Original Article

Zahedan Journal of Research in Medical Sciences

Journal homepage: www.zjrms.ir

Role of Interferon-Gamma in Bronchoalveolar Lavage Fluid on Distinguishing Active Pulmonary Tuberculosis from other Pulmonary Diseases

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Article information

Article history:
Received: 22 May 2013
Accepted: 18 July 2013
Available online: 21 Aug 2013
ZJRMS 2014 Nov; 16(11): 5-8

Keywords:
Pulmonary tuberculosis
Interferon-gamma
Bronchoalveolar lavage fluid

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Abstract

***Background*:** Tuberculosis is a global public health problem in the world. Microscopy of sputum smears is the most widely used method for diagnosing tuberculosis. However, many patients are smears negative for acid fast bacilli. Regarding the pathogenesis of the disease, the effectiveness of interferon-γ (IFN-γ) in bronchoalveolar fluid was investigated for the disease diagnosis.

***Materials and Methods*:** This descriptive study was performed at the Ali Ibn-e-Abitaleb hospital, Zahedan, between 2010 and 2012, to assess the role of IFN-γ level in bronchoalveolar lavage in distinguishing tuberculosis from other pulmonary diseases. In patients who required fiberoptic bronchoscopy as indicated, bronchoalveolar lavage was analyzed in terms of smear acid-fast staining and cytology. The participants were divided into TB patients group (the BK smear of bronchoalveolar fluid or the culture was positive) and pulmonary non-TB patients group (the smear was negative). Yet non-TB disease was definitively diagnosed by other means, as well. The fluid in each group was examined in terms of IFN-γ. Then, Mean IFN-γ levels in BALF were measured in these groups and then compared with each other.

***Results*:** Eighty eight patients were enrolled in the study among which, 31 cases had TB and 57 patients suffered from pulmonary non-TB disease. Mean IFN-γ was 2.85±4.17 pg/mL in pulmonary TB patients and 2.21±1.21 pg/mL in pulmonary non-TB patients.

***Conclusion*:** Lack of significant differences between the two groups in IFN-γ indicate that this factor is not suitable for diagnosis of tuberculosis and differentiating it from other pulmonary diseases.

Introduction

Tuberculosis (TB) mediated by the airborne pathogen *Mycobacterium tuberculosis* remains a global pandemic, with around 8.7 million new cases in 2011 [1]. Control of TB is a high priority and becomes one of the international missions after the increase in the number of cases all over the world including the developed countries [2]. The probability of pulmonary tuberculosis disease is based on the experience of close touch with consumptive person and clinical symptoms and imaging results, but acid-fast bacilli (AFB) smear-positive sputum is usually an initial clue in the diagnosis of pulmonary tuberculosis (TB); its approval by the sensitivity of sputum smear is only 40-70% and its slow growth (4-8 weeks) delays the diagnosis and treatment [3, 4].

Fiberoptic bronchoscopy with transbronchial biopsy and bronchoalveolar lavage have proved a valuable tools in the patients with no sputum or not able to excrete the sputum (with sensitivity 48-80%) [5]. Despite the presence of standard diagnostic methods, diagnosis of TB is still problematic. With respect to the prevalence of the disease in Sistan and Balouchestan (the extent of pulmonary TB outbreak in the province is about 32.27 in each 100,000 comparing to 8.73 in 100,000 in the country) [6] and Prompt diagnosis and early treatment of tuberculosis constitute the most effective intervention in controlling and reducing the transmission of *M. tuberculosis* and since there is no definite laboratory method for TB diagnosis and the low specificity and sensitivity of current diagnostic techniques, it is necessary and useful to find fast and reliable diagnostic methods.

The determination of cytokine concentrations in serum and bronchoalveolar lavage fluid (BALF) may contribute to the diagnosis of tuberculosis since cytokines have been ascribed an important role in TB pathogenesis [7]. The pro-/anti-inflammatory cytokine balance has been shown to play an important role in the pathogenesis and activity of TB including granuloma formation, caseation necrosis and delayed type hypersensitivity. Studies have shown that certain cytokine concentrations in serum as well as bronchoalveolar lavage fluid may also contribute to the diagnosis Among the major factors in the resulted inflammation, the followings can be implied: interferon-γ (IFN-γ), adenosine deaminase, and TNF, and IFN-γ is a key cytokine in the control of *M. tuberculosis* infection. It is produced by both CD4- and CD8-type T cells and

activates macrophages in TB [8] and is one of the main regulators of immune system made by immune cells in response to antigenic and immunological stimulations [7]. IFN-γ increase in much disease for example, granolomatosis disease.

A granuloma is the body's way of dealing with a substance it cannot remove or sterilize. The key association between IFN-γ and granulomas is that IFN-γ activates macrophages so that they become more powerful in killing intracellular organisms.

A granuloma is the body's way of dealing with a substance it cannot remove or sterilize. Infectious causes of granulomas (infections are typically the most common cause of granulomas) include tuberculosis, leprosy, histoplasmosis, cryptococcosis, coccidioidomycosis, blastomycosis and cat. Examples of non-infectious granulomatous diseases are sarcoidosis, berylliosis Wegener's granulomatosis, Churg-Strauss syndrome, pulmonary rheumatoid nodules and aspiration of food and other particulate material into the lung.

IFN-γ induces the anti-mycobacterium inflammatory activity of macrophage which at the time is applied as a diagnostic method in pleural fluid of patients suffering from tubercular pleurisy. In tuberculous pleuritis, diagnosis is established by demonstrating high level of TB markers in pleural fluid (IFN-γ> 140 pg/mL) [3]. In previous studies, IFN-γ levels (blood BALF and peritoneal fluid) were increased in active TB patients [8, 9]. However, there was no significant difference in the serum levels of these cytokines among groups [8]. IFN-γ may be a sensitive and specific marker for the accurate diagnosis of tuberculous peritonitis. The level of IFN-γ may contribute to the accurate differentiation of tuberculosis ascites from non-TB ascites [9]. Elevations of IFN-γ have been found in the affected lung and bloodstream of patients with pulmonary tuberculosis. Shahid reported that measurement of IFN-γ production in blood is helpful to diagnose active tuberculosis, but further research is required [10].

Because the results were inconsistent and incomplete in studies We decided to measure the levels of IFN-γ in bronchoalveolar lavage fluid.

Materials and Methods

This descriptive study took place from 2011 to 2012 at the Ali Ibn-e-Alitaleb hospital, Zahedan on patients who needed bronchoscopy. Participants enrolled in this study gave informed consent. There is no cost to participate in this survey and the study was approved by the hospital ethics committee. Two groups were studied: pulmonary TB patients and pulmonary non-TB patients.

Between 2011 to 2012, in 300 patients needed the fiber optic bronchoscopy as indicated with negative acid-fast sputum smear in triplet, bronchoaveolar fluid was examined in terms of acid-fast staining and cytology. Indications for diagnostic bronchoscopy were suspicion of TB based on clinical and radiological findings and suspicion of lung cancer/obstructive pneumonia atelectasis or reumatologic disease in sputum smear-negative patients. If bronchoaveolar fluid smear was positive and the patients were confirmed as TB by other techniques (including biopsy and positive sputum culture for TB bacilli or BALF smear and/or culture), they were examined in TB group (31 patients). The patients with negative acid-fast bacillus in BALF and sputum, that culture had been negative for TB and/or clinical improvement and radiological resolution on follow-up and who were definitely rejected to be TB using other methods (e.g. cytology and biopsy) and definitely diagnosed as suffering from other diseases were placed in non-TB pulmonary group (57 patients). Patients who did not definitely diagnosis excluded from study. The patients' condition was checked between 6 and 12 months and TB ones who were diagnosed to suffer from other diseases and vice versa exited the study and finally 88 patients were studied.

A written consent was obtained from each patient and after 6 h fasting and the topical application of lidocaine (2%) spray a fiberoptic bronchoscopy was done. Then, normal saline (50 mL) was inserted into bronches and then the liquid was suctioned back, gathered in dry tubes, and submitted to laboratory for examination. If necessary, biopsy was also administered on suspicious and involved areas. Specimens, then, were kept at 21ºC and then examined by means of ELIZA kit (Bender Med Systems company, Austria). In laboratory, the specimens were centrifuged for 15 min with 5000 rmp and the fluid was separated from cell and then examined in terms of acid-fast staining and cytology.

Statistical analysis: A data entry sheet on Microsoft Excel software was used to the data entry, SPSS-20 was used to statistically analyze the data Mann-Whitney *U* test used for comparison between tuberculosis and other pulmonary diseases. *p*-values <0.05 were significant. Receiver operating characteristics (ROC) curves were constructed in order to establish a sensitivity specificity relationship for best cut off values.

Results

During the study period, of the patients undergone bronchoscopy, 88 patients (43 men and 45 women) participated in this study among whom 31 (16 men and 15 women) were diagnosed as pulmonary TB and 57 (27 men and 30 women) pulmonary non-TB. Based on results, mean IFN-γ level in bronchoaveolar fluid was higher in TB patients (2.85±4.17 pg/mL) comparing to non-TB patients (1.21±2.21 pg/mL). Yet, based on Mann Whitney *U* test, there was no statistically significant difference between the two in terms of IFN-γ level. Then, to find the best predictive value in diagnosing pulmonary TB, ROC curve was drawn by SPSS-20 software for determining bronchoalveolar fluid cut-off point.

At cut off 2.05 pg/mL, sensitivity and specificity were respectively gained 32.3% and 57.9% for pulmonary TB diagnosis. Of TB patients, 10 (32.3%) had IFN-γ level ≥2.05 pg/mL, and 21 (67.7%) had <2.05 pg/mL. Of non-TB patients, 24 (42.1%) had IFN-γ level ≥2.05 pg/mL, and 33 (57.9%) had <2.05 pg/mL. Of 34 patients with

IFN-γ level 2.05 pg/mL ≤10 (29.4%) were pulmonary TB and 24 (70.6%) pulmonary non-TB; while of 54 patients with IFN-γ level <2.05 pg/mL, 21 (38.9%) and 33 (61.1%) were respectively pulmonary TB and pulmonary non-TB.

In differentiating pulmonary active TB from pulmonary non-TB patients, the negative and positive predictive values of IFN-γ level in bronchoaveolar fluid were respectively gained 61.1% and 29.4%. Consequently, there was no need to establish cut off levels for these cytokines, indicating that serum cytokines were not reliable diagnostic tools to distinguish TB from other pulmonary diseases.

Discussion

Mean IFN-γ level in bronchoaveolar fluid of tuberculosis patients was higher than in the non-TB patients but no statistical significance was revealed in the present stady.

At cut-off point 2.05 pg/mL, the value of sensitivity and specificity were respectively gained 32.3% and 57.9% which is not high for diagnosing TB and differentiating it from non-TB diseases and indicates that this test is not appropriate for diagnosing pulmonary TB and differentiating it from other pulmonary non-TB diseases. Accordingly, this parameter must not be applied at the time. Animal studies have shown that IFN-γ plays a pivotal and essential role in protective cellular immunity in TB infection [11, 12]. There are many ex-vivo studies showing decreased concentrations of IFN-γ in TB patients [13-16]. Conversely, in previous studies, IFN-γ levels were found to be significantly increased in active TB patients [8, 17-20]. However, there was no significant difference in the serum levels of these cytokines among groups. Our results are similar to other previous ones on IFN-γ level in bronchoaveolar fluid. Studies of Tsao et al. and Kupeli et al. again IFN-γ level in pulmonary BT patients' bronchoaveolar fluid were not different from pulmonary non-BT ones' [21, 22].

In a study by Antonangelo et al. IFN-γ level was significantly higher in bronchoaveolar fluid in pulmonary TB patients' BALF comparing to control group (intact individuals). However, the levels did not have the capability of differentiating TB patients from non-TB ones [23]. Unlike this study, the intact individuals without bronchoscopic indication and or pulmonary TB patients for whom we did not have definite diagnosis were eliminated, in our examination.

The difference between our results and the results of other studies can be related to various methods and different prevalence levels in the area. Disease stage, the time of transmitting the specimens to laboratory and no delay on the way to the lab, the interval between keeping the specimens and carrying out the tests as well as simultaneous diseases (HTN, diabetes melitus,…) which can indicate high or low cytokins level in bronchoalveolar fluid separate from pulmonary disease may also result in the difference between various studies [24, 25].

The weakness of our study is a small number of patients with active tuberculosis. However, it was difficult to find patients with a strong clinical suspicion for active TB and three negative sputum smears for the the organism who needed bronchoscopy and were qualified for entering the study, because many pulmonary TB patients are diagnosed by sputum smear examination. Moreover, disease stage and the existence of simultaneous non-pulmonary diseases require further consideration. Despite carrying out bronchoscopy on pulmonary non-TB patients, again definite diagnosis (based on biopsy specimens and other techniques) was impossible and these patients were not studied. In addition, there was no statistically significant difference between the two groups in IFN-γ level in bronchoaveolar fluid. Hence, IFN-γ level is inappropriate for diagnosing pulmonary TB and differentiating it from other pulmonary non-TB diseases. Accordingly, this parameter must not be applied now, and further studies must be conducted bearing in mind the limitations and challenges of this study.

Acknowledgements

Hereby, I extend my sincere gratitude to all my colleagues whose names are mentioned in the authors' contribution. They all helped me conduct the present study.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Zahedan University of Medical Sciences.

References

1. World Health Organization report. Global tuberculosis control. Geneva Switzerland: 2011.
2. Saif Alfaresi M, Hag-Ali M. Susceptibility Pattern and Epidemiology of Mycobacterium tuberculosis in United Emirati Hospital. Open Microbiol J. 2010;4:1–4.
3. Mario C, Raviglione MC, O'Brien RJ. Tuberclosis. In: Braunwald E, Hauser SL, Longo DI, editors. Harrison´s principles of internal medicine. 17th ed. New York: Mc Graw Hill; 2008. pp. 1016–25.
4. Kayacan O, Karnak D, Delibalta M, Beder S, Karaca L, Tutkak H. Adenosine deaminase activity in bronchoalveolar lavage in Turkish patients with smear negative pulmonary tuberculosis. Respir Med. 2002;96(7):536–41.
5. Anderson RM. The pandemic of antibiotic resistance. Nat Med. 1999;5(2):147–9.
6. Mirhaghani L, Nasehi M. Iranian Ministry of Health Center for Disease Control, editor. National guidelines for TB control. 2002
7. Halvani AB, Binesh F. Adenosine deaminase activity in bronchoalveolar lavage fluid in patients with smear-negative pulmonary tuberculosis. Tanaffos Respir. 2008;7(2):45–9.

8. Belli F, Capra A, Moraiti A, Rossi S, Rossi P. Cytokines assay in peripheral blood and bronchoalveolar lavage in the diagnosis and staging of pulmonary granulomatous diseases. Int J Immunopathol Pharmacol. 2000;13(2):61–7.
9. Su SB, Qin SY, Guo XY, Luo W, Jiang HX. Assessment by meta-analysis of interferon-gamma for the diagnosis of tuberculous peritonitis. World J Gastroenterol. 2013;19(10):1645–51.
10. Hussain S, Afzal N, Javaid K, Ullah MI, Ahmad T, Saleem Uz Z. Level of interferon gamma in the blood of tuberculosis patients. Iran J Immunol. 2010;7(4):240–6.
11. Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme IM. Disseminated tuberculosis in interferon gamma gene-disrupted mice. J Exp Med. 1993;178(6):2243–7.
12. Pearl JE, Saunders B, Ehlers S, Orme IM, Cooper AM. Inflammation and lymphocyte activation during mycobacterial infection in the interferon-gamma-deficient mouse. Cell Immunol. 2001;211(1):43–50.
13. Hirsch CS, Toossi Z, Othieno C, Johnson JL, Schwander SK, Robertson S, et al. Depressed T-cell interferon-gamma responses in pulmonary tuberculosis: analysis of underlying mechanisms and modulation with therapy. J Infect Dis. 1999;180(6):2069–73.
14. Seah GT, Scott GM, Rook GA. Type 2 cytokine gene activation and its relationship to extent of disease in patients with tuberculosis. J Infect Dis. 2000;181(1):385–9.
15. Smith SM, Klein MR, Malin AS, Sillah J, McAdam KP, Dockrell HM. Decreased IFN- gamma and increased IL-4 production by human CD8(+) T cells in response to Mycobacterium tuberculosis in tuberculosis patients. Tuberculosis (Edinb). 2002;82(1):7–13.
16. Turner J, Corrah T, Sabbally S, Whittle H, Dockrell HM. A longitudinal study of in vitro IFNgamma production and cytotoxic T cell responses of tuberculosis patients in the gambia. Tuber Lung Dis. 2000;80(3):161–9.
17. Dlugovitzky D, Torres-Morales A, Rateni L, Farroni MA, Largacha C, Molteni O, et al. Circulating profile of Th1 and Th2 cytokines in tuberculosis patients with different degrees of pulmonary involvement. FEMS Immunol Med Microbiol. 1997;18(3):203–7.
18. Verbon A, Juffermans N, Van Deventer SJ, Speelman P, Van Deutekom H, Van Der Poll T. Serum concentrations of cytokines in patients with active tuberculosis (TB) and after treatment. Clin Exp Immunol. 1999;115(1):110–3.
19. Toossi Z, Kleinhenz ME, Ellner JJ. Defective interleukin 2 production and responsiveness in human pulmonary tuberculosis. J Exp Med. 1986;163(5):1162–72.
20. Torres M, Herrera T, Villareal H, Rich EA, Sada E. Cytokine profiles for peripheral blood lymphocytes from patients with active pulmonary tuberculosis and healthy household contacts in response to the 30-kilodalton antigen of Mycobacterium tuberculosis. Infect Immun. 1998;66(1):176–80.
21. Tsao TC, Huang CC, Chiou WK, Yang PY, Hsieh MJ, Tsao KC. Levels of interferon-gamma and interleukin-2 receptor-alpha for bronchoalveolar lavage fluid and serum were correlated with clinical grade and treatment of pulmonary tuberculosis. Int J Tuberc Lung Dis. 2002;6(8):720–7.
22. Kupeli E, Karnak D, Beder S, Kayacan O, Tutkak H. Diagnostic accuracy of cytokine levels (TNF-alpha, IL-2 and IFN-gamma) in bronchoalveolar lavage fluid of smear-negative pulmonary tuberculosis patients. Respiration. 2008;75(1):73–8.
23. Antonangelo L, Vargas F, Figueiredo V. Interfron-gamma and adenosine deaminase in the bronchoalveolar lavage of patiens with pulmonary tuberclosis and negative sputum. Chest. 2006;23(2):346–60.
24. Boonsarngsuk V, Suwannaphong S, Laohavich C. Combination of adenosine deaminase activity and polymerase chain reaction in bronchoalveolar lavage fluid in the diagnosis of smear-negative active pulmonary tuberculosis. Int J Infect Dis. 2012;16(9):e663–8.
25. Jafari C, Ernst M, Kalsdorf B, Greinert U, Diel R, Kirsten D, et al. Rapid diagnosis of smear-negative tuberculosis by bronchoalveolar lavage enzyme-linked immunospot. Am J Respir Crit Care Med. 2006;174(9):1048–54.

***Please cite this article as*:** Moulaei NA, Niazi AA, Saeidpour-Parizi A, Shahba S. Role of interferon-gamma in bronchoalveolar lavage fluid on distinguishing active pulmonary tuberculosis from other pulmonary diseases. Zahedan J Res Med Sci. 2014; 16(11): 5-8.