Iranian Journal of Pathology (2013) 8 (2), 73- 80

Original Article

Eight-year Study of *Mycobacterium tuberculosis* in Mashhad, Northeast of Iran

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ABSTRACT

Background & Objectives: **Tuberculosis is one of the greatest health problems in Iran. The distribution of the disease is not equal in all parts of the country. The aim of this study was to evaluate the frequency of positive results for *Mycobacterium tuberculosis* in samples referred to an academic hospital in an 8 year period.**

Materials and Methods: **The samples from different wards of Qaem Hospital, Mashhad and samples referred to Outpatient Clinic during the years 2001-2008 and 75 samples from the prison in the same period were analyzed with direct microscopy of smear and culture methods for *M. tuberculosis.* Basic descriptive statistics were performed using SPSS 11.5 software.**

Results: **A total 26817 samples were analyzed and the results showed that the frequency of Mycobacterium positive samples in hospitalized patients' samples was 2412 (9%) with microscopy and 1573 (6%) with culture method. In the outpatients, it was 897 (10.2%) and 417 (4.7%) with microscopy and culture methods, respectively. Form 75 samples from the prison, 9 (12%) were positive with microscopy method. Culture method yielded only one (1.3%) positive result in these samples.**

Conclusion: **The frequency of *M. tuberculosis* was relatively high in the study groups. Therefore it seems continues surveillance is essential to monitor the *M. tuberculosis* in hospitals and community.**

***Keywords: Mycobacterium tuberculosis*, Epidemiology, Iran**

Received: 12 June 2012
Accepted: 02 October 2012
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Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* complex. Tuberculosis can affect almost all organs, but the most common form is pulmonary TB (1). It is a chronic disease affecting mostly the low socioeconomic strata of the society (2). Ninety five percent of the diseases and 99% of deaths due to the disease occur in developing countries (3).

Every second one person in the world becomes infected with the bacillus, every four seconds one person is affected with the disease, and every ten seconds one person dies of the disease (1, 4). One third of world population is infected with tuberculosis (4-5). According to WHO statistics in 2008, most of tuberculosis cases (34.1%) have occurred in South East Asia and about 1.3 million cases died in 2008 (4). In 2008 there were about 9.4 million (8،9 million – 9،9 million) new tuberculosis cases worldwide. Most of the new cases in 2008 were in Asia (55%) and Africa (30%) (6).

Tuberculosis incidence is 23/100000 for Iran (7) and the disease is one of the greatest health problems in the country (8). Considering the high prevalence of tuberculosis in our country, continuous surveillance is essential to monitor the *M. tuberculosis* prevalence and to evaluate its routinely detection methods in our diagnostic laboratories.

The prevalence of tuberculosis is higher in borderline areas of Iran such as Sistan and Baloochistan, Golestan, Khorasan, South Azerbayjan, West Azerbayjan and Kordistan. Khorasan has had the third high incidence after Sistan and Baloochistan and Golestan (9-11). The reported incidence rate in 2004 was 22.7/100000 in Khorasan, 42.97/100000 in Golestan and 43.83/100000 in Sistan & Baloochestan (11-12). According to the importance of tuberculosis, especially in developing countries like Iran, and the emergence of multi drug resistant strains of *M. tuberculosis*, increase of immigrants from Afghanistan in our country, HIV spread, population growth and changes in age pyramid leading to epidemiologic changes in tuberculosis, it is important to evaluate key indices of the disease such as incidence rate, mortality rate and cure rate. So the health system would be provided with good statistics in order to perform better in managing the *M. tuberculosis* epidemic in the country (7).

Several studies were performed on tuberculosis prevalence (2, 5, 13). Our study was performed in Qaem Hospital that is one of the two main academic hospitals in the city and the referral center for the northeast part of the country. It is the only place where culture for *M. tuberculosis* is done. So, the most diagnosis measure practiced in the area is direct microscopy for acid fast bacilli (AFB). The aim of this study was to evaluate the frequency of *M. tuberculosis* in samples referred to Qaem Hospital, Mashhad in an 8 year period. Our study evaluated the questions about the sensitivity and specificity of smear microscopy for different kinds of samples in laboratories without TB culture facilities for diagnosis goals.

Materials and Methods

In this descriptive cross-sectional study, 26817 suspected tuberculosis samples referred to Central Laboratory and Outpatient Clinic of Qaem Hospital, Mashhad (which is the referral center for the North East part of the country) during 2001-2008 were evaluated with two methods of direct microscopy and culture, and the results were compared.

The suspected tuberculosis is a subject who has persistent cough lasting 3 weeks or more that may associate with sputum or bloody sputum. The confirmed tuberculosis is a patient who has at least two positive sputum smear tests, or

one positive sputum smear test and radiological changes to TB, or one positive sputum smear test and one positive sputum culture test (1). It is important to note that there are two main academic hospitals in the city and the selected hospital is the only center in which the culture method is available.

The sampled were divided into groups according to gender, laboratory and method of assessment. The samples from prison were evaluated as a separate group. The study was approved by the Research Deputy of Mashhad University of Medical Sciences regarding methodological and ethical issues.

The specimens were studied with direct microscopy for AFB using Ziehl-Neelsen carbol-fuchsin solution (MERCK, Germany) and culture method in Lowenstein–Jensen medium (MERCK, Germany). Samples were decontaminated and homogenized with equal amount of 4% NaOH. After neutralizing procedures by 2N HCl, five drops of re-suspended sediments were subsequently inoculated to Lowenstein-Jensen solid medium. Media 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 WHO guidelines. Mycabacteria confirmed with phenotypic results.

Basic descriptive statistics were performed using SPSS 11.5 software. The Pearson Chi-Square statistical test was used for comparisons between different groups. Measure of agreement Kappa was used for assessment the agreement between results of direct microscopy method and culture method. A $P<0.05$ was considered statistically significant.

Culture method was considered as the gold standard method and the sensitivity and specificity of the microscopy method (as a screening method) was calculated using the following formula:

$$Sensitivity=\frac{True\ \ Positive}{True\ \ Positive+\ False\ \ Negative}$$

$$Specificity=\frac{True\ \ Negative}{True\ \ Negative+\ False\ \ Positive}$$

Results

In the analyzed samples with microscopy method 2412 samples (9.0%) were positive for AFB. Among the female group 1229 (12.7%) and among male group 1183 (7.7%) were positive. The difference of positive samples in two genders was significant ($P< 0.0001$).

The result of microscopic examination method for detection of *M. tuberculosis* in different clinical specimens including bronchial lavage, pleural biopsy, CSF, ascitis fluid, sputum, and urine were 10.3%, 1.6 %, 1.9% 1.0%, 9.4% and 4.3% respectively. The most positive microscopy results were for samples from bronchial secretions (10.3%) and sputum (9.4%). The difference of positive results between different types of samples was statistically significant (*Pearson chi square test*, $P< 0.0001$).

Our results showed 62.4% of smear positive samples were from Central Laboratory (i.e. sampled from admitted patients), 37.2% were from Outpatient Clinic and 0.4% were from prison. There was a significant difference between these frequencies (*Pearson chi square test*=24.09, P=0.0001).

Among samples from different wards of the hospital, the most referred samples were from thorax (31.6%), Infectious Diseases (25.1%) and Internal Medicine (20.3%) departments, respectively. In microscopic examination, the most positive results were obtained from Infectious Diseases (3.4%), Thorax (2.3%), Internal Medicine (1.2%) and Surgery (0.9%)

departments. The difference of positive results between the wards was statically significant (Pearson chi square test, $P< 0.0001$). In addition, 10.2% of outpatient clinic samples and 11.7% of prison samples were positive by this method.

Our results showed 1573 (5.9%) of samples analyzed with culture method were positive. Among the female group 6.68% (770 of 11530 samples) and among male group 5.25% (803 of 15287 samples) were positive. The difference of positive sampled in two genders was significant (Pearson chi square test, $P< 0.0001$).

The most positive results for different type of samples were for bronchial secretions (10.1%) and CSF (7.4%).The difference of positive results between different types of samples was statistically significant (Pearson chi square test, $P< 0.0001$) (Table 1).

Table 1- The results of culture method for detection of *M. tuberculosis* in different clinical specimens. Pearson Chi-Square statistical test was used for comparing the results in different types of clinical samples.

Culture Method / Sample Type	Negative		Positive		All	
	Number	Percent	Number	Percent	Number	Percent
Bronchial Lavage	6533	89.9	737	10.1	7270	100
Pleural Biopsy	1388	95.7	62	4.3	1450	100
CSF	50	92.6	4	7.4	54	100
Ascitis fluid	200	97.1	6	2.9	206	100
Sputum	15793	95.7	708	4.3	16501	100
Urine	434	97.1	13	2.9	447	100
Others	846	95.2	43	4.8	889	100
All	25244	94.1	1573	5.9	26817	100
Statistical Test			***Pearson Chi-Square*=154.88** P**=0.0001***			

***** Statistically significant

Based on our results, 73.43% of culture positive samples were from Central Laboratory (i.e. sampled from admitted patients), 26.51% were from Outpatient Clinic and 0.06% was from prison. There was significant difference between these frequencies (*Pearson chi square test*, $P< 0.0001$). The most positive results were obtained from Infectious Diseases (3.1%), and Thorax (1.4%) wards. The difference of positive results between the wards was statically significant (Pearson chi square test, $P< 0.0001$). Furthermore, 4.7% of Outpatient Clinic samples and 1.3% of Prison samples were positive by culture method (Table 2).

Table 2- The results of culture method for detection of *M. tuberculosis* in clinical specimens from different wards of the hospital. Pearson Chi-Square statistical test was used for comparing the results in clinical samples from different wards of the hospital

Ward \ Culture Method		Negative		Positive		All	
		Number	Percent	Number	Percent	Number	Percent
Central Laboratory of the Hospital	Thorax	5434	30.3	252	1.4	5686	31.7
	Cardiology	870	4.8	32	0.2	902	5
	Internal Medicine	3504	19.5	149	0.8	3653	20.4
	Surgery	2077	11.6	107	0.6	2184	12.2
	Infectious Diseases	3944	22	557	3.1	4501	25.1
	Pediatric	189	1.1	7	0.1	196	1.1
	Others	582	3.2	27	0.2	609	3.4
	Bronchoscopy	187	1	24	0.1	211	1.1
	Total	16787	93.5	1155	6.5	17942	100.0
Outpatient Clinic		8381	95.3	417	4.7	8798	100.0
Samples from Prison		76	98.7	1	1.3	77	100.0
All		25244	94.1	1573	5.9	26817	100.0

Considering the culture as the gold standard for diagnosis of *M. tuberculosis*, sensitivity, false negative, specificity and false positive results of microscopic examination for different specimens are showed in Table 3. Based on the results, the highest measure of agreement was observed between the microscopy and culture methods of bronchial lavage and the lowest one was observed for pleural biopsy specimens.

Discussion

Although the prevalence of tuberculosis in industrial countries has obviously declined in last decade, TB is still a major problem in developing countries. In Iran, tuberculosis was very common in the past decades. The prevalence has reached 17 cases /100000 in 2002 from 43 cases /100000 in 1992, which is a considerable decrease.

Our data showed that the TB positive results are higher in women than men in our province which is in agreement with the previous studies but is different from another report (14). In addition, our data is different from several studies reported from other countries (15-18). In these studies, they explained the possible reasons leading the difference in sex-specific rate of TB such as biological phenomena. They discussed that women of reproductive age may show tendency for developing TB after infection than men of the same age. Other complications such as HIV infection, diabetes and cirrhosis may also affect the rate of TB occurrence in different genders.

Table 3- The sensitivity, false negative, specificity and false positive results of microscopic examination for different specimens; assuming the culture as the gold standard method; Measure of Agreement Kappa between the results of direct microscopy method and culture method was calculated for different clinical specimens.

Sample Type	Culture Method / Direct Microscopy	Negative		Positive		Measure of Agreement Kappa *P*-Value
		Number	Percent	Number	Percent	
Bronchial lavage	**Negative**	6233	95.4	286	38.8	0.561 **
	Positive	300	4.6	451	61.2	0.0001*
Pleural biopsy	**Negative**	1379	99.4	48	77.4	0.314 ***
	Positive	9	0.6	14	22.6	0.0001*
CSF	**Negative**	50	100.0	3	75.0	0.382
	Positive	0	0.0	1	25.0	0.0001*
Ascitis fluid	**Negative**	200	100.0	4	66.7	0.493
	Positive	0	0.0	2	33.3	0.0001*
Sputum	**Negative**	14771	93.5	173	24.4	0.439
	Positive	1022	6.5	535	75.6	0.0001*
Urine	**Negative**	424	97.7	4	30.8	0.547
	Positive	10	2.3	9	69.2	0.0001*
Others	**Negative**	813	96.1	17	39.5	0.481
	Positive	33	3.9	26	60.5	0.0001*
All	**Negative**	23870	94.6	535	34.0	0.484
	Positive	1374	5.4	1038	66.0	0.0001*

*Statistically significant

**The highest measure of agreement between the microscopy and culture methods.

***The lowest measure of agreement between the microscopy and culture methods.

Several factors may influence in gender rate of TB. In this study, we only explored the frequency of *M. tuberculosis* in referred specimens to our hospital; other epidemiological, clinical, sociological and behavioral information are needed to determine gender differences and further studies should be performed for confirming contradictory reports (15-16).

Previous studies showed the cultures of bronchoscopy specimens were more useful than pre-broncoscopy sputum samples for detecting the *Mycobacterium tuberculosis* in pulmonary tuberculosis patients (17-18). In our study, also the bronchial lavage specimens were the most yielding samples for effective diagnosis in patients with pulmonary tuberculosis (10.3%).

Smear microscopy is an essential method for detecting the AFB in sputum and a rapid test with

low-cost and low-technology which is widely used in developing countries (19). The frequency of positive smears was 9% in our study. This result was among the samples from individuals considered high probable for tuberculosis. One reason for this rather high prevalence could be the high rate of immigration from neighbor countries such as Afghanistan and Pakistan to Khorasan.

Sputum specimens were the most frequent samples in our study. Based on the results of our study, 75.6% of culture positive sputum samples showed positive results in direct microscopy method. Nelson *et al*. showed that AFB was found in only 46% of culture positive smears that is lower than our results (20). A range of 53%-80% of the positive sputum specimens have been showed in other studies (21-24). The frequency of positive results was higher in hospitalized patients than outpatient clinic which may be due to more hospitalization of symptomatic patients. The prevalence of TB in prisons has been reported very high, up to 100 times higher than of its level in civilian population (25-26); however, in our study, only 1.3% of prison referred samples showed TB culture positive results. It is possible that the low positivity is due to limited numbers of the referred samples. Further studies are needed to determine the TB prevalence in the prisons of our country. In our study, the numbers of positive cases were higher in Infectious Diseases and Thorax wards due to more hospitalization of symptomatic TB patients in these wards.

Conclusion

Our data showed the frequency of *M. tuberculosis* was relatively high in the study groups. In addition, based on our results, direct microscopy examination of smears has high specificity and acceptable sensitivity compared with mycobacterium culture as the gold standard.

Acknowledgment

This study was a thesis presented for the degree of Medical Doctor (MD) which supported by Mashhad University of Medical Sciences, Mashhad, Iran (grant No. 87749 and thesis No. 6383). The authors declare that there is no conflict of interest.

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