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Comparison of serum interleukin-10 level of fungal exposure among patients with pulmonary sarcoidosis and healthy people

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Abstract. *Introduction:* Sarcoidosis is a chronic systemic inflammatory disease with unknown etiology. Fungal exposure has been assumed as one of many possible causes of the disease. The prevalence of sarcoidosis is likely to be higher in the Northern Iran compared with other regions. Environmental studies have shown higher levels of fungal spores in the air of this area. Some studies have shown that fungal exposure in patients with sarcoidosis is associated with decreased levels of interleukin-10 (IL-10) serum levels. The aim of present study was comparison of the serum levels of IL-10 in patients with pulmonary sarcoidosis and healthy people. *Objectives and Methods:* In this current analytical, cross-sectional study, 40 patients with pulmonary sarcoidosis compared with 34 healthy individuals as a control group, who had been visited in a pulmonary referral clinic in Rasht (Guilan-Iran). Demographic data were collected by a questionnaire. Serum IL-10 levels were measured by ELISA kit. The data were analyzed by using the SPSS software (version 19). *Results:* The mean concentration of IL-10 serum levels were reported 10.96±9.48 pg/ml^{-1} and 3.77±1.47 pg/ml^{-1} among the patients with pulmonary sarcoidosis and healthy individuals, respectively. The significance difference was demonstrated between patients with pulmonary sarcoidosis and control group ($p<0.0001$). The IL-10 showed a significant difference between the patients older than 40 and those younger than 40. In statistical analysis, 4.75 pg.ml^{-1} was considered the cutoff point to separate patients and control group. *Conclusion:* The results showed that IL-10 was greater among patients who diagnosed as pulmonary sarcoidosis. There was a contrary opinion of the expectations for the role of fungal exposure as a possible cause of greater prevalence of sarcoidosis in Northern Iran. Age and stage of disease showed a significant relationship with the IL-10 serum level and requires further investigation. IL-10 might be a possible predictor of sarcoidosis along with other factors. *(Sarcoidosis Vasc Diffuse Lung Dis 2018; 35: 294-298)*

Key words: blood serum, pulmonary sarcoidoses, fungal exposure, IL-10

Introduction

Sarcoidosis is a chronic systemic inflammatory disease with unknown etiology. It has affected both genders and all races aged 20s-50s, which is often manifested in bilateral hilar lymphadenopathy, pulmonary and ophthalmic manifestations, and skin lesions (1, 2). The most important histologic finding of the disease is non-caseating epithelial cell granuloma

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(2, 3). The prevalence and incidence of sarcoidosis varies widely in the world. The highest incidence is seen in northern European countries (1). Previous studies have shown that the prevalence might be greater in north of Iran (Guilan Province), especially the west part, compared to other regions (4). The etiology of sarcoidosis is not yet fully known; however, infections, inflammatory, environmental and occupational factors along with genetic predisposition are likely to be responsible for sarcoidosis (1, 2). In recent years, progress in different fields, such as biochemistry, genetics, immunology and molecular biology, has dramatically improved our understanding of the disease (5). The studies have shown that fungal exposure could be a risk factor for sarcoidosis (6, 7). Fungal exposure might be due to the contact with pollens, moist house, and water molds. Some studies have proven high levels of fungal exposure in houses of patients diagnosed with sarcoidosis especially those with relapses (7). These findings show that the fungi in the environment be able to play an important role in the progression of the disease (8, 9). The possible mechanism can be attributed to the delayed hypersensitivity reactions to certain (9, 10) factors in fungal cell membrane such as β-glucan or chitin. Inflammatory response in sarcoidosis is characterized by increasing in the levels of various inflammatory mediators such as IL-10, IL-12 and TNF- α (11). Chemokines of T helper cell (Th 1,Th 2) are also effective and IL-10 and IL-12 levels increase in serum and bronchoalveolar lavage (BAL) of patients (12, 13). Among various inflammatory mediators of sarcoidosis, IL-10 is an anti-inflammatory mediator, which suppresses the granuloma formation (14, 15). Therefore, lack of IL-10 have a participatory role in granuloma formation in lungs of patients with sarcoidosis. In-vitro studies, show that the response of peripheral blood mononuclear cells (PBMC) to β-glucan particles among healthy individuals resulted in TNF-α, IL-6, IL-10, and IL-12 secretion (16). Other studies have shown that fungal exposure was associated with reduced serum IL-10 levels. Lower serum IL-10 levels in exposure with fungus suggests that cell wall factors play important role in granuloma formation in sarcoidosis by blocking the immune-IL-10 secretion system (17). On the other hand, the environmental study in Northern Iran showed high concentration of fungal spores in the air, especially in summer (18). Due to the higher incidence of pulmonary sarcoidosis in the Northern Iran, and higher concentrations of fungal spores in this environment. The present study focused on the serum IL-10 level as a possible tracer factor that show role of fungal exposure in patients with pulmonary sarcoidosis and in comparison with healthy individual.

Patients and Methods

This analytical cross-sectional study was achieved on patients with sarcoidosis who referred to a pulmonary referral clinic in Rasht (Guilan-Iran) from October 2015 to October 2016. The diagnosis of patients with sarcoidosis was done based on clinical, radiographical and histopathological evidences. The patients with sarcoidosis who had more than 20 years old included to study. The exclusion criteria were including the patients who had sarcoidosis with no pulmonary involvement, history of pulmonary fungal infection, lung fungal infection in biopsy, and immunodeficiency. The cigarette smokers also exclude of study. The control group included healthy nonsmoker people, more than 20 years old, with no history of respiratory infection, chronic respiratory disease, pulmonary fungal infection and immunodeficiency. Finally, 40 participants were selected as study group and 34 participants as control group. As well, the participants were allocated into four groups based on four stage of chest x-ray (CXR) and scalding standard rating system. Stage 1; Hilar adenopathy alone, stage 2; the combination of adenopathy and lung parenchymal infiltration, stage 3; parenchymal infiltration, and stage 4; Fibrosis and destruction of lung parenchyma. After that, serum IL-10 levels of participants were measured by ELISA test (IBL international, Hamburg, Germany, catalog No: BE53101).

Statistical analysis

The data was analyzed in SPSS software (SPSS Inc. Il, USA)19th version. T-test and non-parametric tests were done to find the relationship of the variables. The mean, standard deviation, ratio, and frequency percentage were used for descriptive statistics. In this study, an alpha of 0.05 is used as the cutoff for significance ($p<0.05$).

Table 1. Demographic characteristics of sarcoidosis patients and healthy people

Feature	Patients	Healthy people
Number	40	34
Gender		
Male (%)	23 (57.5%)	17 (50%)
Female (%)	17 (42.5%)	17 (50%)
Mean Age (Standard Deviation)	41.7 (9.02)	37.26 (7.37)
Age Group		
Older than 40 years old	19 (52.5%)	18 (52.9%)
Younger than 40 years old	21 (47.5%)	16 (47.1%)
Residential Location		
City	36 (90%)	29 (85.3%)
Village	4 (10%)	5 (14.7%)
Apartment	29 (72.5%)	29 (85.3%)
House with Yard (Villa)	11 (27.5%)	5 (14.7%)
Occupation		
Housekeeper	14 (35%)	14 (41.2%)
Employee	6 (15%)	8 (23.5%)
Worker	3 (7.5%)	2 (5.9%)
Other	17 (42.5%)	10 (29.4%)

Results

The demographic characteristics of participants showed that there was no significant differences between age, gender, job, and residential locations of patients with sarcoidosis and control group (Table 1). The CXR finding indicated that 16 participants (40%) were in stage 1, 21 participants (52.5%) were in stage 2, 2 participants (5%) were in stage 3, and one participant (2.5%) was in stage 4. The mean duration of sarcoidosis disease was 3.68±1.9 years.

The mean of serum IL-10 level was 10.96±9.48 $pg.ml^{-1}$ in patients with sarcoidosis and 3.77±1.47 $pg.ml^{-1}$ for control group. Based on statistical analysis, a significant difference was found in serum IL-10 levels of the patients with sarcoidosis and control group (95%-CI) (3.26; 4.28) ($p<0.0001$). Table 2 showed that the mean of IL-10 was almost three times higher in patients with sarcoidosis than control group.

Table 2. Mean IL-10 among patients diagnosed with sarcoidosis and healthy people

Serum IL-10 Level (pg/ml^{-1})	Patients	Healthy people
Mean	10.96	3.77
Standard Deviation	9.48	1.47
Minimum	3.20	1.80
Maximum	53.9	9.20

Figure 1 and 2 demonstrated that serum IL-10 level in the patients with sarcoidosis were higher than control group (95%-CI). Radiological results showed that there is no significant differences between the serum IL-10 levels in patients with sarcoidosis based on the stage of disease; however, IL-10 significantly declined at higher stages (Table 3). The mean of serum IL-10 level in the patients with sarcoidosis was investigated based on gender, job, and residential location. According to results, there was no signifi-

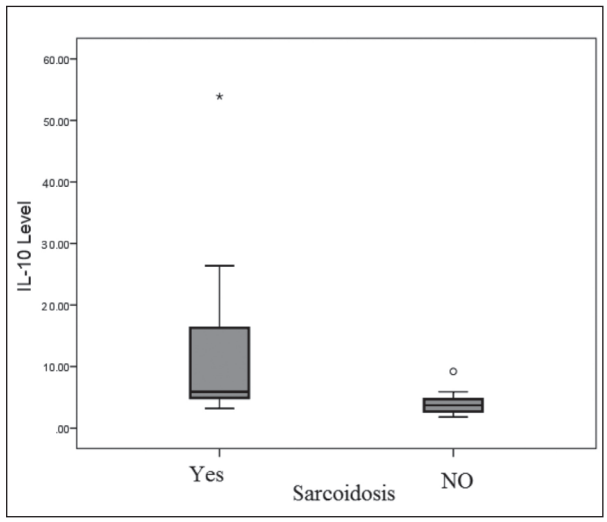

Fig. 1. IL-10 Box Plat in Two Groups

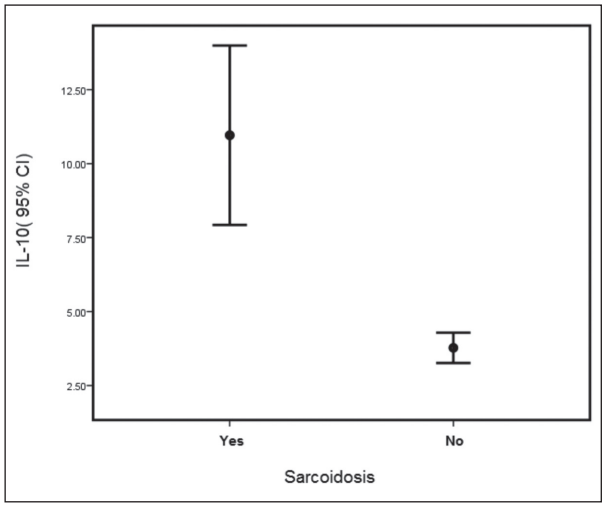

Fig. 2. Error Bar of 95% for IL-10 in Two Groups

Table 3. IL-10 based on stages of disease

	Mean	Standard Deviation	Minimum	Maximum
1	10.39	6.85	3.20	21.90
2	12.33	11.46	4.30	53.90
3	4.85	0.07	4.80	4.90
4	3.50	0	3.50	3.50

cant differences between the serum IL-10 levels with gender, job, and residential location.

The mean of serum IL-10 level were 8.28±6.25 pg.ml^{-1} in participants who were older than 40 years old and 13.41±11.26 pg.ml^{-1} in participants who were younger than 40 years old (p=0.02). No correlation was found between duration of disease and serum IL-10 level (r_s=-0.113 and p=0.488).

Based on significant difference of serum IL-10 level in patients with sarcoidosis and control group, the receiver operating characteristic (ROC) curve was employed to determine the cutoff point to prediction of disease. The area under the curve was 0.899±0.037 for predicting sarcoidosis (p<0.0001) (Figure 3).

Hence, the serum IL-10 level of 4.75 pg.ml^{-1} was the best cutoff point to separateing of the patients with sarcoidosis and healthy individuals (Sensitivity=82.5% and Specificity=82.5%).

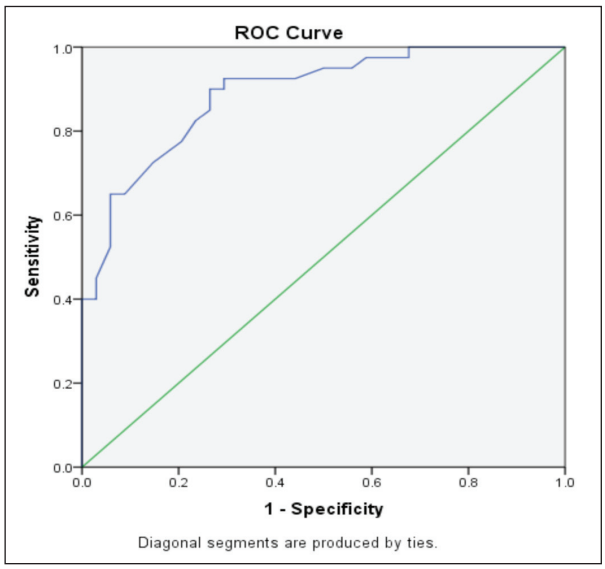

Fig. 3. ROC Curve of IL-10 Predictor Level

Discussion

Our findings showed that the mean serum IL-10 levels were 10.96±9.48 pg.ml^{-1} for the patients with sarcoidosis and 3.26±1.47 pg.ml^{-1} for the control group. The studies showed that the mean serum IL-10 levels could be 16.7±3.8 pg.ml^{-1} for the patients with sarcoidosis and 10.3±2.8 pg.ml^{-1} for healthy individuals (3) Fuse showed that the mean serum IL-10 levels is 23.8±4.0 pg.ml^{-1} for the patients with sarcoidosis and 2.1±0.4 for control group (19). In fact, the different level of serum IL-10 in patients with sarcoidosis and control group might be due to properties of population or poor quality of laboratory diagnostic kits.

There is no evidence in term of any relationship between the fungal exposure and serum IL-10 levels in patients with sarcoidosis. However, many studies have confirmed that the concentration of fungal spores in the Northern Iran is high (18) and the exposure to environmental fungi leads to a decrease in serum levels of IL-10 compared with healthy subjects (3). Therefore, serum IL-10 level was expected to be lower in patients with sarcoidosis. The CXR findings showed that serum IL-10 levels in the patients with sarcoidosis had no significant relationship with the sarcoidosis stages. However, serum IL-10 levels of patients with sarcoidosis were clearly lower at stages 3 and 4. Although, a small number of participants were at stage 3 and 4 of sarcoidosis.

Terčelj investigated the relation between IL-10 levels and the severity of lung granulomas infiltration. In this study lung infiltrations severity evalu-

ated by CXR and classified to five groups: 0 (Normal CXR), 1 (25% of lung parenchymal involvement), 2 (50% involved), 3 (75% involved), and 4 (100% involved). The results showed that pulmonary infiltration had no linear correlation with serum IL-10 levels, so that IL-10 declined at higher stages and the highest serum IL-10 level was reported in group 2 (17). Lower serum IL-10 level that was founded at stage 4 (fibrosis and parenchymal destruction). Due to few participants, the results were not statistically significant. In addition, Fuse has shown that some of drugs such as steroids are able to declined serum IL-10 levels(19). In fact, lower serum IL-10 level at higher stages of sarcoidosis might be due to steroids or the progression of parenchymal involvement.

In this study, direct examination of fungal infections was not done. Likewise, we did not measure the beta glucan effectiveness, antifungal activity of extracellular chitinase, the beta-N-acetyl hexos-aminidase (NAHA) enzyme activity, bronchoalveolar lavage (BAL), and total lymphocyte count due to financial and ethical issues.

According to our findings, the mean serum IL-10 level was higher in patients younger than 40 years old. The question is that; is age an effective factor in chronic disease progression by reducing serum IL-10 level? Similar studies have not been conducted regarding the correlation between serum IL-10 levels and the age of patients with sarcoidosis, and further studies are needed. Based on the statistical analysis, the 4.75 $pg.ml^{-1}$ was cutoff point for normal IL-10 levels between patients and healthy subjects. It is obvious that the serum IL-10 level may be used as a predictor of disease along with other parameters.

Conclusion

The serum IL-10 level is higher among the patients with sarcoidosis despite of expectations about fungal exposure role (with reduced level of IL-10) as a possible cause of higher prevalence of sarcoidosis in Northern Iran. The relationship of age and disease stage with serum IL-10 is notable and further investigation is required to prove it. We suggest that serum IL-10 may be used as a predictor factor of sarcoidosis along with others in the future.

Acknowledgment

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