East African Medical Journal Vol. 76 No 5 May 1999
IS FINE NEEDLE ASPIRATION CYTOLOGY A USEFUL TOOL FOR THE DIAGNOSIS OF TUBERCULOUS LYMPHADENITIS?
A. Getachew, M.D. Pathologist, Department of Pathology, Gondar College of Medical Sciences, P.O. Box 196, Gondar, Ethiopia and Zeamanuel Tesfahunegn, BSc. Laboratory Technologist, Tigrai Health Research and Laboratory Center, P.O. Box 951 Tigrai, Ethiopia

Request for reprints to: Dr. A. Getachew, Department of Pathology, Gondar College of Medical Sciences, P.O. Box 196, Gondar, Ethiopia

IS FINE NEEDLE ASPIRATION CYTOLOGY A USEFUL TOOL FOR THE DIAGNOSIS OF TUBERCULOUS LYMPHADENITIS?

A. GETACHEW and Z. TESFAHUNEGN

ABSTRACT

Objective: **To study the role of fine needle aspiration cytology (FNAC) in the diagnosis of tuberculous lymphadenitis and find a place for FNAC as laboratory diagnostic method in tuberculosis (TB) control programmes.**

Design: **Prospective laboratory study.**

Method: **Duplicate smears from 127 lymphnode aspiration were prepared. Both slides were air-dried. Giemsa stain for cytological examination and Ziehl-Neelsen stain for demonstration of acid fast bacillus(AFB) were used and examined by a pathologist and laboratory technologist respectively.**

Setting: **Tigrai Regional Health Research and Laboratory Centre which is the only unit with microbiological and cytopathological service in the region.**

Subjects: **Patients with one or more enlarged lymph nodes who were sent for FNAC were included.**

Results: **The AFB positivity among the cytologically diagnosed cases of TB lymphadenitis was 56.77%. If we had used culture media for Mycobacterium spp, the positivity would have probably been higher. The positivity rate varied depending on the type of the aspirate. Caseous aspirate showed a higher positivity rate of 60.47% whereas no AFBs were detected in haemorrhagic aspirates.**

Conclusion: **This study has demonstrated the usefulness of FNAC in the diagnosis of TB lymphadenitis and the national TB and leprosy control programmes should encompass FNAC as a diagnostic means instead of biopsy which is more invasive and costly.**

INTRODUCTION

Tuberculosis (TB) is a major health problem in most developing countries. According to the World Health Organisation (WHO), approximately one third of the world population is infected with *Mycobacterium* tuberculosis and 20 million have active disease. The mortality due to TB is close to 30 million annually making it the leading cause of death from a single pathogen(1). The spread of human immunodeficiency virus (HIV) has further aggravated the situation(2). The number of patients infected with both HIV and TB is estimated to be 3.8 million and most of these patients are found in sub-Saharan Africa.

Due to the incompleteness of data collection on TB in Ethiopia, the currently available records are not reliable. However, according to the Ministry of Health of Ethiopia, in 1995 alone TB was a leading cause of outpatient morbidity, ranking fourth with 3.7%; and third reason for hospital admission, constituting 9.4% of all cases admitted in hospital; and the first cause of hospital deaths, constituting 27% of all patients who died in hospital(3).

The available data on TB in the Tigrai region more or less reflect the national picture but extra pulmonary TB (EPTB) accounts for 42% according to the 1997/98 G,C, compiled data in which 7232 patients out of the total 17430 TB patients were diagnosed to have EPTB (Unpublished report of the Tigrai regional health bureau statistics office, 1998). This high percentage of EPTB limits the use of the sputum examination as a diagnostic means only to the remaining 58% of TB patients.

The majority of EPTB is grandular (lymphode) TB which presents clinically as a slowly developing and painless enlargement of lymphnodes, followed by matting and drainage of pus. As stated in the manual of National TB/Leprosy control programme, management of enlarged lymphnodes is based on clinical examination and biopsy(3). Biopsy can play a role in the diagnosis of tuberculous lymphadenitis; but due to the expensive instruments and shortage of trained manpower, it is only done in highly specialised centres. This invasive procedure can be replaced by a fine needle aspiration cytology (FNAC). FNAC is a method of taking a cytology sample by means of needle with a negative pressure supplied by an attached syringe. It is a safe procedure which does not require hospitalisation or

surgery. It is inexpensive and provides rapid diagnosis with an accuracy reaching more than 95%. Furthermore, the aspirate can be cultured(4). Cytological evaluation plays an important role in the identification of pathological entities responsible for lymphadenopathy. With the current emphasis on cost-effective medical care, a rational approach to diagnostic evaluation should begin with the least expensive and least invasive procedure that can provide a reasonable diagnostic accuracy; and should progress to more complex investigation only as needed.

The purpose of this study was to identify the role of FNAC in the diagnosis of tuberculous lymphadenitis and find a place for FNAC as a laborotory diagnostic method in regional and national TB control programmes as well as to compare FNAC to acid fast stain (AFS).

MATERIALS AND METHODS

Duplicate smears were prepared from 127 enlarged lymph node aspirates. Both slides were air dried. Slides for cytological examination were treated with methanol for 30 seconds and stained with Giemsa for three minutes, then washed with water. The other slides were heat fixed and stained with Ziehl-Neelsen stain according to the standard procedure. Both slides were examined independently by a pathologist and medical technologist respectively.

The gross appearance of the aspirate was described as caseous for cheese like/yellow-whitish aspirate; pusy for greenish yellow/yellowish aspirate; haemorrhagic for bloody aspirate and adequate for non-caseous, non-pusy and non-haemorrhagic aspirate. Cytologically, tuberculosis was diagnosed when epitheliod cells and necrosis with or without giant cells were noted and when a pusy aspirate shows sheets of mononuclear inflammatory cells and amorphous necrotic material. Granuloma: if few epitheliod cells only were seen. Reactive: if hetrogenous lymphoid cells populations were seen and abscess when a pusy aspirate yields shoots of neutrophils with granuloma necrotic background.

The Ziehl-Neelsen stain was defined as positive if one or more AFB was seen; and negative if no AFB was seen in 100 oil immersion fields oil the smear.

RESULTS

A total of 117 lymphnode aspirates from 113 patients were subjected for the test. The aspirated lymph nodes were 90 cervical lymph nodes (76.92%), 14 axilliary lymph nodes (11.97%), six submandibular lymph nodes (5.13%), three inguinal lymph nodes (2.56%) and four from other sites (3.42%). Grossly the aspirates were labelled as caseous in 43 cases (36.75%); adequate in 38 cases (32.48%); pusy in 30 cases (25.64%) and haemorrhagic in six cases (5.13%). Based on cytological examination, 74 aspirates were diagnosed as tuberculous lymphadenitis, 16 cases as abscess, 22 cases as reactive and five cases as granulomatous lymphadenitis.

All the 117 smears were examined for acid fast bacilli following Ziehl-Neelsen stain and the results are shown in Table 1.

Table 1

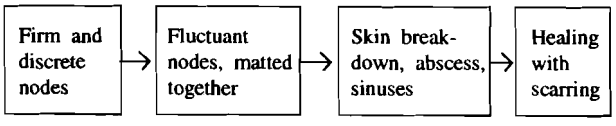
Results of the cytologic diagnosis and Ziehl-Neelsen stain

Aspirate gross appearance	Cytologic Diagnoses	+ve for AFB	-ve for AFB	Total
Pusy	TB abscess	11	3	14
	Abscess	3	13	16
Caseous	TB	26	17	43
	Reactive	0	16	16
Adequate	TB	5	12	17
	Granulomatous	0	5	5
Haemorrhagic	Reactive	0	6	6
	Total	45	72	117

We were able to inocculate 18 cases of pusy aspirates on MacConkey and mannitol salt agar simultaneously (eight TB abscess cases and 10 abscess cases). All TB abscsess cases showed no growth while five out of 10 abscess cases grew Staphylococcus aureus in four cases and Proteus spp in one case.

DISCUSSION

TB lymphadenitis is the most common form of EPTB. The most commonly involved lymph node regions are the cervical lymphnodes(5). This statement is in agreement with our findings. The usual course of TB lymphadenitis is as follows:



The only definitive means of establishing the diagnosis of tuberculous lymphadenitis is by culturing the organism. The identification of acid-fast organism on smear or evidence of caseating granulomas obtained by biopsy of tissue is only presumptive evidence for initiation of therapy(6). According to the manual of National Tuberculosis/Leprosy control programme, diagnosis of TB lymphadenitis relies on clinical examination and biospy. There is no mention of FNAC as an investigation means. FNAC is a quick safe and cheap way of diagnostic pathology. Even the appearance of the aspirate may indicate whether the lesion is TB or not. The appearance of the aspirate varies depending on the course of the disease. In cases of firm and descrete nodes the aspirate tends to be adequate or caseous whereas a fluctuant or matted nodes commonly give rise to caseous or pusy aspirate. Aspirate from a healing lymphadenitis or small and deep seated lymph nodes or when there is technical failure, tends to be haemorrhagic.

The gross appearance of the aspirate may also indicate the nature of the disease. Caseous aspirate is commonly seen in TB except in secondarily carcinoma with extensive necrosis which can occur rarely in the lymphnode. TB abscess appears as a thick whitish liquefied material but sometimes when it is secondarily infected, it may look greenish-yellow. Adequate aspirates are often ambiguous. It has to be examined cytologically as it can be aspirated from all kinds of lymphadenitis.

The AFB positivity following Ziehl-Neelsen stain is high in caseous aspirates (60.47%), followed by pusy aspirate(46.67%), whereas adequate smears show few positivity (13.16%) and no positivity for haemorrhagic aspirates. The above findings indicate that free acid fast bacilli can be more often seen in a necrotic background as the dead phagocytic cells liberate the bacteria into the environment.

In conditions where caseous necrosis is not well developed, the bacilli remain in the cytoplasm of the macrophages and the stain may not reach the bacilli and cannot be visualised under a microscope. We have detected a high AFB positivity in tuberculous abscess(78.57%), even higher than caseous necrosis. Liquefactive necrosis yields more AFB than caseous necrosis. Liquefactive necrosis may sometimes become extensive and mimick abscesses of pyogenic bacterial origin. This could he the reason why we found three AFB positive cases out of simple abscesses.

All in all the AFB positivity among the cytologically diagnosed cases of TB lymphadenitis is 56.77% and if we had used culture media for *Mycobacterium* spp the positivity would have been even higher, as examination by culture increases the number of new cases found, often by about 25-30%. However, claims that culture doubles the number of cases detected bacteriologically have also been made(7). In one study, in patients with a final diagnosis of intrathoracic TB (plumonary, pleural or pericardial) FNA followed by Ziehl-Neelsen staining of extrathoracic lymphnode, even as small as 1.5cm, showed AFB in 87% of patients(8). We were able to inocculate 18 cases of pusy aspirate on a culture medium for the presence of pyogenic bacteria (eight TB abscess cases and ten simple abscess cases). All TB abscess cases and five simple abscess cases showed no growth. Five simple abscess cases grew bacteria (four grew *Staphylococcus aureus* and one grew *Proteus* spp).

FNAC is a useful diagnostic test, especially in case of TB lymphdenitis but its sensitivity and specificity has to be determined by using culture media in our set up. Culture media also help in the determination of the bacilli at species level as well as it can exclude the common contaminating saprophytic mycobacterial species. Diagnosis of TB lymphadenopthy is possible even without laboratory facilities for histology or TB culture. Diagnostic sensitivity of TB lymphadenopathy by aspirate and smear for AFB is 70%. Diagnostic sensitivity increases to 80% if one can excise a lymphnode, look at the cut surface, and do a smear for AFB(9). A newer method based on the use of fluorescent dyes such as auramine or auramine and rhodamine is used by many laboratories(10). Its advantage is that slides can be screened at lower power, the organism exhibiting fluorescence under ultraviolet light. This is not a fluorescent antibody method and thus false positivity are frequent. In inexperienced hands findings need to be confirmed using the Ziehl-Neelsen method. A method using phase contrast microscopy has been described. Its advantages and disadvantages are similar to that of flourescent technique(11).

The Ethiopian National TB and Leprosy control programme should encompass FNA as a diagnostic means for both TB lymphadenitis and pulmonary TB with superficial lymphnode enlargement as a first line investigation. It can also be used in the follow up of patients to monitor therapy. The diagnostic accuracy of FNA may be higher than that of sputum cytology in the diagnosis of mycobacterial lesions(12). The use of biopsy as a diagnostic test does not have any better advantage than FNA cytology in case of TB lymphadenitis as in both cases demonstration of AFB by Ziehl-Neelsen stain is mandatory for confirmation. Biopsy may be helpful whenever exact diagnosis cannot be reached by cytology alone.

A practical approach to investigation of lymphadenopathy in our opinion should be as follows(9):

Procedure	Test	Results	Diagnoses
FNA of LN	Look at material aspirated	Caseation	TB
	Smear for AFB	AFB present	TB
	Smear for cytology	Malignant	Malignancy
If no Dx		Granuloma	
after FNA	Sample for culture/PCR	For definitive diagnosis	
LN biopsy	Look at the cut surface	Caseation	TB
	Smear from cut surface for AFB	AFB seen	TB
	Fresh node sent for TB culture	Positive TB culture	TB
	Node in formalin for histology	Granuloma and AFBs	TB
		Malignant cells	Malignancy

In conclusion, this study has demonstrated the usefulness of FNA cytology in the diagnosis of TB lymphadenitis; however, further studies are required in order to elucidate its sensitivity, specificity and positive predictive value. The above parameters are important measures for the performance of a diagnostic test(13).

ACKNOWLEDGEMENTS

To all the staff of Tigrai Health Research and Laboratory Centre for their support.

REFERENCES

1. Kochi, A. The global tuberculosis and the new control strategy of the World Health Organisation. *Tubercle.* 1991; **72:**1-6.
2. Harries, A.D. Tuberculosis and human immunodeficiency virus infection in developing countries. *Lancet.* 1990; **335:**378-390.
3. National tuberculosis and leprosy control programme, Ministry of health of Ethiopia. (1997). Manual
4. Medical laboratory manual for tropical countries; vol. ii, HIV supplement, Monica Cheesebrough, 1990.
5. P.D.B. Davies, Clinical presentation of Tuberculosis, *Brit. J. Hosp. Med.* June(s). 1971, 749-760.
6. A.S. Banner. Tuberculosis, Clinical aspects and diagnosis. *Arch. Intern. Med.* 1979; **139**: 1387-1390.
7. Fourie P.B., Gatner E.M.S. *et al. Tubercle.* 1979; **61**:71.
8. Anthony D. Hallies. Tuberculosis in Africa, clinical presentation and management. *Pharmacol. Ther.* 1997; **73 No 1:**1-50.
9. World Health Organisation. A clinical manual, Anthony D. Harries, Dermont Maher.
10. Joseph, S.W. and Houk, V.N. Evaluation and application of the fluorochrome stain for microscopic detection of mycobacteria in clinical specimens. *Amer. Rev. Respir. Dis.* 1966; **98**:1044-1447.
11. Zykov, M.P., Roulet, H., Potes, S.J. *et al* Phase contrast microscopy in diagnosis of tuberculosis. *Amer. Rev. Respir. Dis.* 1973; **107**:295-298.
12. Bailey, T.M. Akhtar M, Alima: Fine needle aspiration biopsy in the diagnosis of tuberculosis. *Acta Cytol.*1985; **29**: 732.
13. Toman, K. Sensitivity, specificity and predictive value of diagnostic test. *Bull. Int. Union. Tuberc.* 1981; **56:**18-28.