ORIGINAL ARTICLE

Cytomorphology Versus Conventional Microbiological Tests in the Diagnosis of Tuberculous Lymphadenitis

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ABSTRACT

Objective: To determine the accuracy of Fine Needle Aspiration Cytology (FNAC) in the diagnosis of tuberculous lymphadenitis.

Study Design: Comparative cross-sectional study.

Place and Duration of Study: Department of Pathology, Khalifa Gul Nawaz Teaching Hospital (KGNTH), Bannu, from September 2012 to March 2013.

Methodology: FNAC of enlarged lymph nodes was performed in the Department of Pathology, KGNTH, Bannu. Smears of the aspirates were examined under light microscope after staining with Haematoxylin and Eosin (H & E) stains. In cases of chronic lymphadenitis, the smears were stained with Ziehl-Neelsen (ZN) stain for Acid Fast Bacilli (AFB). If no AFB was visualized, the aspirate was subjected to culture on Lowenstein Jensen (LJ) medium for yield of AFB. The results were analyzed by Microsoft Excel software.

Results: Chronic granulomatous lymphadenitis was found in 110 (46.81%) out of 235 cases. AFB were seen in aspirates of 43/110 (39.09%) cases by direct microscopy. Among the remaining 67 aspirates subjected to LJ medium, only 07 (10.45%) yielded growth of AFB. Smears of 4/15 (3.6%), 13/47 (11.7%) and 33/48 (29.7%) cases with haemorrhagic, inflammatory and caseous background respectively, were confirmed by conventional microbiologic tests. Out of 125 non-granulomatous lymphadenitis cases only 05 were confirmed to be due to tuberculosis by direct microscopy while culture was not positive in any case. Thus accuracy of FNAC was 72.34%.

Conclusion: FNAC has a good accuracy in diagnosing tuberculous lymphadenopathy.

Key Words: *Tuberculosis. Lymph node. Tuberculous lymphadenitis. Fine needle aspiration cytology (FNAC). Ziehl-Neelsen staining. Lowenstein Jensen medium. Acid-fast bacillus.*

INTRODUCTION

Tuberculosis (TB) of the lymph node (tuberculosis lymphadenitis) is the most common form of extrapulmonary tuberculosis.[1] In developing countries like Pakistan, its prevalence is 15%.[2]

The difficult aspect of extrapulmonary tuberculosis is its diagnosis posed by the less number of AFB.[3] Many diagnostic tests are performed for this reason.[4] Generally diagnosis of TB depends specifically on the presence of AFB in ZN stained smears or its growth on cultures. Conventional methods like ZN staining of smears and culture on LJ medium are used in developing countries for the diagnosis of TB. In developed countries new and advanced diagnostic techniques like radiometric and molecular ones are used for this purpose.[5]

Fine Needle Aspiration Cytology (FNAC) is considered a very useful adjunct in the diagnosis of extra pulmonary TB.[6] Because of its rapidity and simplicity, it is becoming a popular test for diagnosis of both pulmonary and extra pulmonary TB. It is convenient for the clinician as well as for the patient. It can be easily performed in the out-patients with minimal pain to them.[7,8] Direct microscopy for AFB smear examination is economical and time honored, but for this technique the specimen should contain between 5000 to 10,000 organisms per ml.[9]

Culture is more sensitive as compared to direct microscopy. It is able to give positive results on a specimen containing as low as 10 - 100 bacilli per ml. The conventional culture method using Lowenstein Jensen medium takes 6 - 8 weeks for primary isolation of the organism. This prolonged period has prompted many workers to look for quicker methods of culture, such as the Rapid Slide Culture (RSC) technique.[10]

The objective of this study was to determine the accuracy of the FNAC in diagnosing tuberculosis in the cases of peripheral lymphadenopathy as a faster method of diagnosis.

METHODOLOGY

It was a comparative cross-sectional study conducted at the laboratory of Khalifa Gul Nawaz Teaching Hospital, Bannu, from September 2012 to March 2013. Patients with enlarged lymph nodes after initial clinical examination by the clinicians were referred for fine needle aspiration

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cytology for cytological evaluation. Non-probability convenience sampling technique was applied for patients' selection. After taking informed consent, the enlarged lymph nodes were aspirated using 23 gauge needle attached to 5 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 of lymphadenitis whether granulomatous or non-granulomatous was used to make two smears, one for cytomorphological examination and the other for ZN staining. If AFB were seen on direct microscopy, the case was confirmed to be due to tuberculosis. If no AFB on ZN staining was seen, the remaining aspirate was inoculated on LJ medium present in screw capped bottles for yield of AFB. Those bottles were kept in an incubator at 37ºC for 3 - 8 weeks. The media were checked for evidence of bacterial growth daily for the first week and weekly for the rest of the time until 8 weeks. If growth of AFB yielded, again the diagnosis of tuberculosis was confirmed, otherwise the specimen was considered to be negative for tuberculosis. Both these conventional microbiologic tests i.e. ZN staining method and culture on LJ medium were used as gold standard for confirming the diagnosis of TB. The whole procedure of diagnosis is shown in Figure 1.

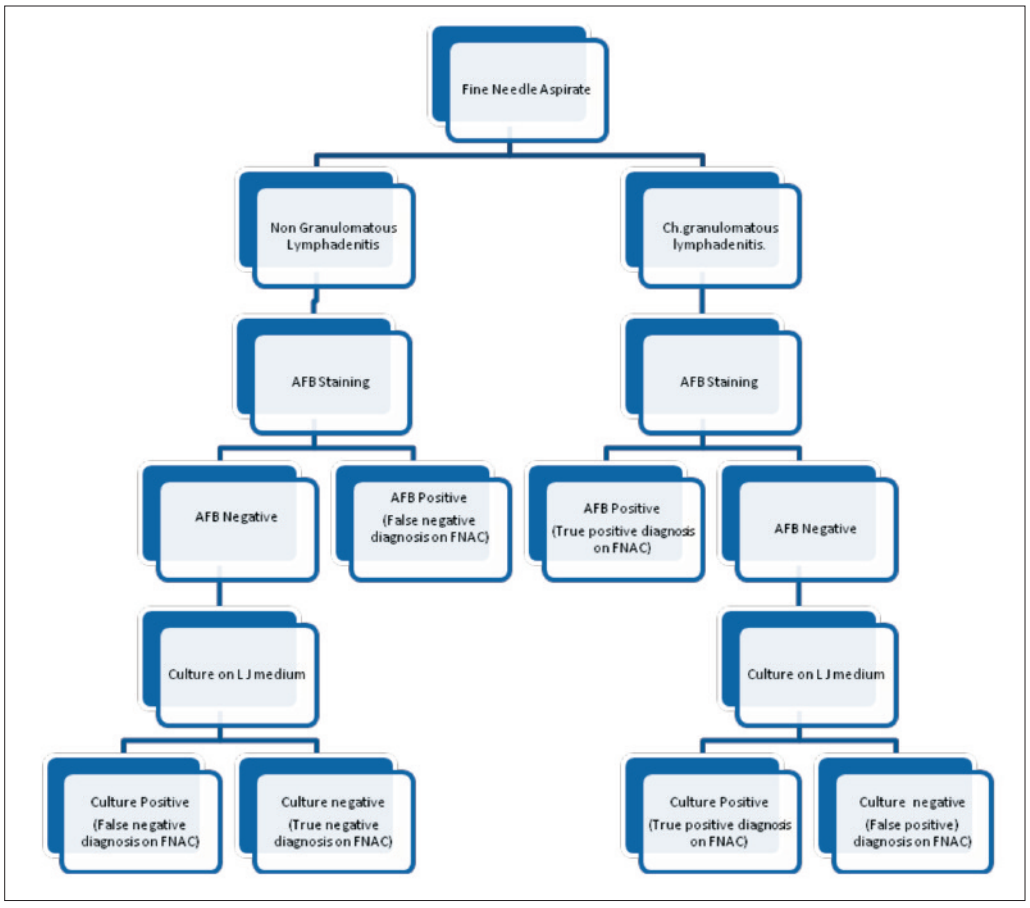

Figure 1: Flow chart for cytomorphological compared to conventional microbiologic diagnosis of superficial lymphadenopathy.

The diagnosis of granulomatous lymphadenitis most probably due to tuberculosis was made on cytomorphological examination of the aspirate in the presence of epithelioid cell granuloma with or without haemorrhage, necrosis or inflammatory cells in the background.

The data was interpreted by using Microsoft Excel. The results of cytomorphologic and conventional microbiologic diagnosis in tuberculous lymphadenopathy were compared. Similarly, specificity, sensitivity, true positive, false positive, true negative, false negative, positive predictive value, negative predictive value and diagnostic accuracy for FNAC of superficial lymph nodes in the diagnosis of TB were derived by their specific formulas and presented in a tabulated form for their frequencies and percentages.

RESULTS

During the study period of 6 months a total of 235 patients of peripheral lymphadenopathy attended the study place for FNAC of lymph nodes. Out of these 235 patients, 110 patients were diagnosed by cytomorphologic examination to have chronic granulomatous lymphadenitis most probably due to tuberculosis while 125 patients were diagnosed to have non-granulomatous lymphadenitis. There were no complications of FNAC except pain at the site of aspiration.

Out of 110 patients of chronic granulomatous lymphadenitis 60 (54.55%) were females and 50 (45.45%) were males ranging in age from 05 years to 70 years with a mean of 27 years. Out of these, 88 (80%) cases belonged to poor families regarding their socioeconomic condition and composed mainly of housewives (n=35, 31.82%) students (n=25, 22.73%), farmers (n=15, 13.64%), shopkeepers (n=6, 5.45%) and others (n=29, 26.36%).

The most frequently involved region in chronic granulomatous lymphadenitis was cervical where left cervical area was involved in 50 (45%) cases while right cervical area in 30 (27%) cases. The next

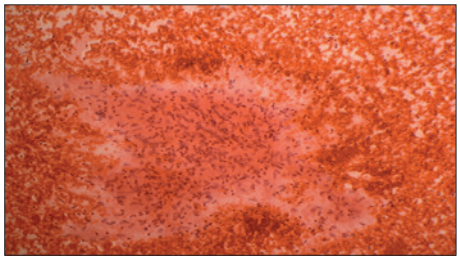

Figure 2: A photomicrograph of a smear prepared from FNA of a lymph node showing granuloma (an aggregate of epithelioid histiocytes) surrounded by a red haemorrhagic background (H & E Stain; x100).

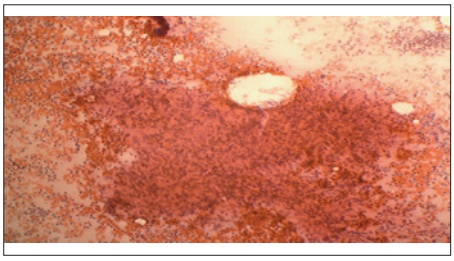

Figure 3: A photomicrograph of a smear prepared from FNA of a lymph node showing granuloma (an aggregate of epithelioid cells mixed with lymphocytes) along with scattered lymphocytes and neutrophils (inflammatory cells) in the background (H & E Stain; x100).

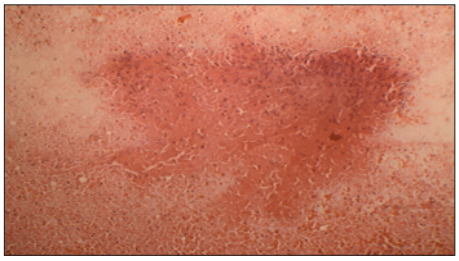

Figure 4: A photomicrograph of a smear prepared from FNA of a lymph node showing a granuloma (an aggregation of epithelioid cells) in the right upper part of the figure with eosinophilic acellular granular caseous necrotic material in the background (H & E Stain; x100).

Table I: AFB positivity on ZN staining and culture method in different types of lymphadenitis cases.

Conventional microbiologic test	Type of lymphadenitis			
	Granulomatous (n=110)		Non-granulomatous (n=125)	
	Frequency of positive cases	Percentage	Frequency of positive cases	Percentage
ZN staining	43	39.09	05	4.0
Culture	07	6.36	00	0.0
Total positive cases	50	45.45	05	4.0

Table II: Statistical values of FNAC (screening test) in comparison to ZN staining and culture on LJ medium (gold standard tests).

True positive cases	50	Sensitivity	90.91%
False positive cases	60	Specificity	63.89%
True negative cases	120	Positive predictive value	45.45%
False negative cases	05	Negative predictive value	96.0%
		Diagnostic accuracy of FNAC	72.34%

frequently involved sites in these cases were axillary and supraclavicular regions comprising of 10 (9.1%) and 8 (7.2%) cases respectively. There were also 6 (5.45%) cases of bilateral cervical and 4 (3.6%) cases of inguinal lymphadenopathy. The remaining 2 (1.8%) cases had lymphadenopathy in other areas. Regarding the duration of lymphadenopathy in these 110 cases, 90 (81.82%) patients reported within 6 months while 20 (18.18%) patients reported to hospital after 6 months for complaint of their lymphadenopathy.

The cytomorphologic diagnosis in chronic granulomatous lymphadenitis cases was divided into three main categories on the basis of background of the smears, the first having granulomas with haemorrhagic background (Figure 2), the second having granulomas with inflammatory cells in the background (Figure 3) and the third having granulomas with caseous necrotic material in the background (Figure 4). In the first category there were 15 (13.64%) cases. The second category had 47 (42.73%) cases while there were 48 (43.64 %) cases in the third category.

Out of the total 110 granulomatous cases, AFB were seen in 43 (39.09%) cases by direct microscopy. The aspirates of the remaining 67 cases in this group were subjected to culture on LJ medium for yield of AFB, where aspirates of only 07 cases yielded growth of AFB. Out of 125 non-granulomatous lymphadenitis cases only 5 cases were confirmed to be due to tuberculosis by detecting AFB in their aspirates after ZN staining of their smears. Culture of the aspirates of the remaining 120 cases in this group did not yield any growth of AFB (Table I).

Regarding background of granulomatous lymphadenitis cases, out of 15 (13.5%) cases having the haemorrhagic background only 04 (3.6%) cases were confirmed by conventional microbiologic tests (all 04 cases confirmed by direct microscopy after ZN staining of the smear), while 11 (9.9%) cases were not confirmed as no AFB was seen by the direct microscopy or after culture of the aspirated material. Similarly, 47 (42.3%) cases having background of scattered lymphocytes and neutrophils only 13 (11.7%) cases (11 cases by direct microscopy and 02 by culture) were confirmed to have tuberculosis by finding AFB in them while 34 (30.6%) cases were not confirmed. Among the remaining 48 (43.2%) cases with a background of caseous material, 33 (29.7%) cases were confirmed (28 by direct microscopy and 05 by culture) to have AFB while 15 (13.5%) cases were not confirmed by application of conventional microbiologic parameters.

On comparing the results of FNAC to conventional microbiologic tests (gold standard) for diagnosis of TB, 50 (45.45%) out of 110 granulomatous lymphadenopathy cases were actually suffering from TB while the remaining 60 (54.55%) cases were not actually suffering from lymph node TB. In contrast, only 05 out of 125 cases of non-granulomatous lymphadenopathy cases were confirmed to be due to tuberculosis by ZN staining and culture tests. Sensitivity, specificity, true positive, false positive, true negative, false negative, positive predictive value, negative predictive value and diagnostic accuracy are shown in Table II.

DISCUSSION

Tuberculosis is an infectious disease and is as old as humanity. It has caused more deaths than any other disease in the human history.[11] Due to emergence of MDR-TB and XDR-TB it is still considered a great threat to humans in spite of the availability of effective anti-tuberculous medicines.[12]

Accurate and early diagnosis is very essential for the treatment of tuberculosis, because it is given for a long duration, is costly and has several side effects.[13] For this purpose, FNAC is gaining wide acceptance especially in peripheral lymphadenopathy due to tuberculosis.

The study highlights the importance of FNAC as a diagnostic test in the management of peripheral lymphadenopathy due to tuberculosis. To accomplish this task cytomorphologic diagnosis was compared with the subsequent conventional microbiologic diagnosis (considered as gold standard and includes ZN staining and culture of the aspirate).

In this study, the male to female ratio was 0.8:1 and age ranging from 05 - 70 years with a mean age of 27 years which is in accordance with different national and international studies.[14,15] The females in developing countries have weaker 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.[16,17] Occupation wise, most of the patients were housewives. Overcrowding and repeated pregnancies also lower their immunity, thus making them an easy prey to tuberculosis.[14] The most frequent site to be involved by tuberculous lymphadenopathy in this study was cervical region (77.45%), which is again in concordance with other studies.[18,19]

The sensitivity and specificity of FNAC in this study was 90.91% and 63.89% respectively. These results are comparable to the study conducted by Khan *et al*.[20]

Maximum number of AFB positivity on direct microscopy was in those smears which had granulomas with caseous necrotic material in the background on cytomorphological examination, that is, 28 out of 48 or 58.33% AFB detection. The least number of AFB positivity was in smears in which there were granulomas with a haemorrhagic background, that is, 4 out of 15 cases. The AFB positivity in smears showing granulomas with inflammatory cells in the background was 11 out of 47 cases. The reason is that the number of tubercle bacilli is more in the aspirates having caseous necrotic material while their number is less in aspirates devoid of necrotic material. This is in accordance with the stages of evolution of tuberculous lymphadenopathy. Combining the results in all the categories the overall positivity for AFB (specificity) was 63.89%. These results are in accordance with a study conducted by Annam *et al*.[16] Some other studies like Abedi *et al*.[15] and Ergete *et al*.[21] have shown high specificity values. This difference may be due to more cases of granulomatous lymphadenopathy without caseation necrosis in this study and the one conducted by Annam *et al*.[16] It is worth mentioning at this stage that for direct smear microscopy to be positive for AFB, 5000 - 10,000 bacilli/ml of the aspirated material should be present.[19] This may be the reason of low AFB positivity in the smears of this study.

The next microbiologic test applied for the diagnosis was mycobacterial culture, which is usually used as a reference method for detection of tubercle bacilli. It is time consuming and requires specialized safety procedures in laboratories. The culture positivity rate in this study was low i.e. 10.45% which is comparable to other international and national studies.[20,22] Several reasons may explain it, including partial treated status, scanty bacilli in the aspirate, harsh decontamination procedures and unrepresentative/inadequate sampling.[5,22] Some other studies[23,24] have shown higher culture positivity rates which once again may be due to low occurrence of the above mentioned factors affecting the results. Similarly, the AFB positivity rate in this study was more in those patients who had lymphadenopathy for less than 6 months. These results are in accordance with the study conducted by Abedi *et al*.[15] These findings are due to less chances of taking anti-TB drugs by patients in the initial few months of their disease and thus resulting in high yield of AFB by conventional methods of AFB detection.

CONCLUSION

Fine Needle Aspiration Cytology (FNAC) is an important investigation in the diagnosis of granulomatous lymphadenitis due to TB. If it is supplemented with light microscopy after ZN staining of the smear and culture of the aspirate for AFB detection, its diagnostic accuracy can be increased. As culture is costly, time consuming, not available in all health facilities and has low yielding results shown in the present study, therefore, emphasis should be put mainly on ZN staining and light microscopy of the smear of aspirate instead of culturing it on LJ medium. In this way if AFB are seen, the diagnosis of TB is confirmed and thus subsequent treatment for TB can be given with full confidence.

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