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Assessment of diagnostic value of different methods (culture, PCR and biopsy) for the diagnosis of tuberculosis in patients with bronchial anthracosis

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Abstract

Introduction: Although several studies have shown the relationship between tuberculosis (TB) and anthracosis, few studies have investigated the diagnostic value of various methods of diagnosis of TB in these patients.
Objectives: This study was conducted to investigate the diagnostic value of different methods including smear, culture and polymerase chain reaction (PCR) of bronchoalveolar lavage (BAL) sample and bronchial biopsy specimens in the diagnosis of TB in patients with bronchial anthracosis.
Patients and Methods: This descriptive cross-sectional study was performed on 67 patients referring for bronchoscopy, and bronchial anthracosis was an incidental finding. The BAL sample and patients' bronchial biopsies were examined for TB infection.
Results: The most affected areas by anthracosis were the right and the left upper lobes. The BAL smear and its culture were positive in 12 (20%) and 10 patients (17%), respectively. The PCR of BAL sample was positive in 37%. Non-specific chronic inflammation followed by bronchial anthracosis was the most common pathologic findings. According to pathological examinations, nine patients (13%) had TB. Sensitivity and specificity of the PCR versus culture were 90% and 70%, respectively, with a diagnostic accuracy of 74%. Sensitivity, specificity and diagnostic accuracy of the pathologic examination of biopsy specimen were 33%, 85% and 74%, respectively.
Conclusion: Findings of this study showed that nearly one fifth of patients with bronchial anthracosis suffer from TB simultaneously. In these patients, PCR of the BAL and pathologic examination of the biopsy specimen had the same diagnostic accuracy, but the sensitivity of PCR was three times higher. However, application of PCR and pathologic examination does not seem to have more diagnostic advantages than BAL culture for the diagnosis of TB, although the results by PCR may help to diagnose the disease sooner.

Introduction

Anthracosis is a bronchoscopic finding which is characterized by formation of black pigments in the airways and mucous membranes of bronchi. Anthracosis can be the result of bronchial injury, metamorphosis, and obstruction. Pathologically, these lesions seem to be due to the deposition of silica, carbon and asbestos particles in the cytoplasm of mucosal and submucosal cells, as well as macrophages (1, 2).

Although initially it was thought that anthracosis had been a type of pneumoconiosis in coal workers (3), later this view was changed, and anthracosis or bronchial anthracofibrosis was considered as a different clinical syndrome (1), which could be due to the spread of urbanization and the presence of particles of carbon, asbestos and

Key point

In patients with bronchial anthracosis who suffer from tuberculosis simultaneously, application of PCR and pathologic examination does not seem to have more diagnostic advantages than BAL culture for the diagnosis of TB, although the results by PCR may help to diagnose the disease sooner.

silica in the air. The prevalence of this disease is low in western countries, North America and Africa, with only a few reports (4-6), but more prevalent in Asian countries. Overall, the prevalence of simple anthracosis in patients who underwent bronchoscopy has been reported 3.4%-21% in various studies, while its cumulative prevalence is 5.7% (0.1-22.5%) (7,8). In a previous study from Iran, its prevalence is reported to be 1.1-31.89 % (9).

Pulmonary tuberculosis (TB) and AIDS are the most important public health problems in developing countries, because TB is a chronic disease and has its own treatment protocol, which imposes a high cost on the health care system. Several studies have reported the relationship between TB and anthracosis (9). In addition, the risk of TB and non-TB pneumonia is higher in patients with anthracosis (10). On the other hand, TB infection in these patients may have an atypical presentation, i.e., it does not have positive TB skin test (purified protein derivative; PPD) or does not form granuloma or may cause by certain subtypes of mycobacterium TB. Thus, the relationship between TB and anthracosis is complex, while there is a debate on their cause and effect relationship. Despite this complicated relationship, few studies have been carried out to assess the diagnostic value of different methods in the diagnosis of TB in patients with bronchial anthracosis (11,12).

Objectives
The aim of this study is to investigate the diagnostic value of different methods (including culture and polymerase chain reaction [PCR] of bronchoalveolar lavage [BAL] sample and pathologic examination of bronchial biopsy specimen) in the diagnosis of TB in these patients.

Patients and Methods
Study design
This descriptive cross-sectional study was performed on 67 patients who referred to the bronchoscopy department of Dr. Masih Daneshvari hospital in Tehran who had undergone bronchoscopy, and bronchial anthracosis (deposition of black pigments) or anthracotic bronchitis (deposition of black pigments with edema, deformity or stenosis of bronchi) were detected during the procedure. The study was explained to all patients before performing bronchoscopy. Patients' information including demographic data (age, gender, occupation and nationality), history of baking and its duration, fuel used for cooking and heating the house, smoking, symptoms and signs, history of TB and radiographic and CT scan findings were recorded. The location of the lesions in bronchoscopy was recorded. BAL samples were examined for smear, culture and PCR of TB and bronchial biopsy specimens were studied pathologically. However, due to some reasons such as bleeding etc., it was not possible to obtain BAL or tissue sample in all patients, additionally in some patients tissue sample was insufficient for evaluation.

Ethical issues
The study was approved by ethical committee of Shahid Beheshti University of Medical Sciences, since written consent was obtained from each patient before the study. In all stages of research, the tenets of the Declaration of Helsinki were observed. Additionally, This study was extracted from fellowship thesis of Seyed Shahabedin

Mohammad Makki at this university (Thesis#141).

Statistical analysis
Data were analyzed by SPSS 13 software. Qualitative variables were described by frequency (percentage) and quantitative variables by using range, mean and standard deviation. In addition, the efficacy (sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy) of PCR of the BAL and the pathologic examination of the biopsy specimens versus the result of BAL culture for TB were calculated.

Results
Thirty-seven patients (55%) were male and 30 (45%) were female. The range and mean (SD) of their age were 41-87 years and 70 ± 10 years, respectively. Thirteen (19%) were foreigners, while most of them (21 patients, 31%) were farmers. Fourteen patients (21%) were smokers, 10 (15%) had a history of TB, seven of which were treated, and all patients had a negative sputum smear. Twenty-six patients (39%) had a history of baking, and the range and mean (SD) of baking time were 3-58 years and 33 ± 17 years, respectively. Gas was the most commonly used fuel for both cooking and heating the house (73% and 64% respectively) (Table 1).

Cough was seen in 61 patients (91%), sputum in 43 (64%), dyspnea in 50 (75%), fever in 25 (37%), sweating in 34 (51%), weight loss in 37 patients (55%) and history of recurrent infection in four (6%). On physical examination, 12 patients (18%) had wheezing, eight (12%) had rales, and 11 (16%) patients had decreased breathing sounds.

The most common findings in chest X-ray (CXR) of patients were infiltration (in 22, 33%) and normal radiography was observed in 12 (18%) patients. Similar to CXR, the most common finding in CT scan was infiltration (in 20, 30%), followed by collapse (in 14%, 21%). Only four (6%) had normal CT scans (Table 2).

Table 3 shows the frequency of mucosal edema, anthracosis, and obstruction in different regions of the lower respiratory tract. The most common involved regions were the left upper lobe (LUL) and the right upper lobe (RUL), since each patient had average of 4.5 anthracotic regions in each lung (other than trachea and carina).

In 59 patients, the BAL sample was adequate for smear and culture. Twelve (20%) were smear positive and 10 patients (17%) had positive culture. Overall, in 10 patients (17%) both smear and culture were positive. In addition,

Table 1. Type of fuel used for cooking and heating the house

Type of fuel	Cooking	Heating the house
Gas	49 (73%)	43 (64%)
Wood	34 (51%)	30 (45%)
Oil	36 (54%)	42 (63%)
Biomass	26 (39%)	20 (30%)

Table 2. Radiographic findings of patients' chest

Findings	CXR	CT scan
Collapse, infiltration and bronchiectasis	-	2 (3%)
Collapse and infiltration	9 (13%)	13 (19%)
Infiltration and bronchiectasis	-	2 (3%)
Collapse	16 (24%)	14 (21%)
Infiltration	22 (33%)	20 (30%)
Others	8 (12%)	12 (18%)
Normal	12 (18%)	4 (6%)

Table 3. Prevalence of involvement in different parts of the lower respiratory tract of patients

Place	Mucosal edema	Anthracosis	Obstruction
Trachea	2 (3%)	2 (3%)	0 (0%)
Carina	3 (5%)	6 (9%)	0 (0%)
RMB	12 (18%)	26 (39%)	3 (5%)
RUL	28 (42%)	47 (70%)	17 (25%)
RML	21 (31%)	43 (64%)	15 (22%)
RLL	18 (27%)	40 (60%)	8 (12%)
LMB	10 (15%)	23 (34%)	1 (2%)
LUL	29 (43%)	56 (84%)	17 (25%)
Lingula	22 (33%)	43 (64%)	15 (22%)
LLL	14 (21%)	35 (52%)	8 (12%)

RMB: Right main bronchus, RUL: Right upper lobe, RML: Right Middle lobe, RLL: Right lower lobe, LUL: Left upper lobe, LML: Left middle lobe, LLL: Left lower lobe, LMB: Left main bronchus.

Table 4. Pathologic findings

Pathologic findings	No. (%)
Chronic non-specific inflammation	29 (44)
Bronchial anthracosis	10 (15)
Granuloma	5 (8)
Granuloma and bronchial anthracosis	2 (3)
Small cell carcinoma	2 (3)
Parenchymal anthracosis	1 (2)
Fibrosis and parenchymal anthracosis	1 (2)
Granuloma and parenchymal anthracosis	1 (2)
Non-small cell carcinoma	1 (2)
Granuloma and silicone nodule	1 (2)
Failure to sampling or insufficient sample for pathological examination	14 (21)

only 25 patients had an adequate biopsy specimen for smear and culture since the latter was positive in only one case. In 60 patients, BAL sample was examined for PCR, which was positive in 22 (37%) patients.

Overall, a total of 62 patients had various samples for pathologic examinations while nine of which (13%) were inadequate. In 53 remaining patients, non-specific chronic inflammation followed by bronchial anthracosis were the most common pathologic diagnosis. In total, nine patients (13%) had granuloma based on a pathological examination (Table 4).

Of 59 patients who had BAL culture, 53 patients had sufficient samples for PCR and 48 had sufficient samples for pathologic examination for TB. Compared to BAL culture, the sensitivity, specificity, and diagnostic accuracy of PCR was 90%, 70%, and 74%, respectively and pathologic examination was 33%, 85%, and 74%, respectively (Table 5).

Discussion
Findings of the study showed that, compared to BAL culture, the diagnostic accuracy of both PCR and pathologic examination for TB was similar, but the sensitivity of the PCR of the BAL sample was about three times more than that of BAL culture.

In most of the studies, association of anthracosis with TB is highlighted. The prevalence of TB in these patients is reported as 31.88% by Mortazavi Moghaddam et al (9). Generally, the cumulative prevalence of TB in patients with anthracosis is reported 7.5% on average (7). However, whether anthracosis is the cause of TB or vice versa, it has not yet been addressed and long-term studies are necessary for this area.

In our study, 17% of patients were diagnosed with TB by BAL culture, while 37% of them were diagnosed with TB by PCR of BAL sample alone. Pathologic examinations revealed 13% of patients had TB. Therefore, considering BAL culture as a gold standard for diagnosis of TB, PCR seems to have had more false positive results. In addition, sensitivity of PCR of the BAL sample in comparison with BAL culture was 90%, its specificity was 70%, and its diagnostic accuracy was 74%. We also found that the sensitivity of tissue biopsy in diagnosis of TB was 33% in comparison with the BAL culture, while its specificity was 85% and its diagnostic accuracy was 74%. In other words, both PCR and pathologic examinations had the same diagnostic accuracy, but sensitivity of PCR was about three times higher. However, both methods had a low positive predictive value (about 40% or less), which means the positive test of each of these two methods indicates a 40% probability of TB in these patients.

In general, sensitivity of culture in diagnosis of TB was reported 80-85%, and its specificity was 95%. Although various studies have examined the prevalence of TB in anthracosis, only a few have assessed sensitivity and specificity of diagnostic methods of TB in these patients. Mirsadraee et al found acid fast bacilli in anthracosis and anthracofibrosis in 17.5% and 25% of patients respectively, and caseous granuloma in 20% and 17.5% of cases, respectively (13). Na et al reported sensitivity and specificity of PCR of bronchial washing specimen in the diagnosis of TB in 30 patients to be 89% and 86%, respectively (12) which is higher than the results of our study. Different PCR methods in these two studies could explain this difference. Mirsadraee et al also examined different methods in diagnosis of TB in patients with

Table 5. Clinical efficacy of PCR of BAL sample and pathological examination of biopsy specimen versus BAL culture for diagnosis of TB in patients with anthracosis*

	PCR of BAL sample (n=53)		Pathological examination of biopsy specimen (n=48)	
	Positive	Negative	TB (granuloma)	Others
Having TB (positive BAL culture)	9	1	3	6
Not having TB (negative BAL culture)	13	30	6	33
True positive	17%		5%	
False positives	25%		13%	
True negative	56%		69%	
False negative	2%		13%	
Sensitivity	90%		33%	
Specificity	70%		85%	
Positive predictive value	41%		33%	
Negative predictive value	97%		85%	
Diagnostic accuracy	74%		74%	

* Of 67 patients, 59 patients had BAL culture, of which 53 were BAL PCR and 48 had a pathologic biopsy specimen for diagnosis of TB.

bronchial anthracosis, and reported the sensitivity and specificity of PCR compared to control group to be 66% and 100%, respectively. However, they detected no significant difference between conventional methods and PCR in the diagnosis of TB in anthracosis patients. Finally, they suggested that PCR should only be performed if there is insufficient expertise to perform traditional techniques or if histopathological examination of the tissue is not possible due to bleeding (11).

Old age and female gender are reported as risk factors for anthracosis (4). Our study showed that the average age of patients was 70 and about half of them were women. Other studies have also suggested that anthracosis is the most common cause of chronic obstructive pulmonary disease in non-smoker females in developing countries (13-15). Like in other studies, only one-fifth of our patients were smokers. Old age of patients may be due to the long-term exposure required for deposition of specific environmental factors in airways. Additionally, physiologic changes in the airways of old people may be a predisposing factor for deposition of particles of biomass fuels. As many patients are non-smoker housewives in rural areas with a history of baking, the long-term history of baking is one of the risk factors for anthracosis and anthracofibrosis. In our study, 39% of patients had a history of baking. Hence, old age and history of baking or prolonged exposure to smoke or biomass fuels, particles of which mainly contain carbon, can help to recognize the pathophysiology of this disease to some extent. However, anthracosis may be an occupational disease, which is mentioned in some studies too (15-17).

Conclusion
Findings of this study revealed that nearly one-fifth of anthracotic patients suffered from TB simultaneously. In these patients, PCR of BAL sample and the pathologic examination of the biopsy sample have similar diagnostic accuracy (both 74%), but sensitivity of PCR is three times higher (90% versus 33%). However, the predictive value

of both methods is around 40%, which means the positive result of these two methods indicates a 40% probability of TB in these patients. However, application of PCR and pathologic examination does not seem to have more diagnostic advantages than BAL culture for the diagnosis of TB, although the results by PCR may help to diagnose the disease sooner.

Limitations of the study
Some of our study limitations were small sample size, single center study, and not enough sample for pathologic examination in some patients.

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