ORIGINAL ARTICLE

The sequential assay of interleukin-10 and 13 serum levels in relation to radiographic changes during pulmonary tuberculosis treatment

Sayyed Gholamreza Mortazavi Moghaddam[1], Mohammad Hasan Namaei[2], Reza Eslami Manoochehri[3], Mahmood Zardast[4]

[1]Departments of Internal Medicine, Division of Pulmonary, School of Medicine, Vali-e-Asr Hospital, Birjand University of Medical Sciences, Birjand, Iran, [2]Infectius Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran, [3]Department of Internal Medicine, School of Medicine, Vali-e-Asr Hospital, Birjand University of Medical Sciences, Birjand, Iran, [4]Department of Pathology, School of Medicine, Vali-e-Asr Hospital, Birjand University of Medical Sciences, Birjand, Iran

Background: We evaluated the sequential changes of interleukin (IL)-10 and IL-13 serum levels with tuberculosis (TB)-related radiographic changes during pulmonary TB (PTB) treatment. **Materials and Methods:** In this cross-sectional study during two consecutive years, forty cases with PTB were recorded, and finally, 24 cases were completed the study. Serum levels of IL-10 and IL-13 were measured on admission time, and 6 months later. Furthermore, chest radiography was performed on admission and 6 months later in the treatment course. **Results:** Radiography at the baseline indicated pulmonary infiltration in all patients (n = 24). Fifteen (62.5%) cases had abnormal and 9 (37.5%) cases had normal radiography at the end of 6 months treatment course. IL-10 and IL-13 upregulated during the treatment time course, and their relationship with radiographic changes shifted from negative (r = −0.14 and P = 0.71) on admission to positive (r = 0.80 and P < 0.001) at the end of 6 months treatment course in normal radiography group. IL-10 level at the start of the treatment was 121.90 ± 88.81 in patients with normal and 82.68 ± 41.50 in patients with abnormal radiography (P = 0.31). **Conclusion:** Sequential increase in IL-10 and IL-13 during PTB treatment course may have a role in clearing the TB-related radiographic infiltration and preventing scar formation.

Key words: Cytokines, diagnostic X-ray, immune system phenomena, mycobacterium

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INTRODUCTION

Abnormal radiography is one of the main pulmonary tuberculosis (PTB) manifestations, and pulmonary complications arising from old tuberculosis (TB) manifest characteristically by scar formation in chest radiography.[1]

Fibrotic phenomenon is an important factor in mortality and morbidity.[2] T-cell-associated cytokines (including interleukin [IL]-13) appear to play an important role in promoting fibrotic reactions.[3] IL-10 (the most well-known anti-inflammatory cytokine) may also play a role in the inhibition of phagosome and also modulate fibrotic reactions.[4,5]

Several studies have shown that patients who undergone a successful treatment course for PTB can suffer from lung fibrosis and abnormal radiological findings.[6-8] In this regard, cytokines such as IL-10 and IL-13 can play an important role.[9,10] There are some reports of increased IL-10 production during active TB.[11] Its production regulates one of the most important mechanisms in protecting the over immune response and tissue damage. The reduced levels of IL-10 increase the risks of damage from immunopathological mechanisms.[12] In contrast to this topic, the greater levels of IL-10 play

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Address for correspondence: Dr. Mohammad Hasan Namaei, Infectius Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran. E-mail: mhnamaei@hotmail.com

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a key role in limiting the immune response and reduce the power to clean pathogens.[13] Little information is available about IL-13 in the course of PTB. Studies in TB patients have shown that rapid responders to antimycobacterial treatment have higher levels of IL-13 in their serum than those of slow responders.[11] On the other hand, in an experimental study, it was hypothesized that transgenic mice overexpressing IL-13 have a higher risk of developing necrosis in granuloma and tissue pathology similar to postprimary TB in humans.[14]

Given the highly dynamic situation of serum cytokines in the course of TB treatment, the remodeling of the lungs as a result of TB and its treatment may be affected by the cytokines and their interaction.[9]

While there are some studies on the role of IL-10 and IL-13 in TB, and despite their essential roles in response to treatment and immune pathogenesis of TB, there are still no consensus on the role and their interaction in relation to immune pathogenesis and complications of TB including lung fibrosis. We investigated the changes of ILs in relation to the radiographic changes during TB treatment.

MATERIALS AND METHODS

In a cross-sectional study, we included all PTB patients (regardless of whether TB is primary or secondary) who were referred to the main TB Center in South Khorasan province (Birjand, Iran) during years 2015–2017. The cases were excluded if they suffered from malignancies or chronic pulmonary diseases such as asthma or chronic obstructive pulmonary disease and being smoker. The sampling method was census and within 2 years of study, forty patients with confirmed lung TB were enrolled, of whom 24 cases had completed our study.

PTB diagnosis was based on the clinical signs and radiographic findings in addition to one of the following conditions:[15]

- Two acid-fast bacilli-positive sputum smear (AFBs+)
- One AFBs + and one positive sputum culture for tubercle bacilli (TBsc+)
- Positive bronchoalveolar lavage (BAL) smear for AFB with a positive culture of BAL or sputum for tubercle bacilli
- One TBsc+ or AFBs+ in patients whose clinical and radiographic findings were strongly suggestive of PTB.

Patients were selected regardless of bacilli density in the smear.

Informed consent was obtained, and 5 ml of blood was taken before the initiation of treatment (Time 1). Blood serums were stored at −70°C. In a similar manner, sampling was repeated at 2 (Time 2) and 6 (Time 3) months later in the treatment course.

Patients were treated based on the World Health Organization protocols.[16] Patients were also examined by pulmonologist every 2 months. After 6 months, patients were evaluated through history and clinical examination. Based on the initial diagnostic method, AFB samples were also repeated to assess getting cured. The time for deciding which patient to stay in the study was the end of treatment. The patients were excluded if there were any evidence of *Mycobacterium tuberculosis* in the smear or culture at the end of 6 months treatment course.

Chest X-ray (CXR) at the start and end of the treatment was evaluated blindly by a radiologist. Regardless of the extension and type of involvement, radiographic changes in favor of PTB were considered.

IL-10 and IL-13 were measured by the ELISA method using the INTEGRA 400 device (the Roche Diagnostics Kit, Germany) and reported quantitatively. Inter and intra assay coefficients of variation for both IL-10 and IL-13 were <12% and <10%, respectively.

Using SPSS 23 software (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.). The normality of data was assessed by Kolmogorov–Smirnov test. We used Mann–Whitney and Friedman tests to compare the means in groups with abnormal distribution. The relationship between data with normal and abnormal distribution was assessed by Pearson correlation and Spearman's rho test, respectively. $P < 0.05$ was considered statistically significant.

RESULTS

Among forty patients with PTB who were enrolled, 24 patients (mean age of 60.87 ± 21.50 years) completed the study (9 (37.5%) males and 15 (62.5%) females).

Radiography at the baseline indicated pulmonary infiltration in all patients. At the end of the treatment, 15 (62.5%) cases had abnormal (abn. CXR group) and 9 (37.5%) cases had normal radiography (nl. CXR group).

The mean serum of IL-10 and IL-13 levels and their comparison between normal and abnormal CXR groups at times 1, 2, and 3 and also the mean changes during the times are presented in Table 1.

The relation between IL-0 and IL-13 at serial time's measurements in normal and abnormal CXR is presented in Table 2.

Table 1: Comparison of the mean serum levels and mean changes of interleukins in abnormal and normal chest X-ray groups at different times of treatment

Cytokine	Mean±SD		*P*
	Abnormal CXR group (*n*=15)	Normal CXR group (*n*=9)	
IL-10 (ng/ml)			
Time 1	82.68±41.50	121.90±88.81	0.31†
Time 2	77.68±39.37	135.21±107.93	0.34†
Time 3	78.66±37.72	147.02±114.66	0.23†
P	0.42	0.89	
Mean changes	−4.02±21.75	+25.12±94.52	0.55†
IL-13 (ng/l)			
Time 1	133.53±54.65	111.63±46.00	0.31†
Time 2	117.86±76.48	124.17±48.75	0.48†
Time 3	107.14±38.74	178.07±150.61	0.23†
P	0.01 (Time1 vs 3)	0.89	
Mean changes	−26.39±50.53	+66.44±138.16	0.03†

†Mann–Whitney test, Time 1=Start of treatment; Time 2=2 months later to start of treatment; Time 3=6 months later to start of treatment; IL=Interleukin; CXR=Chest X-ray

Table 2: The relations between interleukin-10 and 13 in all, abnormal chest X-ray, and normal chest X-ray groups at different times of treatment

	Mean±SD		*r* (*P*)
	IL-10 (ng/ml)	IL-13 (ng/l)	
All patients			
Time 1	97.38±64.56	125.32±51.68	0.3 (0.14)†
Time 2	99.25±76.19	120.22±66.31	0.63 (0.00)†
Time 3	104.29±81.13	133.74±100.17	0.75 (0.00)†
P	0.75	0.11	
Mean change	6.90±60.03	8.42±101.49	0.19 (0.35)†
Abnormal CXR			
Time 1	82.68±41.50	133.53±54.65	0.63 (0.01)†
Time 2	77.68±39.37	117.86±76.48	0.73 (<0.01)†
Time 3	78.66±37.72	107.14±38.74	0.74 (<0.01)†
P	0.42	0.01 (Time1 vs 3)	
Mean change	−4.02±21.75	−26.39±50.53	0.58 (0.02)‡
Normal CXR			
Time 1	121.90±88.81	111.63±46.00	−0.14 (0.71)‡
Time 2	135.21±107.93	124.17±48.75	0.82 (<0.01)‡
Time 3	147.02±114.66	178.07±150.61	0.80 (<0.01)‡
P	0.89	0.89	
Mean change	+25.12±94.52	+66.44±138.16	0.10 (0.79)‡

†Spearman's rho test; ‡Pearson's correlation test, Time 1=Start of treatment; Time 2=2 months later to start of treatment; Time 3=6 months later to start of treatment; IL=Interleukin

DISCUSSION

Chest imaging is one of the main diagnostic tools for PTB, even occasionally in the absence of clinical suspicion.[1] According to the present study, CXR infiltration at the time of PTB diagnosis was universal, but 62.5% of the patients remained to exhibit abnormal CXR at the end of the treatment. There is a wide variety of complications after PTB.[9] Among them, post-TB lung damage is common, and abnormal radiography due to fibrotic changes was reported between 25% and 70% in different study.[17]

The most significant finding in our study was a constant rise in IL-10 and IL-13 during treatment in normal CXR group. This upregulation was significantly more obvious for IL-13 in patients with normal CXR. There was also a significant sequential decrease in IL-13 during the treatment of patients with abnormal CXR. While inhibition of IL-13 was sufficient to protect mice from radiation-induced lung fibrosis,[18] the abnormal radiography (fibrotic sequel) at the end of treatment can be apparently contradictory in the present study as IL-13 declines during treatment. One possibility may be related to type of fibrotic reaction (TB vs. radiation-induced pulmonary fibrosis) and the role of IL-10, where the levels of IL in individuals with abnormal radiography at the end of TB treatment course were lower than those with normal radiography in our study.

Both IL-10 and IL-13 are of type 2 cytokines that contribute but with different effects to fibrotic change in the lung.[19] Targeting IL-10 in mice prevents bleomycin-induced fibrosis.[20] Intravenous injection of IL-10 in rats has modified the fibrosis process even after 2 weeks from bleomycin-induced fibrosis.[21] Subepithelial fibrosis occurs in the respiratory tract when IL-13 expression induced through the transgenic method in mice.[22] A positive and statistically significant correlation between IL-13 and IL-10 in situation with higher levels of IL-13 at initial of PTB treatment was observed in patients with abn. CXR. According to the present study, however, when IL-10 (as an anti-inflammatory cytokine) was higher at the onset of treatment and upregulated proportionate to that of IL-13 (as a profibrotic cytokine) during treatment, residual radiographic scar will be prevented.

Numerous other cytokines have been also studied in relation to inflammatory or fibrotic responses of TB based on chest radiographs after 2 or 6 months of TB treatment.[9,23] By emphasizing on IL-10 and IL-13 as TH-2 regulatory ILs, they can play a decisive role in the course of TB and related sequels including scar formation, whereas IL-10 gives TB bacillus opportunity to proliferate, at the same time, the damage caused by the immune response decreases.[24] In the case of IL-13, it has a modulator role in autophagy as an important protective function during mycobacterial infection.[25] Labeled as the main type 2 cytokine, IL-13 is also suggested to play an essential role in the immune pathogenesis of fibrosis development.[17,21,26] In our study, however, it can be suggested that IL-10 during TB treatment plays a key role in lung fibrosis prevention. While other studies have highlighted the key role for IL-13 in fibrosis, the present study highlights the role of IL-10 in TB-associated pulmonary fibrosis.

Our limitations were relatively small sample size, heterogeneity of the patients, and TB patients recruited from a single center in Iran.

CONCLUSION

Sequential increase in IL-10 and IL-13 during treatment concomitant with a positive relationship between the two ILs during and at the end of treatment in patients who convert to normal radiography, probably reflect the role and interaction of the two ILs in clearing radiographs and preventing pulmonary scar.

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Conflicts of interest

There are no conflicts of interest.

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