

Prevalence of *Mycobacterium tuberculosis* in the samples referred to the tuberculosis research laboratory in Mashhad Ghaem Hospital during 2005-2006

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ABSTRACT

Background: Tuberculosis continues to be a serious public health problem causing nearly three million deaths per year all over the world. Despite major improvement in diagnosis, it is not possible to control the disease in the absence of surveillance treatment, and follow-up programs.

Objectives: This research was designed to study the frequency of *Mycobacterium tuberculosis* among specimens referred to the tuberculosis research laboratory in Qaem Hospital, Mashhad, North- East of Iran in 2005-2006.

Materials and Methods: 3207 samples (1331 sputum, 1209 bronchial lavage, 69 ascitis aspirates, 52 urine samples, 30 CSF samples, 25 joint aspirates and 15 wound secretions) were cultured according to standard procedure and examined microscopically using Ziehl Neelsen staining method.

Results: 536 samples (16.7%) recognized as positive for *Mycobacterium tuberculosis*. (bronchial lavage 18.6%, sputum 17%, gastric lavage 13.3%, CSF 10%, ascitis aspirates 7.2%, wound 6.25%, pleural aspiration 4.5%). No *Mycobacterium tuberculosis* was found in urine samples and joint aspirates.

Conclusion: Considering reported prevalence of 13 cases per 100,000 in the Iranian population, these results are acceptable but more preventive measures should be sought for controlling TB.

Key words: *Mycobacterium tuberculosis*, Ziehl Neelsen staining, prevalence

INTRODUCTION

Tuberculosis (TB) is one of the oldest diseases known to affect mankind (1). Robert Koch identified *Mycobacterium tuberculosis* as the cause of infection during his research in 1882 and introduced it as an infectious disease. Tuberculosis is a chronic infectious disease caused by *Mycobacterium tuberculosis* and one of the leading causes of mortality worldwide (1,2). Almost one- third of the world population (about 2 billion) is infected with *Mycobacterium tuberculosis* and during the past decade even industrialized countries have faced a resurgence of tuberculosis. It is the second major cause of death by infectious disease throughout the world, killing millions (3,4). The

disease mostly involves lung, but almost all organs can be affected by this infection. Extra- pulmonary sites account for 20% of cases seen in HIV- negative subjects but are much more common among HIV seropositive subjects (1).

The disease has high prevalence in Iran and eastern Iran seems to be the most affected region. An estimated, annual cumulative incidence of tuberculosis in this country has indicated 13 cases per 100,000 (3, 5). Since the epidemiological studies of tuberculosis are the main basis for preventive planning (6), the most important aim of this study was to investigate the epidemiology and incidence of TB among samples referred to the Ghaem Medical Center as the largest university hospital in eastern Iran.

MATERIALS AND METHODS

Between 2006 and 2007, 3743 samples were obtained from patients referred to the microbiology

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Table 1. The number and percentile rate of tuberculosis in different samples in Ghaem Hospital in Mashhad on 2005-2006

| Sample | Positive % | Positive | Negative | Total |
|----------------|------------|----------|----------|-------|
| Sputum | %17 | 273 | 1331 | 1604 |
| Bronchial | %18.6 | 226 | 1209 | 1435 |
| Pleural fluids | %4.5 | 18 | 400 | 418 |
| Stomach | %13.3 | 10 | 75 | 85 |
| CSF | %10 | 3 | 30 | 33 |
| Ascites | %7.2 | 5 | 69 | 74 |
| Wounds | %6.25 | 1 | 16 | 17 |
| Urine | 0 | 0 | 52 | 52 |
| Joints | 0 | 0 | 25 | 25 |
| Total | %16.71 | 536 | 3207 | 3743 |

lab of Ghaem Hospital, Mashhad University of Medical Sciences. The data for these samples were recorded and specimens were tested for *Mycobacterium tuberculosis*. Samples were prepared by homogenization and decontamination (7, 8). For this purpose, 4% NaOH was added in an equal volume to the sample, then it was vortexed and was left in room temperature for 15 minutes. Finally, it was centrifuged (3,000 rpm) for 20 minutes (7, 8). The liquid samples (urine and cerebrospinal fluid) were centrifuged and smears were prepared from their sediments (7, 8). Smear was stained with Ziehl- Neelsen staining method (9). The samples were then investigated using the light microscope and acid-fast bacilli were studied (7, 8). In culturing, to neutralize the sample, normal HCl was added to the homogenate. The samples then were cultured on Lowenstein-Jensen slopes in 37°C. The cultures were checked each week for duration of 3- 8 weeks and were checked for growing colonies. Approximately after 8 weeks the results were recorded. *Mycobacterium* was identified by colonial morphology and growth rate (7, 8). We tried to identify *Mycobacterium tuberculosis* in these specimens by acceptable methods (10). The sample was reported positive when culture and/or smear were positive following confirmation by culture.

RESULTS

The results were obtained from 3,743 samples taken from individuals referred to research laboratory of Ghaem Hospital. These specimens consisted of 1,604 sputum, 1,435 bronchial lavage, 418 pleural fluids, 85 stomach, 74 ascitis aspirates, 52 urine

samples, 33 CSF samples, 25 joint aspirates and 17 wound secretions (Table 1).

Among these samples 536 samples (16.7%) recognized as positive for *Mycobacterium tuberculosis*. The prevalence of positive cases in each type of samples is as follow: bronchial lavage 18.6%, sputum 17%, gastric lavage 13.3%, CSF samples 10%, ascitis aspirates 7.2%, wound 6.25%, and pleural aspiration 4.5%. No *Mycobacterium tuberculosis* was found in urine samples and joint aspirates (Table 1).

DISCUSSION

Although the prevalence of tuberculosis in industrial countries has obviously declined in the last decade, TB is still a major problem in developing countries (11-13). In Iran, tuberculosis was very common in the past decades but with interventional health programs and considerable activities against infectious diseases, its prevalence has decreased mostly in the last decades; 42.9 cases in 100,000 people in 1371 was decreased to 17.5 cases/100,000 in 1381 (12, 14).

According to our findings bronchial lavage was the most yielding sample for effective diagnosis in patients with pulmonary tuberculosis (18.6%) and the least yield belonged to urine and joint aspirates in which no results were observed. Our results were in parallel with the results reported by WHO indicating 13 positive smears detected in 100,000 individuals in Iran (14). In our study (Mashhad, Khorasan province with a population of about 3 million), 16.7% positive cases were found among patients which is considered very high. Considering the predictable prevalence, our results indicate a high prevalence in this area. Probably one of the most important reasons for this is

the neighboring of Khorasan province with countries having high prevalence of tuberculosis and high immigration of refugees to this province.

Case finding and accurate treatment are the main steps in decreasing the prevalence of tuberculosis (15). Moreover, in high risk groups, diagnosis and treatment of latent tuberculosis can play an important role in controlling tuberculosis (15). Therefore, it is very important to find symptomatic or asymptomatic tuberculosis patients for appropriate medical interventions (15).

Considering the high immigration rate to this province, we have to organize a local plan for direct sputum smear of suspected immigrants at time of entry to the province and even suspected non-immigrants should be referred for diagnosis, so it would be very feasible to find the patients as soon as possible before spreading the infection to other people and other parts of the country (16-18).

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