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A COMPARISON OF FINE NEEDLE ASPIRATION CYTOLOGY WITH ZIEHL NEELSEN STAINING TECHNIQUE IN DIAGNOSIS OF TUBERCULOUS LYMPHADENITIS.

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ABSTRACT:

OBJECTIVE: *To describe the pattern of presentation of tuberculous lymphadenitis, to correlate the sensitivity of Fine Needle Aspiration Cytology (FNAC) and Ziehl Neelsen (ZN) stain in the diagnosis of Tuberculosis (TB) lymphadenitis and to assess the diagnostic yield of FNAC in cervical lymphadenopathy.*
STUDY DESIGN: *Prospective cross sectional study.*
METHODS: *Total one hundred and sixty four patients of clinically suspected TB lymphadenitis, referred to Dow Diagnostic Reference & Research Laboratory (DDRRL) for diagnostic services for lymphadenopathy from Jan 2008 to Oct 2009 were included. Cytological evaluation for identification of Acid Fast Bacilli (AFB) was done on separate slides with Hematoxylin & Eosin (H & E) & ZN (hot method) stain.*
RESULTS: *Mean age was 24.49; Female were 69%; Male were 31%. Out of 164, tubercular were 117(71.3%) & 17(10.4%) were of reactive nature. The remaining 9(5.5%) cases were of malignant lymphadenopathy, consisting 1(0.6%) case of primary malignancy i.e. lymphoma and 8(4.9%) of metastasis to lymph nodes. Overall 11 cases (6.7%) were found positive for AFB on ZN stain.*
CONCLUSION: *Cytomorphologic diagnostic features of FNAC on H&E stain have significant diagnostic yield. FNAC is an optimally selected, efficient, easy to perform and economical test for initial diagnostic workup in patients with TB Lymphadenitis in Pakistan. Supplementation of ZN stain does not add to the overall Positive Predictive Value of FNAC.*

KEYWORDS: Tuberculosis, lymphadenopathy, fine needle aspiration cytology.

INTRODUCTION.

Tuberculosis is emerging as global problem with nine million new cases and mortality of three million persons every year. Developing countries mostly affected with 25% of all deaths are caused by tuberculosis[1]. Tuberculosis has been declared as a global emergency by WHO mainly in developing countries with 25% of all deaths in adults [2]. Pakistan is a developing country with high burden of tuberculosis and limited resources to combat with disease of such magnitude. Extra-pulmonary ttuberculosis is a major health problem and the most common presentation is cervical lymphadenitis, relatively more common among the Asian populations [3, 4]. Lymph node enlargement could be due to tuberculosis or there may be underlying malignancy, empirical treatment for tuberculosis may lead to diagnostic delay if the cause of lymphadenopathy is malignancy [5]. Tuberculous lymphadenitis usually diagnose on conventional histopathology on basis of caseous necrosis. The chances of identification of acid-fast bacilli in tissue section are les because xylene and formalin can affect the sensitivity of by ZN method to detect mycobacterium tuberculosis in histopathology sections [6].

FNAC is an simple, quicker, reliable and relatively cheap diagnostic modality with minimal trauma and risk of complications [7]. The yield of FNAC of tuberculous lymphadenitis is directly proportional to purulent material in sample[8]. The presence of AFB are commonly seen in purulent samples which may not contain granuloma, caseous necrosis or epitheloid cells, in absence of ZN stain of sample can be diagnosed as acute suppurative lymphadenitis[4]. The aims of this prospective study were to describe the distributional patterns of tuberculous lymphadenitis and to evaluate the correlation between FNAC and the ZN staining in

diagnosing TB lymphadenitis and to assess the FNAC as a useful diagnostic tool in cases of tuberculous lymphadenopathy in a referred laboratory setting.

SUBJECTS AND METHODS.

Study Population and Setting: One hundred and sixty four (164) patients from January 2008 to September 2009 were inducted in this study. Clinically suspected patients of TB lymphadenitis were included in the study. The variables included in the study were age, sex, site of lesion. Relevant history and examination of nodes were recorded. Nodes were aspirated after all aseptic measures with sterile disposable 24 G needle attached with 10 cc disposable syringes. Two smears were prepared with part of aspirated material, one stained with H & E & other unstained. In 94 cases out of 164 were stained with ZN. The slides were examined under oil immersion objective. The remaining part of the aspirate was used to prepare paraffin embedded cell block & after processing sections were stained using H & E. The results of FNAC were further confirmed with tissue blocks. All data grouped and analysed by use of SPSS version 16.0. Descriptive statistical tests were used to analyse the differences within the data.

Procedure: Prepared smears were examined by one microbiologist & one cytopathologist concomitantly for presence of granuloma, necrosis, langerhans giant cells, plasma cells, lymphocytes, macrophages, neutrophils and presence or absence of AFB in ZN stained slides under oil immersion objective.

Presence of sheets of epitheloid cells with lymphocytes and plasma cells with or without multinucleated giant cell were diagnosed as granulomatous lymphadenitis and eosinophilic granular material containing with inflammatory cells and necrotic cell debris was defined as caseous necrosis[8]. The tuberculous abscess was described as degenerate caseous necrosis and/or liquefied necrotic material with marked degenerating and viable inflammatory cell infiltration without epithelioid granulomas [9].

RESULTS.

Aspirates of 164 patients were enrolled in study with suspicion of extra pulmonary tuberculosis. Mean age was 24.49; most of the patients were females (69%) while males were 31% with female to male ratio approximately 3:1. The majority of patients were children and young adults 79.2% less than 30 years of age. (Table I). The most common involved lymph nodes in TB lymphadenitis were cervical 137 (83.5%) followed by submandibular 16 (9.8%) and axillary 9 (5.5%). Table II.

Tuberculous cases were found to be 117(71.3%) and 17(10.4%) were of reactive nature. The remaining 9(5.5%) cases were of malignant lymphadenopathy, consisting 1(0.6%) case of primary malignancy i.e. lymphoma and 8(4.9%) metastasis to lymph nodes. (Table III). Out of 164 cases, ZN stain positivity for AFB was found in 11 cases (6.7%) out of 94 cases.

TABLE I.

Age frequency of FNAC samples

Age (yrs)	Frequency	Percentage (%)
1 to 10	4	2.4
11 to 20	75	45.7
21 to 30	51	31.1
31 to 40	20	12.2
41 to 50	5	3
51 to 60	7	4.3
> 60	2	1.2

TABLE II.

Lymph node involvement

Lymph node site	No. Of cases	Percentage (%)
Cervical	137	83.5
Submandibular	16	9.8
Axilla	9	5.5
Others	2	1.2
Total	164	100

TABLE III.

Cytological and microbiological correlation of lymphadenitis cases

Cytological diagnosis	No. Of cases (%)	AFB smear positive cases (%)	AFB smear negative cases (%)
CGI suggestive of TB	107 (65)	5 (9.4)	48 (90.5)
Acute and CI	11 (6.7)	3 (33.3)	6 (66.6)
CN	09 (5.4)	3 (33.3)	6 (66.6)
RC	17 (10)	0 (0)	6 (100)
CI	03 (1.8)	0 (0)	2 (100)
MC	08 (4.8)	0 (0)	7 (100)
CAI AI compatible with abscess	02 (1.2) 03 (1.8)	0 (0) 0 (0)	2 (100) 3 (100)
Lipoma	02 (1.2)	0 (0)	2 (100)
ASI	01 (0.6)	0 (0)	1 (100)
Lymphoma	01 (0.6)	0 (0)	0 (00)
Total	164	11	83

CGI, Chronic granulomatous inflammation; CI, chronic inflammation; CN, caseous necrosis; RC, reactive changes; MC, metastatic ca; CAI, chronic active inflammation; AI, acute inflammation; ASI, acute suppurative inflammation.

DISCUSSION.

In Pakistan, TB burden is ever increasing with added problems like poor socio-personal conditions, poverty, improper health care delivery system and poor education level. This collectively leads to an escalation of TB burden in Pakistan. Rise in incidence of HIV/AIDS is complicating the picture further. Pakistan is ranked as sixth among the 22 countries of the EMRO region with the highest burden of TB [10, 11]. In 2001, the Government of Pakistan declared TB as a National Emergency. The diagnosis of extrapulmonary TB still remains to be more of a clinical decision. Not many clinically sensitive tests are available in Pakistan to assist the treating physician. For accurate diagnosis of Mycobacterium tuberculosis, isolation and culture of organism is gold standard but as Mycobacterium tuberculosis is slow growing organism so culture on conventional Lowenstein-Jensen (LJ) medium takes 6 to 8 weeks. Middlebrook medium isolates growth of organism comparatively more rapidly. Mean duration to yield positive culture is about 3 weeks. But the disease like TB, this is long enough to wait for results as it is necessary to start treatment at the earliest. Therefore, comparatively rapid diagnostic strategies need to be established for diagnosis of tuberculous lymphadenitis [12]. FNAC is a well-established diagnostic technique in the evaluation of lymphadenopathy. In highly prevalent areas of mycobacterial infections, a diagnosis of tuberculosis can be made with confidence when its cytomorphological criteria are met. FNAC is cost effectiv , safe and rapid method of diagnosing not only TB lymphadenitis but also reveals other pathologies. It also avoids the possible physical and psychological complications of an excisional biopsy [13, 14].

In this study we examined 164 cases for diagnosis of TB lymphadenitis on FNAC. The majority of the patients were less than 30 years of age, which correlates the findings of other studies [5, 15-17]. Females were more in number (69%) than males (31%), which were conversely reported by other researchers [5, 18].

Most common site involved was cervical region (83.5%), similar findings were reported previously [5, 8, 19]. CGI was the commonest finding (65%), followed by reactive changes in 10% of cases. Over all ZN positivity was identified in only 11out of 94 cases (11.7%) low concentration of mycobacteria could be the reason. ZN positivity was only 9.4% in cases of CGI & it was more (33.3%) in CN.

In pulmonary tuberculosis, the sensitivity of ZN stain depends upon the concentration of mycobactrium in sputum sample; more than ?10 ^{4} organisms/mL results in positive reaction [20]. This is also a reason for sensitivity of ZN stain in aspirates from tuberculous lymphadenitis as suggested earlier [12]. Some studies have reported ZN positivity of 37.4% to 59.4% [5, 21, 22].

CONCLUSION.

FNAC may be an important diagnostic tool for TB lymphadenitis in developing countries with limited diagnostic and therapeutic resources. It is an excellent method, even with negative ZN stains for AFB, the cytomorphological features on FNAC is sufficient for diagnosis of TB lymphadenitis.

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