HOW DOES CLINICAL DIAGNOSIS OF MYCOBACTERIAL ADENITIS CORRELATE WITH HISTOLOGICAL FINDINGS?

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ABSTRACT

Objective: To describe and compare histopathological findings with clinical criteria in diagnosis of TB adenitis.

Methods: Lymph node biopsies were obtained from 213 patients. Specimens were processed for culture and histopathologic examination, using standard methods. One hundred blocks with good preservation of tissue morphology were selected for detailed histological examination.

Results: About 75% of 213 patients had granulomas. In the remaining 25%, neither histopathological nor microbiological evidence of mycobacterial disease was found. Of 100 blocks selected for detailed examination, 79 blocks had granulomatous changes. The granulomas were well organised in 24%, mixed in 33%, and poorly organised in 43%. Langhans giant cells and acid-fast bacilli were observed in 88.6% and 21.5% of the 79 blocks, respectively. Cultured specimens were positive in about 10% of 79 biopsy specimens.

Conclusions: Histological evidence of mycobacterial disease was only found in three quarters of patients that were clinically diagnosed and started on empirical treatment for tuberculous adenitis. Neither histological nor mycobacteriological evidence was found in a quarter of the patients who were already on treatment for TB, basing on clinical criteria. These findings call for new research on simple diagnostic tools for patients who seek care for symptoms of extra-pulmonary TB.

Key Words: Tuberculosis adenitis, Granuloma, Treatment, Tanzania

Introduction

Tuberculosis remains a leading cause of death from infectious diseases (1). In 2001 alone, about 61,000 tuberculosis (TB) cases were reported in Tanzania, a six-fold increase from 1979. Extra-pulmonary tuberculosis (EPTB), which is 20% of all reported cases in the year, had increased almost 10 folds from 1979 (2). Mycobacterial adenitis is the most common form of EPTB, and is frequently reported in Arusha and Mbeya regions (2). The majority of the inhabitants in the two regions are subsistent farmers and cattle keepers, and they are at high risk of getting extrapulmory TB due to consumption of raw or not well cooked animal products. Tuberculosis can be transmitted from animals to humans through drinking or eating raw milk and other animal products.

In developing countries diagnosis of EPTB is more difficult than pulmonary tuberculosis (PTB). The diagnosis of mycobacterial adenitis depends on lymph node biopsy with histological and cultures examination (3). Most of the rural health facilities do not have the capacity to perform biopsy and histological examinations. At district level, the district hospitals are able to perform biopsy procedures but histopathology services are not available. About 70% of the population can only access health services at district and rural settings (4). Because of this diagnostic problem, the National Tuberculosis and Leprosy Control Programme (NTLP) has adopted clinical guidelines for diagnosis of EPTB, including mycobacterial adenitis (5). However, diagnosis which is based on clinical features might lead to over-diagnosis of mycobacterial adenitis .

Consequently, this could lead to over-treatment and, therefore, could cause development of mycobacteria resistant strains, over-reporting and over-burdening the TB services. The aim of this study is to describe and compare histopathological findings with clinical criteria in diagnosis of TB adenitis.

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Methods

Study areas and study procedures

Data collection was carried out in three districts of Manyara region and one district of Arusha region from 1999 through 2001. The study population included all patients who presented to health facilities with symptoms suggestive of extra-pulmonary TB according to the NTLP clinical guidelines (5). The guidelines define a case of extra-pulmonary tuberculosis as a patient with tuberculosis of other organs than lung, including lymph nodes. Diagnosis is based on strong clinical evidence consistent with active extra-pulmonary disease (including lymph nodes), followed by decision by a clinician to treat with a full course of anti-tuberculosis chemotherapy. Clinical evidence includes large lymph nodes (about 4 cm in diameter or more, or history of rapidly growing lymph nodes), tenderness, matted or fluctuant, and constitutional features like fever, weight loss, and night sweat. A total of 213 patients were enrolled in the study. All patients gave verbal consent to participate in the study.

Collection of biopsy specimens

Open biopsy specimens were taken from all patients fulfilling the clinical diagnostic criteria before starting anti-TB chemotherapy. Laparotomy was indicated for patients

presenting with peritonitis at Haydom and Dareda hospitals. For peritonitis cases, the cut surfaces of lymph node biopsies were examined for evidence of caseatious necrosis. If such materials were observed, a diagnosis of tuberculosis was made, and the patients were included in the study. The biopsy specimen from each patient was cut into two halves, in which one half was processed for culture and another half for histology. The half for culture was placed in a universal container and stored in a deep-freezer. The half for histology was fixed in 10% formalin and stored at room temperature.

Specimen processing and histological examination

The deep frozen biopsy specimens were processed for culture at the Central Tuberculosis Reference Laboratory. Inoculation was made on two Lowenstein Jensen media, one media contained glycerol and another pyruvate. Lowenstein Jensen media were incubated at 37^0C for 6 to 12 weeks while observing for signs of growth weekly.

The formalin fixed specimens were embedded in paraffin blocks, cut and stained with haematoxylin and eosin (H&E). All sections were stained by the Ziehl-Neelsen (ZN) method for acid fast bacilli (AFB), supplemented by periodic acid Schiff (PAS). Histological examinations using microscopy for the presence or absence of granulomas were performed by two independent pathologists. The pathologists were blinded for clinical information and culture results. On one slide, the first pathologist report indicated presence of granuloma while the second pathologist report was inconclusive. After re-examination it was agreed that the quality of the staining was poor but the inflammation had features consistent with granuloma.

Hundred slides, corresponding to specimens from 100 patients, were selected for a detailed histological examination by one pathologist. The selection criterion was based on the good preservation of morphology to enable detail microscopic examination of inflammatory cells. In lymph nodes where granulomas were present the pattern was classified into well organised, mixed and poorly organised. Well organised granulomas were characterised by varying amounts of eosinophilic necrosis (from little to extensive) surrounded by granulomas composed of mature epithelioid macrophages, mainly Langhans type giant cells and a mantle of lymphocytes and fibrous tissue. The mixed pattern was seen in lymph nodes also containing well organised granulomas but in between these were granulomas with coarse central necrosis often with nuclear debris, ill-defined mantle with epithelioid and immature macrophages, lymphocytes and plasma cells, varying numbers of giant cells with fewer Langhans type and a varying amount of fibrosis. Poorly organised granulomas were those characterised by central areas of sparse coarse necrosis with nuclear debris and often polymorphonuclear granulocytes. Also, the granulomas had an ill-defined mantle with mixed cells composed of macrophages, lymphocytes, and plasma cells. Only a few giant cells were seen and there was little or no fibrosis.

Role of the funding source and conflict of interest
The study sponsors had no role in study design; in collection, analysis, and interpretation of data or in writing of the report. There is no conflict of interest related to this article.

Results

The association of socio-demographic characteristics with histopathological findings of the study patients is described in table 1. The level of agreement between the two pathologists on presence or absence of granuloma was 100%. Granulomatous inflammation was found in 74.6 % of the study patients. Similarities in proportion of the granulomatous inflammation were observed between the subgroups of districts (74.3%, 136/183), gender (74.7%, 136/182), and age groups (74.6%, 135/181). The proportion of granulomatous inflammation was significantly higher ($\chi^2 = 4.1$, $p = 0.04$) in cervical adenitis (76.2%, 138/181) than in other sites (50.0%, 6/12).

Table 2 describes the histology of lymph nodes examined. Granulomatous inflammation was seen in 159 (74.6%) of 213 lymph nodes. In the remaining 54 the diagnosis was reactive hyperplasia in 18 (8.5%), squamous cell carcinoma in 1 (0.5%) and others category in 35 (16.4%). The category "others" included inflammatory changes with no features suggestive of tuberculosis which included mixed chronic inflammation, lymphoid tissue, fatty and fibrous tissue and keratin layer of skin.

Table 1: Socio-demographic characteristics of the study patients

socio-demographic characteristics	Histological features	
	Granulomatous % (n/N)	Other changes[i] (% n/N)
Districts (n = 183)		
Babati	72.8 (59 /81)	27.2 (22/ 81)
Mbulu	78.7 (37/ 47)	21.3 (10 /47)
Hanang	71.4 (30/ 42)	28.6 (12/ 42)
Karatu	76.9 (10/ 13)	23.1 (3/ 13)
Gender (n =182)		
Males	76.1 (70/ 92)	23.7 (22 /92)
Females	73.3 (66/ 90)	26.7 (24/ 90)
Age group (n =181)		
1-19	74.7 (67/90)	25.3 (23/90)
20-39	74.6 (50/67)	25.4 (17/67)
>39	75.0 (18/24)	25.0 (6/24)
Children (1-15 years)	72.0 (54/75)	28.0 (21/75)
Adenitis sites (n = 193) [ii]		
Cervical	76.2 (138/181)	23.8 (43/181)
Others sites [iii]	50.0 (6/12)	50.0 (6/12)

[i]Reactive lymphadenitis, mixed chronic inflammation, lymphoid tissue, malignancy (Squamous cell carcinoma)
[ii] Pearson Chi-Square = 4. 1, p = 0.04
[iii] Inguinal (3/6), Mesenteric (2/5), axillary (1/1),

Tables 2: Histological features among adenitis patients

Inflammatory Features	% (n/N)
(a) All blocks, (n = 213)	
Granulomatous inflammation	74.6 (159/213)
Reactive hyperplasia	8.5 (18 /213)
Squamous cell carcinoma	0.5 (01/213)
Others	16.4 (35/ 213)
(b) Selected blocks, (n = 100)	
Granulomatous inflammation	79.0 (79/100)
Well organized granulommatous	24 (19/79)
Mixed Organized granulommatous	33 (26/79)
Poorly organized granulommatous	43 (34/79)

Of the 100 blocks selected for a more detailed histological examination, 79 showed granulomatous inflammation consistent with TB. Of these, 19 (24%) showed only well organised granulomas, 26 (32.9%) showed a mixed pattern and 34 (43%) showed only poorly organised granulomas. Examination of ZN staining slides, revealed only occasional bacilli and their number varied from 1 to 2 per 100 high power fields. AFB were identified in 18/79 cases (22.8%) and were all in the group with granulomatous inflammation consistent with TB. PAS staining was negative in all cases examined including the poorly organised granulomas group.

Of 213 specimens cultured, 24 (11.3%) were culture positive, 134 (63.9%) were culture negative and 2 (0.9%) were contaminated. Among the 100 specimens of the selected blocks, 8 were culture positive and 69 culture negative.

Discussion

The frequency of extra-pulmonary tuberculosis including TB lymphadenopathy is increasing, especially in countries with high prevalence of HIV infection (3,6). The clinical criteria are widely used in most health facilities in Tanzania, except for the referral centres. Diagnostic tools for TB adenitis are limited while molecular methods are expensive. This leaves no choices to developing countries except relying on histological features in referral health facilities and culture in reference TB laboratories.

The diagnosis of EPTB is difficult and gets worse in rural health facilities, where about 70% of people live. Culture and histology services are neither available at region nor at district health facilities. Hence, diagnosis depends on clinical guidelines (1,5) that may present a dilemma in making diagnostic decisions and may cause over-diagnosis. In this study we used histological features and culture to evaluate the effectiveness of clinical criteria in the diagnosis of TB adenitis. The study found that, in 25% of the patients diagnosed using clinical criteria, had neither histopathological nor bacteriological evidence of mycobacterial adenitis. This could reflect an oversensitivity of the clinical criteria in diagnosing these patients.

Although the granulomas are highly suggestive of mycobacterial infection, there is a possibility that a few patients could have other diseases than mycobacterial disease. However, tuberculosis is endemic in developing countries, and is the most common cause of granulomatous inflammation in these countries. Therefore, granulomatous inflammation is presumed to be tuberculosis and treated as such (7). Though expensive, molecular methods could give more precise comparison analysis and should be considered in future studies.

The over diagnosis of about one quarter suggested by this study, indicated that patients in developing countries, like Tanzania, are started on unnecessary TB treatment. This practice could results in waste of resources, and is a potential risk for development of resistant strains to anti-tuberculosis drugs. Although the level of resistant strains in routine sample in the country is low (2), the increasing HIV infection rate (8) makes it difficult to predict the pattern in the future.

Since the treatment of tuberculosis takes up to six months, over-diagnosis would increase the burden to NTLP workers that are already over-stretched by increasing number of pulmonary tuberculosis. Consequently, this could reduce attention to infectious cases. Moreover, over-diagnosis would result in unnecessary over-reporting of extra-pulmonary tuberculosis in regional and national reports.

In poor resource countries, it is difficult to decentralise histopathogical services to health facilities in district and rural settings. Although the performance of the clinical criteria could be considered adequate, for the reasons discussed above, efforts to research on new and simple diagnostic tools are of paramount importance. Epidemiological and clinical research to refine clinical criteria and develop diagnostic tools applicable at district level should be encouraged.

The histological pattern described by this study varied from well organised to poorly organized granulomas. Although 43% of 79 lymph nodes with a granulomatous inflammation showed a poorly organised pattern, over half showed well organised granulomas or a mixed pattern. Only a few AFB were found and these features suggest that most of the patients had an almost normal immunological response to the mycobacterial infection.

The poorly organised granulomas and microabscesses are features that could occur in non-tuberculosis mycobacterial disease, in some other infections and in poor immunological response like in HIV infection (9,10). This study was carried out in rural districts where HIV infection is low.[8] In 2000, the numbers of AIDS cases reported from Babati, Mbulu, and Oldean hospitals were 32, 6, and 14 respectively (11). The histological features like microabscess and poorly organised granulomas have also been described in non-tuberculous mycobacteria infection (9,10).

The 22.8% of acid fast bacilli detection in the present study is as low as proportions reported in other studies that

range from about 20.0% to 50.0% (12). The scanty AFB in this study, broad clinical diagnostic criteria, and long transit time could explain low culture positivity in this study.

Conclusions

Histological evidence was found in three of every four Mycobacterial adenitis patients that were started on empirical TB treatment. Neither histological nor mycobacteriological evidence was found in a quarter of the patients started on TB treatment on clinical criteria. These findings call for new research on simple diagnostic tools for patients who seek care for symptoms of extra-pulmonary TB.

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