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Original Article

Study the Relation of Serum Level of IL-33 with Severity of COPD Disease

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Abstract

Background: Cytokines are important in many pathobiological processes of chronic obstructive pulmonary disease (COPD). This study aimed to determine the relationship between serum levels of interleukin-33 (IL-33) and the severity of COPD disease.

Method: In this cross-sectional research, the study population consisted of all COPD patients referring to the pulmonary clinic of Imam-Ali Hospital of Zahedan city. Sixty patients were selected using the available sampling method. Serum IL-33 levels were measured by the quantitative ELISA method.

Results: Of 60 patients, 23 (38.3%) and 37 (61.7%) subjects were male and female, respectively. Analysis shows a significant difference between serum IL-33 of the two groups with regard to the severity of COPD disease. There was a statistically significant negative relationship between the serum level of IL-33 and the severity (decrease of forced expiratory volume in one second (FEV1)) of COPD disease.

Conclusion: Our results indicate a systemic release of IL-33 correlated with the severity of COPD.

Keywords: Cytokine; IL-33; Severity; Chronic Obstructive Pulmonary Disease; COPD

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Introduction

Chronic obstructive pulmonary disease (COPD) is a serious public health problem. It is the end result of a sensitive lung exposed to adequate environmental stimuli. COPD, caused primarily by tobacco smoke and domestic air pollution (HAP), is a silent killer in low- and middle-income countries: an estimated 328 million people worldwide have COPD, and is predicted to be the leading cause of death in the future (1). The disease has different types, such as emphysema and chronic bronchitis, which

are divorced only on the condition that there is a chronic obstruction (blockage) of airflow. Chronic bronchitis is not considered COPD without chronic airway obstruction (2, 3). The disease is diagnosed clinically by a pulmonologist, who will examine and observe the results of spirometry tests with or without bronchodilators. Finally, a questionnaire will be used to determine the presence of respiratory symptoms (4).

The spirometry results for COPD diagnosis are based on a decrease in the volume of exhaled air during the first second (FEV1) and the ratio of the volume of exhaled air during the first second to the mandatory critical capacity (FEV1 / FVC) less than 70% (5, 6). Identifying risk factors for the disease is an important step in preventing, improving symptoms, and policy-making for the disease. As an important risk factor for COPD, it has changed health policy in many respects; although there have been many studies on smoking and its association with COPD, this is not the only risk factor for COPD, and many studies have shown COPD has also been reported in non-smokers (4, 7).

For instance, working in coal mines, silica, gold, and the cotton textile industry has been suggested as a risk factor for chronic airway obstruction (1). Little is known about the role of cytokines in the pathogenesis of COPD and the clinical manifestations of the lung. A significant role of inflammatory cytokines, including interleukins one beta, interleukins 6, 17, 18 and 32, 33, interferon-gamma, TNFα and growth factors, including beta-modifying growth factor (TGFβ) in the pathobiological course of COPD has been reported (8).

The level of IL-33 in lung tissue was significantly increased in mice exposed to COPD. The role of innate immunity in chronic obstructive pulmonary disease based on long-term exposure of epithelial progenitor cells to high levels of IL-33 has been showed (13-9).

Nowadays, in order to identify predisposing factors in the pathogenesis of COPD, it is recommended to study the role of inflammatory factors such as cytokines, especially pro-inflammatory cytokines such as interleukins 1, 6, 8, and 35 and tumor necrosis factor (TNFα). These factors may play a role in the development of COPD. The role of inflammatory cytokines produced during the activity of lymphocyte cells in patients with severe COPD showed the role of these cells in the production of pro-inflammatory and post-inflammatory cytokines (10).

Therefore, the present study aimed to determine the relationship between serum concentrations of IL-33 as a pro-inflammatory cytokine in patients with COPD and the relationship with the severity of the disease.

Methods

This cross-sectional study was conducted on all patients with a definitive diagnosis of COPD who were referred to the outpatient clinic of Ali Ebne Abitaleb Hospital in Zahedan, which formed the study population. The exclusion criteria were other inflammatory diseases such as gastritis, rheumatoid arthritis, Infection with other infectious diseases. After taking the history and physical examination of patients referred to the lung clinic of Ali Ebne Abitaleb Hospital and performing spirometry, patients with COPD were identified by a pulmonologist. Then, the severity of the disease was determined based on spirometry results so that patients with FEV1 were more than 50% of the severe form and FEV1 were considered more than 50% of the non-severe form of COPD. Patients were then asked to participate in the project, and patients who consented to enter the study were given informed written moral consent. Five milliliters of whole blood were taken from each patient to measure the serum level of IL-33 by ELISA. All the above data were recorded in the design information form. The design data were entered in SPSS statistical software version 18 and analyzed.

Sample size and sampling method

Because no similar study has been conducted with this title so far, as well as the limitations of project implementation costs, the maximum sample size of this study was set at 60 people. Descriptive tests were used to describe the data, and independent t-tests and the Spearman correlation coefficient were used to analyze the data. *P*-value was considered 0.05.

Results

Of our patients (60 individuals), 37(61.67%) were females. The mean of age was 59.21±10.20 years, ranging from 41 to 90 years.

Table 1 shows the changes of IL-33 and FEV1 in different levels of severity of COPD. The table revealed that 46 patients had severe levels of COPD. The level of IL-33 was 61.89±26.17 and 27.63±22.54 in the severe and non-severe COPD. The level of IL-33 was significantly higher in severe levels of COPD (t=5.02, *P<0.001*). The levels of FEV1 were 32.17±7.29 and 57.71±4.78 in the severe and non-severe COPD; the levels of FEV1 were lower in the severe level of COPD (t=-12.22, *P<0.001*).

Table 2 shows the changes of IL-33 and FEV in gender; IL-33 had higher levels but was non-significant in females (54.34±33.02) compared to the males (45.87±28.47). FEV1 had non-significantly lower levels in males (39.30±11.52) compared to the females (37.41±13.67) .

Table 3 shows the correlation between Age, IL-33, and FEV1. Only IL-33 had a negative correlation with FEV1 (r= -0.663 and *P<0.001*).

Table 1. The changes of IL-33 and FEV in different levels of severity on COPD

Variable	COPD	Mean	SD	t-value	*P*-value	Mean	SD	Max	Min
IL 33	Severe	61.89	26.17	5.02	<0.001	51.09	31.38	125	2
	Non-Severe	27.63	22.54						
FEV1	Severe	32.1739	7.29171	-12.228	<0.001	38.13	12.82	65	20
	Non-Severe	57.7143	4.77862						

COPD, Chronic obstructive pulmonary disease; SD, Standard deviation; IL, Interleukin; FEV1, Forced expiratory volume in one second

Table 2. The changes of IL-33 and FEV in sex

Variable	Gender	N	Mean	SD	t-value	*P*-value
IL33	Female	37	54.34	33.02	1.016	0.314
	Male	23	45.87	28.47		
FEV1	Female	37	37.41	13.67	-0.555	0.581
	Male	23	39.30	11.52		

N, Number; SD, Standard deviation; IL, Interleukin; FEV1, Forced expiratory volume in one second

Table 3. Correlation between age, IL-33, and FEV in the patients

Variables	Statistics	IL33	FEV1
Age	R	-0.16	.189
	P	0.23	.149
IL33	R	1	-.663
	P		.000

IL, Interleukin; R, Rate; P, *P*-value; FEV1, Forced expiratory volume in one second

Discussion

The results of studies have shown that the control and reduction of specific cytokines can be very effective in controlling the disease and reducing emphysematous and fibrotic processes. Numerous studies have been performed to clarify the role of cytokines in the pathogenesis of COPD, but very few studies presented the role of IL-33 (9, 11, 12). Hacker et al. (11) studied 128 individuals equally grouped in patients with COPD and controls. They also divided the patients into two groups: non-smokers and healthy smokers, COPD with severity 1 and 2 and COPD with severity 3 and 4 (according to Gold criteria), which were the levels of COPD. The results of their study showed that the serum level of the IL-33 receptor was higher in mild to moderate COPD than in smokers with normal lung function. Byers et al. (9) examined the role of long-term production of IL-33 in lung tissue in mice with COPD after viral infection and in humans with severe COPD. They achieved an increase in *IL-33* genes and its receptor in airway serous cells and type 2 alveolar cells in mice and an increase in *IL-33* gene expressions in human airway basal cells. They concluded that the role of innate immunity in COPD was based on long-

term exposure of epithelial progenitor cells to high levels of IL-33. In the study of Xia et al. (12), serum levels of IL-33 and its receptor were measured by ELISA in COPD patients and compared with healthy individuals. The results confirmed the hypothesis of this study based on increasing the expression of IL-33 and its role in airway inflammation and systemic inflammation and, thus, the onset and progression of COPD. Analysis of flow cytometric results showed that the increase in peripheral blood lymphocytes in COPD patients leads to increased expression of the *IL-33* gene. Also, analysis of immunofluorescence evidence showed that the main source of IL-33 is in the lung tissue of bronchial epithelial cells. In this study, it was shown that cigarette smoke and lipopolysaccharides can increase the ability of peripheral blood lymphocytes and bronchial epithelial cells to express the *IL-33* gene.

A study by Kearley et al. (13) compared transgenic mice with and without the *IL-33* gene exposed to secondhand smoke from cigarettes and respiratory viral infections showed that the lack of this gene had a protective effect on inflammation caused by these factors. Confirmed the key role of this cytokine in the pathogenesis and exacerbation of COPD. The results of this study also showed that increasing the level of IL-33 in the face of cigarette smoke by increasing its receptor on macrophages and natural killer cells and in the face of viral infections by increasing the inflammatory response of helper immune cells plays an important role in pathogenesis, progression and flammability of COPD. Today, the role of IL-33 as a new cytokine of the interleukin family (14) in inflammatory disease has been considered, as it has been reported to be produced by innate immune helper cells (Th) and leads to severe tissue inflammation (15). In various studies, the expression of the *IL-33* gene has been reported in smooth muscle cells, bronchial epithelial cells (16), and endothelial cells of the intravascular layer (17). Several studies have been performed on the role of IL-33 in the pathogenesis of COPD, but very limited research is available on the association of IL-33 with the severity of COPD. The present study aimed to investigate the relationship between serum levels of IL-33 and the severity of COPD and showed that the level of IL-33 in patients with severe COPD was significantly higher than in patients with non-severe COPD. There was a reverse correlation between IL-33 levels and the severity of COPD (decreased FEV1). In other study, Hacker et al. (11) reported their findings based on higher serum levels of IL-33 receptor in mild to moderate COPD compared to the normal-smokers. Also, Xia et al. (12). reported the increase serum and tissue levels (airways) of IL-33 and its receptor in COPD patients compared to the healthy individuals. Wang et al. demonstreated (18) a significant effect of IL-33 on the development of chronic inflammation caused by air pollution and exposure. It was compatible with multi-walled carbon nanotubes. Consistent with study by Byers et al. (9), we showed that the *IL-33* gene and its receptor are present in airway serous cells and type 2 alveolar cells in mice and in human airway basal cells with COPD. An increase in the level of IL-33 in the severe form of the disease in our study indicates the systemic inflammation and airway inflammation in the patients. Our findings are consistent with the study by Qiu et al. (19) demonstarted suppression of airway inflammation by using neutral anti-IL-33 antibodies. The results of a study by Kearley et al. (13) also confirmed the lack of protective effect of the *IL-33* gene on inflammation caused by exposure to cigarette smoke and viral respiratory infections. The association of IL-33 with disease severity may be related to its key role in the pathogenesis and exacerbation of inflammation and COPD, suggesting a prognostic factor for COPD patients. Qiu et al. (19) survayed the effect of IL-33 on smoking-induced inflammation in mice with COPD. The mice were exposed to cigarette smoke for three hours within four consecutive days. The results showed an increase in the expression of the *IL-33* gene and its soluble receptor in the lung tissue of mice. Exposure to cigarette smoke also induced neutrophil and macrophage infiltration and expression of inflammatory cytokines (IL1β, TNFα, IL17) in the airways. All of these changes were significantly controlled by treatment with neutral anti-IL-33 antibodies. Wang et al. (18) evaluated the effect of IL-33 on inflammation and lung damage due to exposure to multi-walled carbon nanotubes in mice. For this purpose, pulmonary histological and functional changes in mice with and without *IL-33* gene were evaluated after 30 days of exposure to multicolored carbon nanotubes. The results showed an increase in neutrophil and macrophage cells in the lavage fluid of mice expressing the *IL-33* gene as well as increased inflammation and fibrosis of the upper airways, while inflammation was not observed in non-expressing *IL-33* mice. Thus, the remarkable effect of IL-33 on chronic inflammation caused by air pollution and exposure to multi-walled carbon nanotubes was implanted.

Conclusion

The study concluded that there was a difference between the level of IL-33 in patients with severe and non-severe COPD. There is also a significant negative linear relationship between IL-33 levels and the severity of COPD (decreased FEV1). The findings of this study emphasize the importance and the role of cytokines, especially IL-33, in the severity of COPD inflammation. It is also suggested for future studies to measure IL-33 levels in the airways and serum simultaneously to provide a clearer view of the role of systemic and localized IL-33 in CPOD severity. Measurmen of IL-33 can be considered as a good indicator in determining the inflammatory status of lung tissue and the prognosis of the disease due to the higher concentration of IL-33 in people with severe COPD.

Conflict of interests

All the authors approved that they have no conflict of interest.

References

1. Quaderi SA, Hurst JR. The unmet global burden of COPD. Glob Health Epidemiol Genom. 2018;3:e4.
2. Wheaton AG, Liu Y, Croft JB, VanFrank B, Croxton TL, Punturieri A, et al. Chronic Obstructive Pulmonary Disease and Smoking Status - United States, 2017. MMWR Morb Mortal Wkly Rep. 2019;68(24):533-8.
3. Syamlal G, Doney B, Mazurek JM. Chronic Obstructive Pulmonary Disease Prevalence Among Adults Who Have Never Smoked, by Industry and Occupation - United States, 2013-2017. MMWR Morb Mortal Wkly Rep. 2019;68(13):303-7.
4. Clancy J, Turner C. Smoking and COPD: the impact of nature-nurture interactions. Br J Nurs. 2013;22(14):820, 2-6.
5. Wilt TJ, Niewoehner D, Kim C, Kane RL, Linabery A, Tacklind J, et al. Use of spirometry for case finding, diagnosis, and management of chronic obstructive pulmonary disease (COPD). Evid Rep Technol Assess (Summ). 2005(121):1-7.
6. Roberts SD, Farber MO, Knox KS, Phillips GS, Bhatt NY, Mastronarde JG, et al. FEV1/FVC ratio of 70% misclassifies patients with obstruction at the extremes of age. Chest. 2006;130(1):200-6.
7. Behrendt CE. Mild and moderate-to-severe COPD in nonsmokers: distinct demographic profiles. Chest. 2005;128(3):1239-44.
8. Caramori G, Adcock IM, Di Stefano A, Chung KF. Cytokine inhibition in the treatment of COPD. Int J Chron Obstruct Pulmon Dis. 2014;9:397-412.
9. Byers DE, Alexander-Brett J, Patel AC, Agapov E, Dang-Vu G, Jin X, et al. Long-term IL-33-producing epithelial progenitor cells in chronic obstructive lung disease. J Clin Invest. 2013;123(9):3967-82.
10. Jin Y, Wan Y, Chen G, Chen L, Zhang MQ, Deng L, et al. Treg/IL-17 ratio and Treg differentiation in patients with COPD. PLoS One. 2014;9(10):e111044.
11. Hacker S, Lambers C, Pollreisz A, Hoetzenecker K, Lichtenauer M, Mangold A, et al. Increased soluble serum markers caspase-cleaved cytokeratin-18, histones, and ST2 indicate apoptotic turnover and chronic immune response in COPD. J Clin Lab Anal. 2009;23(6):372-9.
12. Xia J, Zhao J, Shang J, Li M, Zeng Z, Zhao J, et al. Increased IL-33 expression in chronic obstructive pulmonary disease. Am J Physiol Lung Cell Mol Physiol. 2015;308(7):L619-27.
13. Kearley J, Silver JS, Sanden C, Liu Z, Berlin AA, White N, et al. Cigarette smoke silences innate lymphoid cell function and facilitates an exacerbated type I interleukin-33-dependent response to infection. Immunity. 2015;42(3):566-79.
14. Akdis M, Burgler S, Crameri R, Eiwegger T, Fujita H, Gomez E, et al. Interleukins, from 1 to 37, and interferon-γ: receptors, functions, and roles in diseases. J Allergy Clin Immunol. 2011;127(3):701-21.e1-70.
15. Küchler AM, Pollheimer J, Balogh J, Sponheim J, Manley L, Sorensen DR, et al. Nuclear interleukin-33 is generally expressed in resting endothelium but rapidly lost upon angiogenic or proinflammatory activation. Am J Pathol. 2008;173(4):1229-42.
16. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity. 2005;23(5):479-90.
17. Baekkevold ES, Roussigné M, Yamanaka T, Johan-

sen FE, Jahnsen FL, Amalric F, et al. Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. Am J Pathol. 2003;163(1):69-79.
18. Wang X, Shannahan JH, Brown JM. IL-33 modulates chronic airway resistance changes induced by multi-walled carbon nanotubes. Inhal Toxicol. 2014;26(4):240-9.
19. Qiu C, Li Y, Li M, Li M, Liu X, McSharry C, et al. Anti-interleukin-33 inhibits cigarette smoke-induced lung inflammation in mice. Immunology. 2013;138(1):76-82.