on ZN stain, and FNAC are important for arriving at an initial diagnosis of TB lymphadenitis. Based on provisional diagnosis clinicians can initiate early treatment before a final diagnosis can be reached on culture and biopsy. In Pakistan the burden of TB is increasing due to poor socioeconomic conditions, inappropriate health care delivery conditions and low literacy rate. The conventional methods are slow and time consuming and there is need to establish newer diagnostic techniques that are rapid, safe and cost effective. FNAC is a well-established technique with high level of accuracy in diagnosing TB lymphadenitis especially in endemic areas like Pakistan where diagnosis can be made with confidence if cytomorphological criteria is met. In the present study we aimed to determine the role of FNAC and ZN staining in the diagnosis of TB lymphadenitis.

In the present study the highest frequency of TB lymphadenitis was found among age group 16-40 years with an increased preponderance in female patients. About 67% of the cases were females as compared to only 33% of male cases with a male to female ratio of 2:1. Although previous studies have shown an increased frequency of pulmonary TB among women than men, TB lymphadenitis was reported more commonly in female patients as compared to male patients[17]. The reason for increased risk among women is not clear, one possible explanation could be occupational or cultural practices (e.g milking cows, consumption of unpasteurized milk) predisposing them to oropharyngeal exposures. Other contributing factors might include hormonal influences and patients with poor nutritional status and a compromised immune system (e.g HIV co-infection)[18]. Involvement of cervical lymph nodes is reported in majority (45%–70%) of cases with 12%–26% in the supraclavicular region[19]. Consistent with reports from previous studies we found posterior triangle nodes involvement in 43% of cases, followed by anterior triangle and supraclavicular region in 24% and 11% of cases respectively.

Based on cytomorphological findings, out of total 125 lymph node aspirates, 89% cases revealed cytomorphological features suggestive of tuberculous lymphadenitis. ZN stain was found to be positive for AFB in 65% of the cases, which are in accordance with previous studies. The most common cytomorphological pattern in our study was caseous necrosis with or without epithelioid granulomas. Interestingly we also observed a higher rate of AFB positivity (54%) in cases with necrosis both with or without epithelioid cells. In case of epithelioid granuloma without necrosis, frequency of AFB positivity was low (11%). These findings are to some extent in line with previous studies. Gupta *et al.*[20], reported a higher rate of positivity (75%) in smears with necrosis as compared to smears with no necrosis. In the same study frequency of AFB positivity on ZN stain was 65% which is comparable to results of current study with overall AFB positivity as 65%. In contrast Aggarwal *et al*. observed cytomorphological findings suggestive of TB lymphadenitis in only 41.3% of cases and a relatively low (19.6%) rate of AFB positivity in ZN stained smears[21]. Therefore, FNAC can be a beneficial and convenient first line investigation in diagnosis of patients with TB lymphadenitis. In patients presenting with lymphadenopathy especially in developing countries where TB is endemic and where advanced diagnostic facilities are not available, combination of FNAC and ZN staining is an alternate non-invasive and cost- effective technique

for initial diagnosis and early treatment of tuberculous patients.

REFERENCES

1. Taghizade Moghaddam H EMZ, Khademi Gh, Bahreini A, Saeidi M. Tuberculosis: Past, Present and Future. Int J Pediatr. 2016;4(1):1243-54.
2. Zumla A, George A, Sharma V, Herbert RH, Baroness Masham of I, Oxley A, et al. The WHO 2014 global tuberculosis report--further to go. The Lancet Global health. 2015;3(1):e10-2.
3. Golden MP, Vikram HR. Extrapulmonary tuberculosis: an overview. American family physician. 2005;72(9):1761-8.
4. Thakur B, Mehrotra R, Nigam JS. Correlation of various techniques in diagnosis of tuberculous lymphadenitis on fine needle aspiration cytology. Pathology research international. 2013;2013:824620.
5. Campbell IA, Dyson AJ. Lymph node tuberculosis: a comparison of various methods of treatment. Tubercle. 1977;58(4):171-9.
6. Singh ML, I A. Poddar, CK. . Efficacy of fine-needle aspiration technique, Ziehl-Neelsen stains and culture (BACTEC) in diagnosis of tuberculous lymphadenitis in tertiary care hospital, Gaya, India. J Evid Based Med. 2017;4(70):4164-9.
7. Weiler Z, Nelly P, Baruchin AM, Oren S. Diagnosis and treatment of cervical tuberculous lymphadenitis. Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons. 2000;58(5):477-81.
8. Wamala D, Asiimwe B, Kigozi E, Mboowa G, Joloba M, Kallenius G. Clinico-pathological features of tuberculosis due to Mycobacterium tuberculosis Uganda genotype in patients with tuberculous lymphadenitis: a cross sectional study. BMC clinical pathology. 2014;14(1):14.
9. Nhu NT, Heemskerk D, Thu do DA, Chau TT, Mai NT, Nghia HD, et al. Evaluation of GeneXpert MTB/RIF for diagnosis of tuberculous meningitis. Journal of clinical microbiology. 2014;52(1):226-33.
10. Chand P, Dogra R, Chauhan N, Gupta R, Khare P. Cytopathological Pattern of Tubercular Lymphadenopathy on FNAC: Analysis of 550 Consecutive Cases. Journal of clinical and diagnostic research : JCDR. 2014;8(9):FC16-9.
11. Borah AD, B. A study correlating positivity of acid fast bacilli (AFB) in cytologic aspiratesshowing granulomatous inflammation. J Evolution Med Dent Sci. 2016;5(51):3278-82.
12. Kumar SN, Prasad TS, Narayan PA, Muruganandhan J. Granuloma with langhans giant cells: An overview. Journal of oral and maxillofacial pathology : JOMFP. 2013;17(3):420-3.
13. Guler M, Simsek A, Ofluoglu R, Celenk Erguden H, Capan N. Are all granulomatous lesions tuberculosis? Respiratory medicine case reports. 2012;5:42-4.
14. Muyanja D, Kalyesubula R, Namukwaya E, Othieno E, Mayanja-Kizza H. Diagnostic accuracy of fine needle aspiration cytology in providing a diagnosis of cervical lymphadenopathy among HIV-infected patients. African health sciences. 2015;15(1):107-16.
15. Gupta AK, Nayar M, Chandra M. Critical appraisal of fine needle aspiration cytology in tuberculous lymphadenitis. Acta cytologica. 1992;36(3):391-4.
16. Hegde S, Rithesh KB, Baroudi K, Umar D. Tuberculous lymphadenitis: early diagnosis and intervention. Journal of international oral health : JIOH. 2014;6(6):96-8.
17. Fontanilla JM, Barnes A, von Reyn CF. Current diagnosis and management of peripheral tuberculous lymphadenitis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2011;53(6):555-62.
18. Aaron L, Saadoun D, Calatroni I, Launay O, Memain N, Vincent V, et al. Tuberculosis in HIV-infected patients: a comprehensive review. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2004;10(5):388-98.
19. Polesky A, Grove W, Bhatia G. Peripheral tuberculous lymphadenitis: epidemiology, diagnosis, treatment, and outcome. Medicine. 2005;84(6):350-62.
20. Gupta R DD, Suri J. Study of incidence and cytomorphological patterns of tubercular lymphadenitis in a secondary care level hospital of Jammu Region. Indian J Pathol Oncol 2015;2:161-4.
21. Aggarwal P, Wali JP, Singh S, Handa R, Wig N, Biswas A. A clinico-bacteriological study of peripheral tuberculous lymphadenitis. The Journal of the Association of Physicians of India. 2001;49:808-12.