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Chapter 10

Diagnosis of tuberculous lymphadenitis in Butajira, rural Ethiopia.

Diagnosis of Tuberculous Lymphadenitis in Butajira, Rural Ethiopia

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Tuberculous lymphadenitis (TBLN) is a diagnostic challenge in sub-Saharan Africa, where there is a high rate of human immunodeficiency virus (HIV) infection. This study aimed to find ways to improve the diagnosis in Butajira, rural Ethiopia, where TBLN constitutes 40% of the total tuberculosis (TB) diagnosis. Among 147 clinically suspected cases, 107 (72.8%) were confirmed as TBLN by fine-needle aspiration (FNA) cytology and acid-fast bacillus (AFB) smear examination. Of the remaining 40 cases, denoted non-tuberculous lymphadenitis (NTBLN) after this smear examination. 37 (92.5%) showed a cytological

INTRODUCTION

Tuberculous lymphadenitis (TBLN) is the most common form of extrapulmonary tuberculosis (1, 2) and its recent rise in sub-Saharan countries is probably associated with the alarming increase in the prevalence of human immunodeficiency virus (HIV) infection (3, 4).

TBLN is difficult to diagnose. The sensitivity of conventional methods used in mycobacteriology laboratories, such as acid-fast bacillus (AFB) smear examination and culture, may not exceed 40% (2, 3, 5, 6). Moreover, algorithms particularly requiring excision biopsy, as suggested by different groups, could be difficult to apply in rural areas with very limited resources (7, 8).

Butajira is a rural site located 130 km South of Addis Ababa, the capital city of Ethiopia. TBLN, identified in the local dialect as Anget Berut (literally meaning 'metallic neck'), has been a major problem in the area, constituting 40% of the total tuberculosis (TB) patients diagnosed in 2 years preceding this study. As is the case in many other rural centres in Ethiopia, the health centre in the area did not provide a cytology/histology service, so it was impossible to follow the diagnostic algorithm of the National TB Control Program which, among other things, requires excision biopsy. This study was done with the following objectives: (i) to investigate the accuracy of clinical diagnosis of TBLN; (ii) to investigate the importance of AFB smear and cytological examination in supporting the diagnosis of TBLN; and (iii) to improve the diagnosis of TBLN in Butajira using methods that may be used in a rural setting.

MATERIALS AND METHODS

Patients and clinical diagnosis

The study was conducted between August 1998 and December 2000, at Butajira Health Centre, after obtaining ethical clearance from the Armauer Hansen Research Institute (AHRI) and the Ethiopian Science and Technology Commission (ESTC). The centre serves about 500,000 people, the majority being farmers (9). Patients visiting the centre with a major complaint of at least 1 chronic enlarged lymph node of more than 1 cm in diameter were eligible for further follow-up.

A clinical diagnosis of TBLN was considered probable when the patient had chronic enlarged lymph nodes with or without constitutional symptoms and lack of response to a 2 week course of broad-spectrum antibiotics. 177 patients fulfilling these criteria were included in the study and relevant clinical data were recorded on a questionnaire. Fine-needle aspirates (FNA) and blood samples were collected. 30 patients out of 177 had incomplete data (14 because of an inadequate FNA specimen for analysis and a further 16 because no AFB result was available) and were excluded from analysis.

The algorithm suggested by the National TB and Leprosy Control Program (8) was not used in this study, but the findings were analysed in such a way as to strengthen the algorithm and possibly to increase its applicability in similar settings.

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Cytological examination of the aspirate

FNA of enlarged superficial lymph nodes were obtained by a physician at Butajira Health Centre using a 21-gauge needle under sterile conditions. From each patient the specimen was smeared on 2 slides for microscopic examination. The smears were air-dried. All slides, blood samples and completed questionnaires were transported to a research laboratory (AHRI, Addis Ababa). At AHRI 1 slide was stained by the standard Ziehl–Neelsen method for AFB and the other by the haematoxylin–eosin method for cytological analysis. An experienced pathologist examined all of the stained slides.

FNA were considered diagnostic of TBLN when they contained a thick, yellowish material showing any of the following 3 cytological criteria: (i) the presence of a necrotic background associated with the presence of lymphohistiocytic cells and absence of a significant polymorphonuclear cell population; (ii) the presence of elements of a granulomatous inflammatory reaction consisting of giant cells, and/or epithelioid cell clusters and a lymphohistiocytic cell population; (iii) positivity for AFB on Ziehl–Neelsen staining.

The remaining cases without cytological features diagnostic of TBLN were classified into 2 groups: (i) pyogenic lymphadenitis when neutrophil aggregates were seen; and (ii) chronic non-specific inflammation.

Polymerase chain reaction

For 71 samples, where the amount of FNA was adequate, mycobacterial DNA was extracted (10) and a set of primers was used to amplify a segment of the IS6110 gene (11). The polymerase chain reaction (PCR) was done in a Hybaid PCR machine (Omnigene, Teddington, UK) and the products were analysed by electrophoresis in 2% agarose gel (Sigma) containing ethidium bromide. The reaction was considered positive when a 984 base-pair product was seen that matched with the size of the PCR product of a similarly amplified DNA extract from Mycobacterium tuberculosis (ATCC 35836), as positive control.

HIV immuno-deficiency virus serology

Venous blood (5 ml) was collected from each patient using a 10 ml Vacutainer tube. HIV sero-status was determined anonymously using a commercially available micro-enzyme-linked immunosorbent assay kit (Vironostika, HIV Uni-Form 11 Plus O; Organon Teknika, Eppelheim, The Netherlands) according to the manufacturer’s instructions.

Statistical analysis

Statistica 6 software was used to compute the statistical significance of the difference in proportions. Actual values are given in the text and p-values < 0.05 were considered significant.

RESULTS

Of 147 patients in this study, 107 (72.8%) were confirmed as cases of TBLN by FNA cytology and AFB smear examination. 105 cases (71.4%) had a cytology suggestive of TB; 28 (19%) were positive by AFB smear, 26 of these had a positive cytology and the diagnosis of 2 cases of TBLN was confirmed on the basis of a positive AFB smear alone. Of the 40 cases without cytological features diagnostic for TBLN, referred to as non-NTBLN (NTBLN), 37 (92.5%) showed marked neutrophil aggregates and 3 (7.5%) were diagnosed as chronic non-specific lymphadenitis based on the cytology results.

Table I shows the frequency of selected variables in the group confirmed as positive for TBLN compared with the NTBLN group. The 2 groups did not differ in the age distribution. There were more females than males in the group with confirmed TBLN.

Table II shows the frequency of selected variables in the group confirmed as positive for TBLN compared with the NTBLN group. The 2 groups did not differ in the age distribution. There were more females than males in the group with confirmed TBLN.

Of 107 cases with TBLN (22.4%) and 9 of 40 NTBLN cases (22.5%) were HIV seropositive. There was no statistically significant difference in HIV seropositivity between TBLN and NTBLN cases (p > 0.5). HIV status did not affect the cytology (p > 0.5) or AFB results (p > 0.5).

Moreover, HIV-seropositive and HIV-seronegative TBLN cases did not differ in the general manifestations, including weight loss (15/24 vs 56/83, p = 0.54) and cough (6/24 vs 14/83, p = 0.38). However, the manifestation of night sweat was more common in HIV-coinfected cases (17/24 vs 37/83, p = 0.02).

The duration of enlarged lymph nodes was > 16 weeks for 67 of 107 TBLN (62.6%) and 32 of 40 NTBLN cases (80%) (p = 0.04), confirming the chronic nature of the lymphadenitis at presentation even in the NTBLN group.

Cervical lymph nodes were the most affected in both TBLN and NTBLN cases. 88 of 107 TBLN (82.2%) and 30 of 40 NTBLN (75%) cases had cervical lymphadenopathy. The types of lymph nodes affected (cervical, submandibular, axillary, sternal and inguinal) did not vary in the TBLN and NTBLN groups (p > 0.5). In the age group < 18 y, 33 of 35 TBLN cases (94.3%) and 8 of 10 NTBLN cases (80%) had cervical lymph-node enlargement.

Since the clinical features were similar in the TBLN and NTBLN groups defined by cytology and AFB staining, it appeared that the latter group might also contain patients with tuberculous infection. Sufficient FNA material was available for DNA extraction and amplification by PCR in 71 patients, and the results are shown in Table II. In the TBLN group defined by cytology, 34 of 48 (70.8%) were positive for M. tuberculosis complex DNA, while 15 of 23 (65.2%) were positive in the NTBLN group (p = 0.5).

Table III shows the recommendations from this study to strengthen the algorithm suggested by the National TB Control Program. The steps that have to be followed in the suggested algorithm are shown along with key findings from this study.

DISCUSSION

The results showed that TBLN is a major problem in Butajira, Ethiopia. 107 of the 147 suspected patients (72.8%) had cytological and/or AFB smear results indicative of TB. In this study cytology supported the diagnosis of TB in 98.1% of all confirmed cases, while AFB smear was positive in 26.2% of these cases. A similar sensitivity of AFB smears was reported previously (2). In another study from Zambia, where 84% of cases were coinfected with HIV, AFB smear had a fairly low sensitivity (8%), although it was reported that HIV co-infection may increase the sensitivity of the AFB smear (4). In the present study, the diagnosis of
**Table I.** Clinical and epidemiological variables in the group confirmed positive for tuberculous lymphadenitis (TBLN) by cytology and the non-TBLN (NTBLN) group

<table>
<thead>
<tr>
<th>Variable</th>
<th>TBLN (n=107)</th>
<th>NTBLN (n=40)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>51 (47.7)</td>
<td>26 (65)</td>
<td>0.07</td>
</tr>
<tr>
<td>Female</td>
<td>56 (52.3)</td>
<td>14 (35)</td>
<td></td>
</tr>
<tr>
<td>Human immunodeficiency virus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>24 (22.4)</td>
<td>9 (22.5)</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td>Negative</td>
<td>82 (76.6)</td>
<td>32 (77.5)</td>
<td></td>
</tr>
<tr>
<td>Duration of enlarged lymph nodes (weeks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 8</td>
<td>19 (17.8)</td>
<td>4 (10)</td>
<td>0.24</td>
</tr>
<tr>
<td>8–16</td>
<td>21 (19.6)</td>
<td>4 (10)</td>
<td>0.16</td>
</tr>
<tr>
<td>&gt; 16</td>
<td>67 (62.6)</td>
<td>32 (80)</td>
<td>0.04</td>
</tr>
<tr>
<td>Lymph-node scar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35 (34.6)</td>
<td>28 (70)</td>
<td>0.0002</td>
</tr>
<tr>
<td>No</td>
<td>72 (66)</td>
<td>12 (29.3)</td>
<td></td>
</tr>
<tr>
<td>Generalized manifestations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Night sweat</td>
<td>54 (50.5)</td>
<td>22 (55)</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td>Weight loss</td>
<td>71 (66.4)</td>
<td>24 (60)</td>
<td>0.5</td>
</tr>
<tr>
<td>Cough</td>
<td>20 (18.7)</td>
<td>4 (10)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Data are shown as n (%).

**Table II.** Mycobacterium tuberculosis specific polymerase chain reaction (PCR) result on samples of tuberculous lymphadenitis (TBLN) and non-TBLN (NTBLN) cases

<table>
<thead>
<tr>
<th>PCR result</th>
<th>TBLN (n=48)</th>
<th>NTBLN (n=23)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>34 (70.8)</td>
<td>15 (65.2)</td>
<td>0.6</td>
</tr>
<tr>
<td>Negative</td>
<td>14 (29.2)</td>
<td>8 (34.8)</td>
<td></td>
</tr>
</tbody>
</table>

Data are shown as n (%).

TBLN was established on the basis of the smear microscopy result for AFB alone in 2 of the 107 confirmed cases (1.87%). The HIV infection rate in the Butajira community is incompletely known. However, 22% of TBLN-confirmed cases in this study were HIV positive. The HIV prevalence among both TBLN and NTBLN patients in this study was higher than the national average HIV infection rate of 7.5% (12), indicating that both forms of lymphadenitis may be linked with the HIV epidemic. However, the rates obtained in the study groups were lower than the HIV coinfection in pulmonary TB cases (about 50%) reported from different places in Ethiopia (13) and are markedly low compared with similar reports from countries in sub-Saharan Africa where the HIV coinfection rate among TBLN cases was > 80% (4, 7).

Previous studies showed that clinical manifestations including constitutional symptoms are important in supporting the diagnosis of TBLN (2, 14, 15). However, it is difficult to differentiate clinically TBLN from NTBLN (16). In the present study the constitutional symptoms in TBLN

**Table III.** Summary of the algorithm recommended by the National TB Control Program for the diagnosis of tuberculous lymphadenitis (TBLN) in patients not responding to 3 weeks of treatment with broad-spectrum antibiotics and the relevance of findings in this study to strengthen the recommendations

<table>
<thead>
<tr>
<th>Stepwise clinical work-up</th>
<th>Indications for anti-TB treatment</th>
<th>Indications for biopsy</th>
<th>Findings from this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discharging sinus</td>
<td>√</td>
<td></td>
<td>Very rare, 1/107 (0.93%) had a discharging sinus</td>
</tr>
<tr>
<td>Patient age &gt; 18 y</td>
<td>√</td>
<td></td>
<td>Biopsy not used in the area because of lack of expertise. FNA cytology and AFB smear can be used</td>
</tr>
<tr>
<td>Cervical lymph node involved for patients &lt; 18 y</td>
<td></td>
<td></td>
<td>33/35 TBLN (94.3%) and 8/10 (80%) NTBLN cases below 18 y of age had cervical lymphadenitis. Cytology and AFB should be used for all suspected cases but the NTBLN cases must be worked out further with other diagnostic methods</td>
</tr>
<tr>
<td>Yes</td>
<td>√</td>
<td></td>
<td>Biopsy not used in the area because of lack of expertise. FNA cytology and AFB smear can be used</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TB, tuberculosis; FNA, fine-needle aspiration; AFB, acid-fast bacillus; NTBLN, non-tuberculous lymphadenitis.
cases were not different from those in NTBLN cases. Night sweat was the only constitutional symptom affected by HIV status, being more common in HIV-seropositive cases. The majority (92.5%) of patients in the NTBLN group had a cytology showing neutrophil aggregates suggestive of pyogenic infection. The differential diagnosis of patients in the NTBLN group may include malignancy and reactive lymphadenopathy (2, 4, 6, 12, 17).

The TBLN and NTBLN groups were no different in terms of many features listed in Table I, raising the concern that a significant number of patients in the NTBLN group could be missed cases of TB. This possibility was confirmed by the PCR result, where 65% of the NTBLN cases were found to be positive for M. tuberculosis. PCR could have a sensitivity as high as 94% (18) and its application can be expanded to include the rapid detection of drug-resistant TB (19) or species identification (10). The PCR results, particularly in the NTBLN group, could have 2 explanations. The first and direct one is that the PCR-positive cases in the NTBLN group were actually cases of active TB who could not be diagnosed because of a low sensitivity of cytology. The second explanation could be that at least some of the PCR-positive cases were cases with latent TB localized in the lymph nodes affected by a pyogenic infection; this is difficult to prove, but remains a theoretical possibility in areas where latent TB is common (20).

The National TB and Leprosy Control Program of Ethiopia has formulated an algorithm for the diagnosis of TBLN (8). The algorithm may be further improved with the findings from this study, as summarized in Table III. Even after 2 weeks of treatment with broad-spectrum antibiotics, cases diagnosed as NTBLN with cytology suggestive of pyogenic infection did not respond satisfactorily. A considerable number (65.2%) of these NTBLN cases were positive by M. tuberculosis specific PCR, indicating the need to use other diagnostic methods for these cases before the diagnosis of TB is ruled out.

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