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Original Article

Frequency of Smear-Negative Tuberculosis in Northwest Iran

Mahya Pourostadi[1], M-Pill;
Jalil Rashedi[2,3], MS;
Behroz Mahdavi Poor[2,4], MS;
Hossein Samadi Kafil[5], PhD;
Maral Hariri-Akbari[6], MS;
Mohammad Asgharzadeh[7], PhD

[1]Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran;
[2]Department of Laboratory Science, Faculty of Paramedicine, Tabriz University of Medical Sciences, Tabriz, Iran;
[3]Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran;
[4]Department of Medical Parasitology, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran;
[5]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran;
[6]Adjunct Lecturer, Faculty of Health and Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran;
[7]Biotechnology Research Center and Faculty of Paramedicine, Tabriz University of Medical Sciences, Tabriz, Iran;

Correspondence:
Jalil Rashedi, MS;
Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Golgasht Ave., Azadi St., Tabriz, Iran
Tel: +98 41 33392633
Fax: +98 41 33371971
Email: Rashedijalil@gmail.com
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What's Known

- In some TB detection centers, laboratory assays are conducted only via direct smear examination, leading to some false negatives particularly among smokers, asthmatics, and extra-pulmonary TB patients.

What's New

- In this study, the rate of smear-negative TB (22.8%) in northwest Iran was evaluated for the 1st time. Use of appropriate detection methods, alongside culture, to rapidly diagnose smear-negative TB patients is vitally important.

Abstract

Background: Microscopic smear examination is the most common test in tuberculosis (TB) detection. It is, however, not strong enough to identify TB in the majority of afflicted individuals; thus, a significant number of TB patients are smear negative and capable of transmitting the infection. The aim of this study was to evaluate the rate of smear-negative TB in northwest Iran.

Methods: In this cross-sectional study, 329 TB-confirmed patients were evaluated through culture up to March 1, 2015, in northwest Iran. The demographic and clinical features of the smear-negative and smear-positive TB patients were compared. The χ^2 test was used to compare the frequency of the variables. All the statistical analyses were conducted using SPSS, version 16 (Chicago, IL, USA).

Results: Seventy-five cases were smear negative and 254 were smear positive. Smokers, asthmatics, and extra-pulmonary TB patients were primarily among the smear-negative cases. The rate of mortality was also relatively higher among the smear-negative TB patients.

Conclusion: Totally, 22.8% of the TB cases in northwest Iran were smear negative, with a relatively higher rate of mortality than those with positive smears. A delay in these patients' return to TB diagnosis and treatment centers increases the chance of transmission to others. This is a very sensitive issue in centers where there is no equipment for TB cultivation. Thus, it is essential to equip centers without TB cultivation facilities and to use appropriate diagnostic techniques in centers with those facilities to help rapidly detect smear-negative cases.

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Introduction

For all the various methods and universal efforts to control tuberculosis (TB), 9,600,000 new cases of TB occurred all over the world in 2014 and 1.5 million died because of the disease, 400,000 of these cases being HIV-positives.[1] These people were infected primarily by individuals afflicted by untreated active pulmonary TB. Actually an untreated TB-affected person may infect up to 10 people over a year. Two appropriate techniques in TB detection are microscopic examination and cultivation. The microscopic investigation of sputum is considered the basic method of acid-fast bacterium detection all over the world. The diagnostic

threshold, the least amount, via light microscopy is 5,000 to 10,000 bacilli per 1 mL of sputum.[2] The sensitivity of microscopic examination is between 30% and 80% by comparison with cultivation,[3] and the sensitivity in HIV-positive individuals and children is less than 20%.[4] To identify pulmonary TB, cultivation requires 10 to 100 bacilli per 1 mL of sputum, while the infecting dosage of *Mycobacterium tuberculosis* is estimated to be fewer than 10 bacilli.[5] As sputum cultivation on the Lowenstein–Jensen medium takes 6 to 8 weeks, using the method as 1st-line detection will create some limitations. Although the sputum smear test is not capable of identifying 50% of TB patients, it still remains the most useful test to detect TB.[6] Therefore, a significant number of TB patients will be smear negative. It has been demonstrated that people are at risk of infection if they come into contact with smear-negative TB patients.[7] For example, a study in San Francisco reported that 17% of TB cases were due to transmission from negative smears.[8] Smear-negative patients are considered less infective prior to HIV epidemics, and they are believed to have better outcomes than smear-positive patients.[9] Overwhelming epidemics of HIV in different regions of the world have increased smear-negative TB disease, particularly in high prevalence HIV, and given rise to high rates of mortality.[10] This is associated with immune deficiency in the afflicted individuals. The prevalence of HIV is on the rise in Iran. It reached from 0.01 in 100,000 people in 1984 to 19.6 in 100,000 people in 2006.[11] The number of HIV-positive patients has been increasing in northwest Iran, in tandem with the other parts of Iran. HIV infection is deemed a risk factor for TB, particularly smear-negative TB. Accordingly, we conducted this epidemiological evaluation of smear-negative pulmonary TB so as to better understand the TB transmission process with a view to helping make preventive decisions to reduce the TB dissemination in the country.

Patients and Methods

The present cross-sectional study included TB cases confirmed through cultivation up to March 1, 2015, in northwest Iran. Specimens were prepared from the patients to detect acid-fast bacilli, and Ziehl–Neelsen staining was applied for the microscopic examination of the sediments of the specimens after homogenization (digestion and decontamination) via 4% NaOH and centrifugation. Cultivation was done on the Lowenstein–Jensen medium (HiMedia, Bombay, India) according to the Petroff method with incubation at 37 °C for 6 to 8 weeks. Clinical and radiological evaluations were also performed. Cases considered as laboratory cross-contamination, according to the standard criteria, were eliminated.[12] A patient would be regarded as smear positive if at least 1 smear was positive. A patient would be considered smear negative if all of the prepared specimens (smears), at least 3 to 6 were negative in microscopic examination, while at least 2 of them were culture positive. The demographic features and the clinical variables of the smear-negative and smear-positive patients were compared. The features and variables comprised age, sex, use of anti-TB drugs, underlying diseases (e.g., diabetes, asthma, cardiac failure, renal failure, anemia, and liver cirrhosis), hospitalization in the preceding year, smoking, site of the disease, history of any contact with TB patients, being HIV-positive, death, imprisonment, and addiction.

Statistical Analysis

All the patients in the study were categorized to 2 groups of negative and positive smears. The categorical data were compared using the χ^2 test, and a P value less than 0.05 was considered significant. All the statistical analyses were conducted using SPSS, version 16 (Chicago, IL, USA).

Results

In 2 centers in northwest Iran, 331 cases of *Mycobacterium tuberculosis* were confirmed. Out of this total, 2 cases were detected as laboratory cross-contamination. From the remaining 329 cases, 254 (77.2%) were smear positive and 75 (22.8%) were smear negative. The demographic and clinical data of the patients were evaluated (table 1). In the young people (<16 y), although obtaining sufficient and adequate sputum was relatively difficult, the rate of smear-negative TB cases was not statistically significant (P=0.26); it was, however, more frequent than that in the adults. The rate of smear-negative TB among the individuals aged at least 70 years was not significant. In the older people, the rate of smear-negative TB was higher. HIV was not seen among the smear-negative TB cases. Four (5.33%) smear-negative cases died during the therapy process, while the rate of mortality in the smear-positive cases was 3 (1.18%). The rate of extra-pulmonary TB in the smear-negative patients was high compared with the smear-positive patients (P<0.001). The rate was also high among the smear-negative cases (44%) compared to the smear-positive cases (29.53%) among the smokers (P=0.02).

Table 1: Demographic and clinical characteristics of TB patients in northwest Iran with smear results

Characteristics	n (%)		P
	Smear-Negatives	Smear-Positives	
Sex			
Male	41 (54.67)	123 (48.43)	0.34
Female	34 (45.33)	131 (51.57)	
Age (y)			
≤15	2 (2.67)	5 (1.97)	0.26
16-30	8 (10.66)	55 (21.65)	
31-50	20 (26.67)	59 (23.23)	
51-70	25 (33.33)	84 (33.07)	
70 ≤	20 (26.67)	51 (20.08)	
Previous history of anti-TB drug consumption			
Yes	7 (9.33)	37 (14.57)	0.24
No	68 (90.67)	217 (86.43)	
Hospitalization during the preceding year			
Yes	35 (46.67)	81 (31.89)	0.02
No	40 (53.33)	173 (68.11)	
Smoking			
Yes	33 (44)	75 (29.53)	0.02
No	42 (56)	179 (70.47)	
Contact with TB patients			
Yes	14 (18.67)	43 (16.93)	0.73
No	61 (81.33)	211 (83.07)	
Site of TB			
Pulmonary	62 (82.67)	249 (98.03)	<0.001
Extra-pulmonary	13 (17.33)	5 (1.97)	
HIV-positive	0 (0)	2 (0.79)	
Death	4 (5.33)	3 (1.18)	
Imprisoned	3 (4)	5 (1.97)	
Addiction	1 (1.33)	6 (2.36)	

TB: Tuberculosis

Twenty-one (28%) smear-negative patients had an underlying condition, while 62 (24.4%) smear-positive cases had an underlying condition. Asthma was more frequent among the smear-negative cases (table 2).

Discussion

The results of the present study indicated that a significant number of TB cases confirmed by cultivation were detected through acid-fast microscopic examination. In addition, the majority of the patients were smear positive, while only 22.8% were smear negative (19.9% sputum-related). As the sensitivity of microscopic examination under ideal condition is 80%,[3] we performed microscopic examination on samples that were decontaminated, digested, and concentrated according to the Petroff method.[13] Hassanzadeh et al.[14] showed that 19.3% of their pulmonary TB patients were smear negative and that 2.3% of their entire study population was HIV positive. Cavanaugh et al.[15] conducted a study in the United States and reported that 40% of their HIV-positive TB patients had smear-negative sputum. In Ethiopia, the rate was 37% in HIV-positive pulmonary TB-patients.[16] In contrast in our study, there were only 2 HIV-positive TB patients, both of whom were smear positive (table 1). Thus, unlike other studies,[10,16] no relationship was noticed between being smear negative and HIV-positive. Currently, the HIV prevalence is low in the northwest of Iran: Perhaps, patients have not been identified or some of those afflicted hide their condition to avoid encountering problems in society. Consequently, a better management of TB requires the application of the HIV detection test in central laboratories. In the present study, unlike that by Shah et al.[6] in the United States, liquid media were not used; consequently, the result of direct smear examination and solid cultivation may have been negative in cases in which the number of acid-fast bacilli was low. However, the issue of cultivation on solid media can imply improvement in TB detection in

Table 2: A comparison between smear-positive and smear-negative TB patients regarding underlying diseases

Underlying diseases	Frequency (%)		P
	Smear-negative	Smear-positive	
Diabetes			
Yes	10 (13.33)	37 (14.57)	0.79
No	65 (86.67)	217 (85.43)	
Febrile convulsion	-	2	
Hypertension	-	1	
Cancer	-	1	
Renal failure	2	2	
Psychoses	-	1	
Heart failure	2	1	
Asthma			
Yes	8 (10.67)	6 (2.36)	<0.001
No	67 (89.33)	248 (97.64)	
Chemically wounded veteran	-	1	
Autoimmune thyroiditis	-	1	
Anemia	-	5 (1.97)	
Osteoporosis	-	1	
Gastritis	-	1	
Cirrhosis	-	2	
Cervical adenitis	-	1	

A smear-negative patient had both asthma and diabetes, and another smear-positive patient had both anemia and cirrhosis.
TB: Tuberculosis

northwest Iran. At present, as the HIV prevalence is relatively low in the region, the percentage of smear-negative TB among children and the elderly in comparison with smear-positive TB cases of the whole population under the study is high, albeit not significantly (P=0.26). The percentage of smear-positive TB cases among children is low. This is because the primary disease usually appears in children without extensive cavitations and the paucibacillary condition occurs with a limited number of bacilli in sputum. Meanwhile, collecting sufficient amounts of specimen from children and the elderly is difficult. Obtaining induced sputum using nebulizers and a precise recording of clinical manifestations can be ultimately helpful. With increasing HIV, smear-negative pulmonary TB will be converted from a slow advancing and well-prognosis disease into a disease which would infect mostly the lower lobes of the lungs[17] and cause a high rate of mortality.[10] This would result in increasing numbers of smear-negative cases and thereby cause bias. Since in the present study the TB patients were confirmed by cultivation, the possibility of bias was low. Cultivating the bacteria requires a temperature of 37 °C for 6 to 8 weeks.[18] This along with the diagnosis period and delay in treatment allows the patient to disseminate the infection. Examples are the smear-negative and culture-positive patients in San Francisco[8] and Vancouver.[19] The delay in diagnosis can also threaten the lives of HIV-positives, particularly children.[20] Therefore, detecting smear-negative cases will lead to more sufficient isolation and treatment of the patients and reduce the transmission. It seems that the use of other sampling methods such as induced sputum[17] and lymph node aspiration[21] as well as other detecting techniques like the modified Ziehl–Neelsen,[22] fluorescent microscopy examination (Auramine–Rhodamine staining) in cases where the 1st and the 2nd samples are negative according to the Ziehl–Neelsen staining, broth-based cultivation, and polymerase chain reaction (PCR) will be very helpful in obtaining desired results. Hence, initiating treatment without diagnosing the disease should be avoided (at least in this region of Iran) due to drug resistance, which will be the main problem in the future. Meanwhile, appropriate detection of non-TB lung disorders will also reduce the wrong diagnosis. The use of the broth medium in cultivation and PCR to rapidly detect infection, particularly in smear-negative cases, will raise the possibility of cross-contamination. In detecting cross-contamination cases, molecular methods such as the mycobacterial interspersed repetitive unit (MIRU) and IS6110-RFLP[23] should be used in smear-negative cases. In the current study, the percentage of extra-pulmonary TB in the smear-negative patients was high in comparison to that of the positive-smear patients. As in extra-pulmonary TB specimens such as

lesion discharges (pus), cerebrospinal fluid, ascites fluid, urine, gastric juice, and synovial fluid are investigated, and the amount of the bacilli in them is typically less than that in sputum, the percentage of smear-negatives is high. The rate of smear-negative cases among the patients who previously received anti-TB drugs, compared with the patients without a history of TB, was low (16% vs. 24%). This could have been due to the high amount of sputum and septic mucus (instead of saliva) in the patients with a history of TB. Considering that, one of the numerous factors causing a sample to be smear negative in pulmonary TB is delivering the patient's saliva instead of septic sputum to the laboratory. Patients with a history of TB with previous long-term pulmonary annoyance usually have a sensitive and full secretion pulmonary physiology. This issue along with having the experience of preparing suitable septic sputum helps the patient to deliver an appropriate sample (instead of saliva) to the laboratory. Another salient point is that observing even one acid-fast bacillus in any of 3 or even 6 sputum samples of patients with a history of TB is just reported with a high emphatic by laboratory technical personnel and more importantly the physician has taken it into consideration and the patient's test result is considered positive, whereas for patients without history of TB there is no such a high sensitivity and most likely, in such cases (for example, a bacillus in 6 numbers of sputum) the physician will wait for the result of the culture to a final decision to be taken as a result of cultivation. Since one of the risk factors for TB is smoking,[24] it was taken as a risk factor in this study. Our results demonstrated that smear-negative TB was higher among the smokers than among the nonsmokers. As the chronic inhalation of cigarette smoke is associated with mucus hypersecretion,[25] the accumulation of bacilli in the lungs happens in smaller amounts over time; therefore, the progress of TB in these individuals will be slow. These patients will be more often smear negative than nonsmokers. Among prisoners in Iran, chiming in with the study by Shah et al.[6] in the United States, the prevalence of smear-negative TB in comparison to that of smear-positive TB was high. Most of the prisoners were within 31 to 50 years old and were smokers. Like other smokers, their rate of bacilli excretion through sputum was low. Similar to a study by Harries et al.[10] in sub-Saharan African countries, the relative rate of mortality in the smear-negatives was high compared to the smear-positives. This was probably because of delay in the return of the patients to the physician or the laboratory; therefore, the detection of the disease was delayed and the disease was already active and in progress. In the study by Cavanaugh et al.[15] in the United States, the HIV-positive patients had availability to antiviral treatment and the rate of mortality was low. In this study, out of 75 smear-negative patients, 21 (28%), and out of 254 smear-positive patients, 62 (24.41%) had underlying diseases. Thus, a significant difference was not observed regarding the underlying diseases. Trivial underlying diseases were more frequent among the smear-negatives. Especially, asthma was higher in the smear-negatives (10.67%) than among the smear-positives (2.36%) ($P<0.001$), which may have been related to corticosteroid therapy and the subsequent reduction in sputum secretion. In the process of preparing the specimens, these patients gave saliva rather than sputum.

There are some limitations in the present study. Not all the TB patients detected by the direct method were referred to the central laboratory, precluding the cultivation of the specimens. Another drawback of note is that cultivation was performed only in a solid medium rather than in a liquid one. The fact that genetic host factors and pathogens were not investigated counts as another weak point. These factors can affect the process and the severity of the disease along with the demographic and clinical features and the rate of negative and positive smears. It is, therefore, essential that future research be conducted with a view to better understanding genetic host factors and disease-inducing agents in TB transmission through smear-negative patients.

Conclusion

The results of the present study revealed that 22.8% of the TB cases in northwest Iran were smear negative. The percentage was higher among the extra-pulmonary TB patients, smokers, those hospitalized in the preceding year, and asthmatics. The rate of mortality among the smear-negative patients in comparison to the smear-positives was also relatively high. The delay in the return of smear-negative TB patients to TB diagnosis and treatment centers increases the chance of transmission to others. This issue in centers devoid of TB cultivation facilities is of great significance. Therefore, using appropriate detection methods to rapidly diagnose smear-negative TB patients is vitally important.

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References

1. World Health Organization [Internet]. Global tuberculosis report 2015. WHO/HTM/TB/2015. Geneva, Switzerland. WHO, 2015. Available from: http://apps.who.int/iris/bitstream/10665/191102/1/9789241565059_eng.pdf
2. Getahun H, Harrington M, O'Brien R, Nunn P. Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection or AIDS in resource-constrained settings: informing urgent policy changes. Lancet. 2007;369:2042-9. doi: 10.1016/S0140-6736(07)60284-0. PubMed PMID: 17574096.
3. Levy H, Feldman C, Sacho H, van der Meulen H, Kallenbach J, Koornhof H. A reevaluation of sputum microscopy and culture in the diagnosis of pulmonary tuberculosis. Chest. 1989;95:1193-7. PubMed PMID: 2656111.
4. Steingart KR, Ng V, Henry M, Hopewell PC, Ramsay A, Cunningham J, et al. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis. 2006;6:664-74. doi: 10.1016/S1473-3099(06)70602-8. PubMed PMID: 17008175.
5. Nicas M, Nazaroff WW, Hubbard A. Toward understanding the risk of secondary airborne infection: emission of respirable pathogens. J Occup Environ Hyg. 2005;2:143-54. doi: 10.1080/15459620590918466. PubMed PMID: 15764538.
6. Shah NS, Cavanaugh JS, Pratt R, Cain KP, Wells C, Laserson K, et al. Epidemiology of smear-negative pulmonary tuberculosis in the United States, 1993-2008. Int J Tuberc Lung Dis. 2012;16:1234-40. doi: 10.5588/ijtld.11.0794. PubMed PMID: 22748057.
7. Elwood RK, Cook VJ, Hernandez-Garduno E. Risk of tuberculosis in children from smear-negative source cases. Int J Tuberc Lung Dis. 2005;9:49-55. PubMed PMID: 15675550.
8. Behr MA, Warren SA, Salamon H, Hopewell PC, Ponce de Leon A, Daley CL, et al. Transmission of Mycobacterium tuberculosis from patients smear-negative for acid-fast bacilli. Lancet. 1999;353:444-9. PubMed PMID: 9989714.
9. Dutt AK, Stead WW. Smear-negative pulmonary tuberculosis. Semin Respir Infect. 1994;9:113-9. PubMed PMID: 7973170.
10. Harries AD, Hargreaves NJ, Kemp J, Jindani A, Enarson DA, Maher D, et al. Deaths from tuberculosis in sub-Saharan African countries with a high prevalence of HIV-1. Lancet. 2001;357:1519-23. doi: 10.1016/S0140-6736(00)04639-0. PubMed PMID: 11377627.
11. Fallahzadeh H, Morowatisharifabad M, Ehrampoosh MH. HIV/AIDS epidemic features and trends in Iran, 1986-2006. AIDS Behav. 2009;13:297-302. doi: 10.1007/s10461-008-9452-7. PubMed PMID: 18770025.
12. Jasmer RM, Roemer M, Hamilton J, Bunter J, Braden CR, Shinnick TM, et al. A prospective, multicenter study of laboratory cross-contamination of Mycobacterium tuberculosis cultures. Emerg Infect Dis. 2002;8:1260-3. doi: 10.3201/eid0811.020298. PubMed PMID: 12453353; PubMed Central PMCID: PMCPMC2738534.
13. Rafi A, Moaddab SR. Principles of mycobacteriology. 1st ed. Tabriz: Sotude publications; 2003.
14. Hassan zadeh J, Nasehi M, Rezaianzadeh A, Tabatabaee H, Rajaeifard A, Ghaderi E. Pattern of reported tuberculosis cases in iran 2009-2010. Iran J Public Health. 2013;42:72-8. PubMed PMID: 23514975; PubMed Central PMCID: PMCPMC3595631.
15. Cavanaugh JS, Shah NS, Cain KP, Winston CA. Survival among patients with HIV infection and smear-negative pulmonary tuberculosis - United States, 1993-2006. PLoS One. 2012;7:e47855. doi: 10.1371/journal.pone.0047855. PubMed PMID: 23110113; PubMed Central PMCID: PMCPMC3479118.
16. Yassin MA, Takele L, Gebresenbet S, Girma E, Lera M, Lendebo E, et al. HIV and tuberculosis coinfection in the southern region of Ethiopia: a prospective epidemiological study. Scand J Infect Dis. 2004;36:670-3. doi: 10.1080/00365540410020848. PubMed PMID: 15370654.
17. Colebunders R, Bastian I. A review of the diagnosis and treatment of smear-negative pulmonary tuberculosis. Int J Tuberc Lung Dis. 2000;4:97-107. PubMed PMID: 10694086.
18. Rashedi J, Mahdavi Poor B, Rafi A, Asgharzadeh M, Abdolalizadeh J, Moaddab SR. Multidrug-resistant tuberculosis in north-west of Iran and Republic of Azerbaijan: a major public health

concern for Iranian people. J Res Health Sci. 2015;15:101-3. PubMed PMID: 26175292.
19. Hernandez-Garduno E, Cook V, Kunimoto D, Elwood RK, Black WA, FitzGerald JM. Transmission of tuberculosis from smear negative patients: a molecular epidemiology study. Thorax. 2004;59:286-90. PubMed PMID: 15047946; PubMed Central PMCID: PMCPMC1763818.
20. Chintu C, Mwaba P. Tuberculosis in children with human immunodeficiency virus infection. Int J Tuberc Lung Dis. 2005;9:477-84. PubMed PMID: 15875917.
21. Monkongdee P, McCarthy KD, Cain KP, Tasaneeyapan T, Nguyen HD, Nguyen TN, et al. Yield of acid-fast smear and mycobacterial culture for tuberculosis diagnosis in people with human immunodeficiency virus. Am J Respir Crit Care Med. 2009;180:903-8. doi: 10.1164/rccm.200905-0692OC. PubMed PMID: 19628775.
22. Zheng LH, Jia HY, Liu XJ, Sun HS, Du FJ, Pan LP, et al. Modified cytospin slide microscopy method for rapid diagnosis of smear-negative pulmonary tuberculosis. Int J Tuberc Lung Dis. 2016;20:456-61. doi: 10.5588/ijtld.15.0733. PubMed PMID: 26970153.
23. Asgharzadeh M, Kafil HS, Roudsary AA, Hanifi GR. Tuberculosis transmission in Northwest of Iran: using MIRU-VNTR, ETR-VNTR and IS6110-RFLP methods. Infect Genet Evol. 2011;11:124-31. doi: 10.1016/j.meegid.2010.09.013. PubMed PMID: 20951237.
24. Asgharzadeh M, Samadi Kafil H, Pourostadi M. Source case identification and control of tuberculosis by molecular epidemiology. J Mazandaran Univ Med Sci. 2014;24:181-92.
25. Dye JA, Adler KB. Effects of cigarette smoke on epithelial cells of the respiratory tract. Thorax. 1994;49:825-34. PubMed PMID: 8091331; PubMed Central PMCID: PMCPMC475133.