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The Study of *Mycobacterium tuberculosis* in Iranian Patients With Lung Cancer

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A B S T R A C T

Background: *Mycobacterium tuberculosis* has the ability to invade type II alveolar epithelial cells. As a result, the associations between invasion of alveolar epithelial cells and pathogenesis of lung infection seem strong.
Objectives: The current study aimed to evaluate the presence of *M. tuberculosis* in patients with lung cancer.
Patients and Methods: This cross-sectional study was performed on samples collected from 380 patients with lung cancer who referred to two state-run hospitals in Mashhad, Iran. Microscopic and cultural methods were utilized to assess the presence of *M. tuberculosis* in the patients` specimens.
Results: The subjects included 252 (66.3%) males and 128 (33.7%) females. Based on cultural and microscopic methods, *M. tuberculosis* infection was observed in twenty six (6.8%) of cases.
Conclusions: Results of the current study showed the high prevalence of *M. tuberculosis* among the patients with lung cancer; therefore, it seems that continuous surveillance is essential to monitor the *M. tuberculosis* in the patients with lung cancer.

Keywords: Mycobacterium tuberculosis; Lung Cancer; Epidemiology

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Implication for health policy/practice/research/medical education:
It seems necessary for clinicians and health care systems to be fully aware of *Mycobacterium tuberculosis* in patients with lung cancer and continuous surveillance is essential to monitor the patients for proper management.

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1. Background

Worldwide, lung cancer constituted 13% (1.6 million) of the total cases of cancer in 2008(1). Lung and bronchus cancers caused death of an estimated 156,940 in the United States in 2011(2). In Iran, it is the fifth most common cause of cancer and according to the two reports published in 2002 and 2003, its prevalence was considered to be very low (3, 4).

Overall, it is estimated that one-third of the world's population is infected with *Mycobacterium tuberculosis* (MTB). The incidence is directed toward Asia, where China, India, Bangladesh, Indonesia and Pakistan constitute over 50% of the total cases (5). In Iran, in 2005 it was reported that the prevalence of tuberculosis (TB) was high in border-line provinces such as Sistan and Balouchestan, Golestan, Khorasan, Hormozgan and Khouzestan (6).

Several studies have explained the possible mechanisms behind the role of MTB infection and lung cancer in inducing genetic damage. Nalbandian *et al*. findings in 2009 provided evidence for the role of chronic MTB infection in cell dysplasia and squamous cell carcinoma (SCC) (7). Lung cancer is supposed to be related to pulmonary inflammation; since inflammatory cells release activated oxygen molecules, inflammatory mediators and proteolytic enzymes promote molecular changes leading to cancer (8). It was suggested that TB intensifies the probability of lung cancer due to the fact that inflammation and fibrosis may result in genetic damage (9).

2. Objectives

The current study was conducted to assess the presence of *M. tuberculosis* in patients with lung cancer.

3. Patients and Methods

This cross-sectional study was carried out on samples collected from 380 patients with lung cancer who referred to two state-run hospitals in Mashhad between May 2009 and October 2011. Patients underwent bronchoscopy and evidence for lung cancer was observed. Informed consent was obtained from participants. After preparing bronchoalveolar lavage fluid for pathological observations to confirm the previous diagnosis, extra lavage fluid was employed in the study for microscopic investigation and also for the bacterial culture. It is critical to note that no extra samples were obtained for the research. One hundred and thirty seven of the total cases lack cytological results. Regarding the fact that verifying lung cancer requires cytological studies by at least two pathologists, two surveys were employed. Initially, 243 patients with thorough cytological results, and subsequently, the total 380 patients were examined.

For bacteriological tests, samples were decontaminated and homogenized with equal amount of 4% NaOH. After neutralizing procedures by 2 N HCl, five drops of re-suspended sediments were subsequently inoculated to Lowenstein-Jensen solid medium and then incubated in a slanted position for about two months. Two drops of the sediments were used for indirect smear preparation (Ziehl- Neelsen staining). Slide reading followed the recommendations outlined in World Health Organization (WHO) guidelines and Mycobacteria confirmed with phenotypic results.

Statistical analysis was performed using Pearson's Chi-square and in cases when more than 20% of the expected frequencies were less than five, Fisher's exact test was used. In order to interpret multivariate statistics, Logistic regression was carried out. SPSS v.20 (SPSS Inc., Chicago, IL, USA) was employed for the statistical analysis. Differences with $P<0.05$ were considered to be statistically significant.

4. Results

In the current study, 380 patients clinically diagnosed with lung cancer were employed. The subjects included 128 (33.7%) female and 252 (66.3%) male. *M. tuberculosis* was positive by culture in 26 cases out of 380.

No significant statistical differences were observed between the culture results and sex (P value=0.068), age groups (P value=0.637), Marital status (p-value=0.170), Literacy (P value = 0.223), Smoking (P value=0.476) (*Table 1*). The data showed statistically significant differences between addiction and the positive results in culture method (P value=0.007) (*Table 1*). Nineteen (5%) of the total subjects were taking anti-tuberculosis drug, whereas 361 (95%) were non-users and the differences were not statistically significant (*P* value = 0.999). In the current study, the correlation between culture and microscopic methods indicated 57.8% consistency which was statistically significant (P value = 0.0001) (*Table 2*). The sensitivity, specificity, agreement and disagreement of the microscopic method in comparison with culture method were 69.2%, 98.5%, 54.5% and 97.7%, respectively.

5. Discussion

Although some studies such as those reported in Canada in 1986 and 2001 suggested no significant relationship between *M. tuberculosis* and lung cancer, and also lung and heart diseases (10), several studies pointed out the existence of association between *M. tuberculosis* and lung cancer. According to a study by Kurasawa in 1992, lung cancer is 1-2% linked with tuberculosis (11). However, a similar study conducted by Tamura, showed a linkage of 1-5% (12).

Table 1. Socio-Demographic Data of the Patients With Lung Cancer Based on Positive/Negative Results of the Bacteria Culture

Socio-demographic variables	Number	Number of positive culture, No. (%)	Number of negative culture, No. (%)	*P* value
Sex				
Male	252 (66.3)	13 (5.2)	239 (94.8)	0.068
Female	128 (33.7)	13 (10.2)	115 (89.8)	0.068
Total	380 (100)	26 (6.8)	354 (93.2)	0.068
Age, y				
< 30	10 (2.63)	0 (0.0)	10 (100.0)	0.637
30-39	15 (3.95)	0 (0.0)	15 (100.0)	0.637
40-49	38 (10.00)	1 (2.6)	37 (97.4)	0.637
50-59	95 (25.00)	6 (6.3)	89 (93.7)	0.637
> 59	222 (58.42)	19 (8.6)	203 (91.4)	0.637
Total	380 (100)	26 (6.8)	354 (93.2)	0.637
Marital status				
Single	8 (2.1)	1 (12.5)	7 (87.5)	0.170
Married	352 (92.6)	22 (6.3)	330 (93.8)	0.170
Divorced/widowed	20 (5.3)	3 (15.0)	17 (85.0)	0.170
Total	380 (100)	26 (6.8)	354 (93.2)	0.170
Literacy				
Illiterate	204 (53.7)	14 (6.9)	190 (93.1)	0.223
Elementary school	111 (29.2)	11 (9.9)	100 (90.1)	0.223
Secondary school	24 (6.3)	0 (0.0)	24 (100.0)	0.223
High school diploma	29 (7.6)	0 (0.0)	29 (100.0)	0.223
Academic education	12 (3.2)	1 (8.3)	11 (91.7)	0.223
Total	380 (100)	26 (6.8)	354 (93.2)	0.223
Drug addiction				
Drug addicts	120 (31.6)	2 (1.7)	118 (98.3)	0.007[a]
Non-drug addicts	260 (68.4)	24 (9.2)	236 (90.8)	0.007[a]
Total	380 (100)	26 (6.8)	354 (93.2)	0.007[a]
Smoking				
Smokers	201 (52.9)	12 (6.0)	189 (94.0)	0.476
Non- smokers	179 (47.1)	14 (7.8)	165 (92.2)	0.476
Total	380 (100)	26 (6.8)	354 (93.2)	0.476

[a] Statistically significant

Table 2. Consistency Results For Direct Microscopic Method in Comparison With the Culture Method in Patients With Clinical Lung Cancer Manifestations

Results [a, b]	Positive, No. (%)	Negative, No. (%)	Total, No. (%)
Positive	18 (69.2)	15 (4.2)	33 (8.7)
Negative	8 (30.8)	339 (95.8)	347 (91.3)
Total	26 (100)	354 (100)	380 (100)

[a] Probability = Measurement of Agreement (Kappa) = 0.578
[b] P value = 0.0001

A study carried out by Wu, suggested that infection with *M. tuberculosis* increases the risk of developing lung

cancer (13). Another study by Yu *et al.* concluded the same notes and indicated that the incidence of lung cancer in patients with *M. tuberculosis* is 10.9 times more than that of non-tuberculosis subjects (14).

In the present study, positive *M. tuberculosis* culture was found in 6.8% of total samples, which was more than that of Kurasawa (1-2%) (11), Tamura (1-5%) (12) and Cicenas (2.1%) (15). This was probably caused by samples taken from low socioeconomic families because they live in areas with lower sanitation and have populated family environment with limited house space.

Engels *et al.* pointed out that the increase in risk of developing lung cancer following infection with *M.tuberculosis* is biologically conceivable. Since respiratory symptoms are present months before clinical manifestations, during which, *M. tuberculosis* induces production of tumor necrosis factors (TNFs) by leukocytes. Immune responses, therefore, generate various types of oxygen and nitrogen molecules that in turn may bind to DNA and alter the genome (16).

Cicenas *et al.* believe that the reason behind coexistence of tuberculosis and lung cancer in patients should be divided into three categories: 1. No association exists between tuberculosis and lung cancer. 2. Alterations made by tuberculosis lead to deformation of alveoli and bronchiole as well as dysplasia of respiratory epithelial cells. This, in turn, would raise the probability of developing lung cancer. 3. During the process of lung cancer progression, an old foci of tuberculosis reactivates and leads to dissemination of *M. tuberculosis* (15).

In the present study, 83.4% of the subjects were in the age group 50-59 and over 60. This is justifiable considering the high incidence of lung cancer between the age 55 and 65 and the current study results is similar to those of previous studies; the study conducted in 2011 by Wang *et al.* put forward the notion that regarding each region, the age group accounting for much of the burden of lung cancer differs and that the lung cancer was most prevalent in age groups 50-59, 60-69 and 70-79 years old (17). Similar results were observed in a study by Schottenfeld which indicated that most cases of lung cancer were patients aged 60-80 (18).

Totally, 82.7% of our subjects had low education level. This fact could be interpreted in two ways; either the subjects referred to state-run hospitals, where the current study was conducted, because of their low socioeconomic status, or that the risk of lung cancer among low socioeconomic class has elevated. Dalstra *et al.* reported that cancer, in general, had a higher frequency among lower educated subjects when the age group 25-59 was taken into account which is similar to the results of the current study; however, the trend was opposite among those of 60-79 age group (19). According to the study by Devesa and Diamond, no strong association was found between education and elevated risk of lung cancer among females of both races (black and white) (20).

The current study is the first study in Iran which considers *M. tuberculosis* frequency in patients with lung cancer and can be a turning point for such studies. In comparison with other cross-sectional studies carried out in the world, the sample size recruited in the current study was acceptable. The researchers suggest bronchoalveolar lavage specimen as an appropriate alternative to sputum specimen because in the former, no extra sample is obtained from the patients.

This study had crucial limitations such as being cross-sectional, therefore not proposing a cause and effect relationship between lung cancer and *M. tuberculosis,* and also there was insufficient access to histology documents of patients. Another limitation was that samples were taken solely from two state-run hospitals and were not random. The present study encourages further investigations, such as cohort and case control studies, to reveal stronger evidence supporting the associations between these two significant diseases.

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Authors' Contribution

None declared.

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