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Acid fast staining in formalin-fixed tissue specimen of patients with extrapulmonary tuberculosis

Mohammad Nassaji[1], Ramin Azarhoush[2], Raheb Ghorbani[3], Fateme Kavian[4]

[1]Associate professor of Infectious diseases Research Center for Social Determinants of Health, Semnan University of Medical Sciences, Semnan, Iran
[2]Associate professor of Clinical Pathology, Department of pathology, Golestan University of Medical Sciences, Gorgan, Iran
[3]Professor of Biostatistics, Research Center for Social Determinants of Health ,Department of Community Medicine, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran
[4]Medical students, Students' Research Committee, Semnan University of Medical Sciences, Semnan, Iran

Abstract- Diagnosis of extrapulmonary tuberculosis (EPTB) is difficult owing to low number of bacilli in the specimens, lack of adequate sample and non-uniform distribution of bacteria in tissues. The aim of this study was to investigate the utility of acid-fast bacilli (AFB) staining in biopsy specimens with typical granulomatous inflammation in patients with extrapulmonary tuberculosis and some related predictors.This study included 226 tissue biopsies of patients with EPTB showing typical granulomatous inflammation. Ziehl-Neelsen staining was performed for acid fast bacilli on paraffin embedded sections of tissue blocks. The most common site of involvement was pleura followed by vertebral and lymph nodes. Past history of pulmonary tuberculosis was positive in 46% of patients. The overall AFB positivity in specimens was 26.1%. The most positivity was in pleural TB (35.2%) and the least was in bone and joints TB (4.8%). There was significant association between site of involvement and AFB positivity (p=0.042). In multivariate logistic regression model, previous history of pulmonary tuberculosis was strongly associated with AFB positivity. Our study showed somewhat higher rate of smear positivity for acid fast bacilli in tissue specimen with typical pathology in some types of EPTB especially in patients with history of pulmonary tuberculosis. Despite low sensitivity, this method should be performed in patients suspected to EPTB especially in developing counties where new modality is not routinely available.

Index Terms- Acid-fast bacilli, Extrapulmonary tuberculosis, Formalin-fixed tissue, Ziehl-Neelsen staining

I. Introduction

Tuberculosis (TB) remains one of the most important infectious diseases affecting human. Despite longstanding intense efforts to conquer tuberculosis, this disease still is an important global health problem and is a major cause of death worldwide.[1]

Extrapulmonary tuberculosis (EPTB), like pulmonary TB is a significant health problem in both developing and developed countries. Over the last several years, the number and proportion reported cases with EPTB was increasing.[2] Human immunodeficiency virus infection has been identified as a major risk factor that is associated with increase in EPTB incidence.[3] EPTB accounts for 15-20% of tuberculosis cases in areas with low HIV prevalence. It can increase up to 50% in patients with concurrent AIDS and tuberculosis. The frequencies of different clinical sites affected by tuberculosis vary from one to another population.[4] Clinical manifestations of EPTB are so protean that diagnosis is often delayed and depends on the physician considering the possibility of disease in patients. Laboratory diagnosis of EPTB is also difficult owing to low number of bacilli in the most specimens, lack of adequate clinical sample and non-uniform distribution of bacteria in tissues.[5]

Several methods are used for the diagnosis of EPTB such as imaging, pathological examination, smear microscopy, mycobacterial culture and nucleic acid amplification tests.[6] Culture is the standard method for diagnosing of mycobacterial infection from tissue, but still require many weeks for final results. The first step in the diagnosis of mycobacterial infection is microscopic examination of a smear prepared from a specimen and stained for acid-fast bacilli (AFB). The stained smear is the easiest and quickest procedure that can be performed for diagnosis of TB and is economical especially in developing countries.[7] The Ziehl-Neelsen (ZN) staining method has commonly been used for AFB demonstration in smears around the world. In this method, a fixed smear covered with carbol-fuchsin is heated, rinsed, decolorized with acid-alcohol, and counterstained with methylene blue.[8]

In EPTB pathological findings and histological detection of acid-fast bacilli (AFB) in tissue is two way to support or confirm the presence of disease.[9] Despite widely use of smear microscopy in the diagnosis of EPTB, it has limitations due to low and variable sensitivity and specificity values. Various studies reported different sensitivity for this method.[10]

Another way for diagnosis of EPTB is by histopathological examination of tissue sample for the presence of granulomatous inflammation and caseous necrosis. Typical granuloma of TB infection consisting of central, infected macrophages, surrounded by epithelioid macrophages, foam cells, and multinucleated giant cells of the Langhans type, with peripheral fibrous capsule.[11]

Several previous studies have examined the utility of AFB staining in EPTB. However, these studies reported different rate of smear positivity. Some of the studies included only cytology smear specimens especially in patients with tuberculosis lymphadenopathy. On the other hands most previous study conducted on small number of patients and or on one type of EPTB.[12-16]

The purpose of this study was to evaluate demonstration of acid fast bacilli in tissues section showing typical histopathological features of tuberculosis (highly suggestive) in patients with EPTB using ZN staining method. Another aim was to evaluate some probable predictors of staining positivity in these patients.

II. MATERIAL AND METHODS

The study material comprised tissue specimens from patients with EPTB collected during the period from 2003 until 2012. The study was performed in a university affiliated hospital in Golestan province north-east of Iran that is referral center for EPTB. This province has high prevalence of tuberculosis. Diagnosis of EPTB was based on clinical, imaging, typical histological findings (highly suggestive) of TB in tissue and response to treatment in accordance with WHO diagnostic criteria.[4] All histological tissues that showed the presence of of granulomatous inflammation with epithelioid cells, Langerhans type giant cells, and caseation necrosis were considered as typical (highly suggestive).

The clinical and demographic records were also retrieved about patients included age at diagnosis, gender, co-morbidity, site of involvement by EPTB and previous history of pulmonary tuberculosis. Previous co-morbidities variables included in study were diabetes, malignancy, collagen vascular diseases and chronic renal failure.

Formalin-fixed, paraffin-embedded tissue blocks from patients with EPTB and typical pathologic findings were included in this study. All samples were obtained from the patients before treatment with anti-tuberculosis drugs.

Ziehl-Neelsen staining (Merck, Germany) was performed for acid fast bacilli on sections of tissue blocks. All ZN-stained smears were examined under a conventional light microscope (oil immersion objective) by one pathologist. Cases with any missing information or inappropriate tissue specimen were excluded.

The study protocol was approved by Research Council and Ethical Committee of the Semnan University of Medical Science.

III. STATISTICAL ANALYSIS

Data were analyzed by using Chi-square test and logistic regression analysis using SPSS Version 16.00(SPSS, Inc., Chicago, IL). The p-value less than 0.05 were considered statistically significant.

IV. RESULTS

We finally studied 226 blocks of formalin-fixed, paraffin-embedded tissue from same numbers of patients with diagnosis of EPTB. From all patients that fulfilled inclusion criteria 105 (46.5%) were male. The mean (± SD) age of patients was 38.9 ±19.5 years (range 4-85years). Co-morbidities were found in 48 (21.8%) patients.

Previous history of pulmonary tuberculosis was positive in104 (46%) of patients. History of TB was more common in patients with peritoneal (71.4%, 161/226), spinal (60.5%, 136/226) and pleural (50.8%, 114/226) involvement. The least history was positive in patients with gastrointestinal TB (18.8%, 42/226).

Pleura was the most common site of involvement followed by vertebral. Table 1 outlines the various sites of involvements for the patients with EPTB. The overall AFB positivity by ZN staining in specimens was 26.1% (95% CI: 20.4%-31.8%). The most AFB positivity was in pleural TB (35.2%) and the least was in bone and joints TB (4.8%). There was significant association between site of involvement and AFB positivity (p=0.042).

In patients with previous history of pulmonary tuberculosis AFB was positive in 50% (113) of samples. Whereas, in those without history only in 5.7 % (13) was positive (p<0.001). Table 2 shows AFB positivity and its correlation with patients' characteristics.

After adjustment for potential confounders in a multivariate logistic regression model, previous history of pulmonary tuberculosis remained strongly associated with AFB positivity (OR=15.77, 95% CI: 6.68-37.26, p<0.001).

V. DISCUSSION

The diagnosis of EPTB is a challenging problem for clinicians. In most types of EPTB conventional diagnosis of mycobacterial infections is based on the detection of acid-fast bacilli in histological specimens. But, searching for tubercle bacilli in tissue sections is laborious because the numbers of bacilli are usually few.[17] Positive smear results in conjunction with clinical and radiologic findings can be used for presumptive diagnosis of tuberculosis, which is the common practice in many developing countries.[9]

The distribution of various types of EPTB has varied among different populations and countries. In our study among the patients that selected by typical pathology the most common site of involvement was pleura followed by vertebra and lymph nodes. In contrast to our finding most previous studies reported that tuberculous lymphadenitis is the most common type of EPTB.[18-21] Others studies reported different results. In a study on Turkish patients the most commonly seen types of EPTB were genitourinary (27.2%) and meningeal TB (19.4%).[22] Another study performed on 85 culture-proven EPTB cases in Arkansas showed that bone and/or joint tuberculosis was the most common type of extrapulmonary tuberculosis (27.1%).[23] Methodological differences, such as the difference in the inclusion criteria and type of sample selection can be possible reasons for the various distribution of EPTB in different population. We only included tuberculosis lymphadenopathy that was detected by excisional biopsy and typical pathology. But most other researcher used fine needle aspiration for diagnosis of tuberculous adenitis in their study.

Previous history of pulmonary tuberculosis was positive in 46% of our patients. Cagatay et al in their study reported that of 252 patients, 55 (21.8%) had a previous history of pulmonary tuberculosis.[19] In another study that conducted on 312 patients with EPTB, I5.4% of patients had history of pulmonary TB.[24]

Various studies conducted in the past have shown different sensitivities for ZN staining in diagnosis of EPTB. The overall AFB positivity in our specimens was 26.1%. Most previous studies reported lower rate of AFB positivity in patients with

EPTB. In a study on 252 immunocompetent adult patients with extrapulmonary tuberculosis, staining was positive in 17.8% of patients.[19] In another study the overall sensitivity of conventional smear microscopy was only 3.9% (three of 76) for detecting EPTB.[5] Salian et al have shown 25% (15/60) positivity by acid-fast staining of histological specimens.[25] A study conducted on 182 samples from suspected EPTB cases for testing of M. tuberculosis by different methods. Results showed that ZN staining was positive in 3.3% of samples.[26]

Few studies reported somewhat higher sensitivity. Mahaisavariya et al evaluated one hundred and thirty-one tissue blocks with AFB staining and PCR .The causative organisms were identified by AFB staining in pathologic sections in 31.29% of specimens.[27] Another study conducted on the archival formalin fixed paraffin embedded tissue sections of patients with EPTB showed that AFB positivity was observed in 36.1% of tissue with tuberculous granulomas.[28] Different sample selection can be a reason explaining this varied results.

Our study illustrated that site of involvement is significantly associated with rate of AFB positivity. The AFB staining was more positive in specimens of pleural TB (35.2%) and least in bone and joints (4.8%). In Cagatay et al study the most positivity was in pleural TB (33.3%) and the least in peritoneal (10%).[19] more previous studies reported lower sensitivity for pleural TB. Chakravorty et al in their study evaluated 99 extrapulmonary specimens collected from 87 patients with different methods. Pleural tissue smear was positive in 12.5% of specimens.[5] In another study from 36 patients with pleural TB, 9.3% had positive direct examination.[29] Hasaneen et al reported that ZN staining results of biopsy specimens were positive in only 1 of 26(3.8%) patients with pleural tuberculosis.[30]

Second rate of positivity in our study was lymph node biopsy (31%). This finding is compatible with some previous studies. In Patwardhan et al study, biopsies of 65 patients with tubercular lymphadenopathy were evaluated for comparison of various laboratory diagnostic modalities. Twenty (30.7%) of tissue samples were positive on ZN staining.[14] In a retrospective study clinical and laboratory data of 141 patients with EPTB were evaluated by authors. Acid-Fast Stain staining was positive in 30% of Lymph node samples.[31] In contrast in a study conducted on 100 patients with diagnosis of lymph node tuberculosis by histopathology, only 3% of biopsies were positive in the ZN staining.[13]

Vertebral biopsy showed AFB in 30.2% of ours cases. Similarly, in a study that included patients with tuberculous spondylitis, 30% of biopsies were positive for acid-fast bacilli on staining.[32] In another study 25% of surgical biopsies compatible with vertebral tuberculosis were positive for AFB.[33]

In cutaneous specimens AFB was positive in 12.5% of our cases. In comparison with this finding, one study on skin specimens demonstrated that 13.8% of biopsy specimens from patients with lesions compatible with tuberculosis of the skin had a positive AFB.[15] Other authors reported 5.8%, 15.8% and 25.8% AFB positivity in patients with cutaneous tuberculosis.[34, 16, 35]

One striking finding in our study was the significant association of previous history of pulmonary tuberculosis with AFB positivity. Half of the samples from patients with this history were positive in staining. Also multivariate analysis showed statistically significant association between AFB positivity and history of pulmonary tuberculosis. This may be to immunological status of patients that experienced two episode of tuberculosis. More research about this finding is recommended in future studies.

The strength of study was the selection of tissue compatible with typical tuberculosis histology. In this study, culture was not used as a reference for comparison of sensitivity and specificity of AFB staining. This is one limitation of this study.

VI. CONCLUSION

Although our study showed somewhat higher rate of smear positivity for detecting AFB in tissue specimen with typical pathology in some types of EPTB, nevertheless, it is low when compared with culture and PCR. Past history of pulmonary TB may be considered as predictor factor that increase the sensitivity of AFS. Despite low sensitivity, acid-fast staining should be performed on all tissue in patients suspected to EPTB especially in developing countries where new modality like PCR and mycobacterial culture is not routinely available. Another reason for use of the acid-fast staining is the rapid time and low cost compared with culture and PCR. A positive AFS helps the clinician for initiating treatment much earlier while waiting for culture results. Finally, it is better to utilize a combination of all the available tests for the diagnosis, which provide maximum useful information to the clinicians.

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Table 1. Distribution of organ involvement in patients with extrapulmonary tuberculosis

organ	Number	Percentage
Pleural	65	28.8
Vertebral	43	19.0
Lymphadenopathy	29	12.8
Bone and joints	21	9.3
Gastrointestinal	16	7.3
Cutaneous	8	3.6
Peritoneal	7	3.2
Genital	5	2.3
breast	4	1.8
Urinary	3	1.4

Others	25	11.0

Table 2. Acid fast bacilli positivity and its correlation with patients' characteristics.

Characteristic	n	Acid fast Positive n (%)	p-value
Gender			
Male	105	25(23.8)	NS(0.464)
Female	121	34(28.1)	
Age(year)			
<20	33	8(24.2)	
20-29	51	15(29.4)	
30-39	37	11(29.7)	NS(0.464)
40-49	25	4(16.0)	
50-59	37	7(18.9)	
60-69	24	5(20.8)	
70≥	19	9(47.3)	
TB History			
+	104	52(50.0)	< 0.001
-	122	7(5.7)	
Co-morbidity			
+	48	17(35.4)	NS(0.098)
-	178	42(23.6)	
TB Location			
Pleura	65	23(35.2)	
Vertebral	43	13(30.2)	
Lymph node	29	9(31.0)	
Bone & Joint	21	1(4.8)	0.042
Gastrointestinal	16	1(6.2)	
Cutaneous	8	1(12.5)	
Peritoneal	7	2(28.6)	
Other site	37	5(13.5)	

NS: not significant

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AUTHORS

First author-Mohammad Nassaji*, Associate professor of Infectious diseases, Research Center for Social Determinants of Health, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran.

Second author- Ramin Azarhoush, Associate professor of Clinical Pathology, Department of pathology, Golestan University of Medical Sciences, Gorgan, Iran.

Third author- Raheb Ghorbani, Professor of Biostatistics, Research Center for Social Determinants of Health, Department of Community Medicine, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran.

Fourth author- Fateme Kavian, Students' Research Committee, Semnan University of Medical Sciences, Semnan, Iran.

Corresponding Author: Mohammad Nassaji, Kowsar Hospital, Basij Blvd, Semnan, Iran, hnassaji@yahoo.com, mnzmohammad@gmail.com
Tel: +98 2333437821, Mobile: +98 09121318640