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Original Article

Down-regulation of transforming growth factor-beta and interleukin-6 serum levels in the idiopathic chronic obstructive pulmonary disease

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Abstract: Background: Idiopathic chronic obstructive pulmonary disease (ICOPD) is a prevalent human disease. The etiology of the disease is yet to be clarified. The main aim of this project was to explore serum levels of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and transforming growth factor-beta (TGF-β) in the ICOPD patients in comparison to healthy controls. Methods: In this cross-sectional study, serum levels of IL-6, TNF-α and TGF-β were evaluated in the 70 non-smoker ICOPD patients and 70 sex and age matched controls, using ELISA technique by the commercial kits from Karmania Pars Gene Company. Analysis of data was performed by parametric independent and Pearson correlation test. Results: Serum levels of IL-6 and TGF-β, but not TNF-α, were significantly decreased in the ICOPD patients in comparison to controls. Serum levels of IL-6, TNF-α and TGF-β were not altered in the ICOPD male in comparison to female and also in mild when compared to moderate ICOPD patients. Conclusions: Down-regulation of TGF-β may be the main risk factor for deterioration of inflammation in the ICOPD patients. Decreased IL-6 may be related to the idiopathic type of COPD.

Keywords: IL-6, TNF-α, TGF-β, idiopathic chronic obstructive pulmonary disease

Introduction

Chronic obstructive pulmonary disease (COPD) is a human disorder and can be induced after several pro-inflammatory-related diseases, such as asthma [1]. The disorder belongs to a group of lung diseases that cause breathing difficulties, including emphysema and chronic bronchitis [2]. COPD is a common condition that mainly affects middle-aged or older adults who smoke. The COPD-related breathing problems can be gradually worse over time [3].

Several genetic and environmental parameters, such as stress and chemical contamination, may affect the severity and also the onset of the diseases [2, 4]. Based on the fact that COPD can be considered as a potential risk factor for several diseases such as lung cancers [5], neuromuscular disease [6], cognitive impairments [7], hence, the main risk factors participate in the induction or deterioration of the disease need to be elucidated. However, in several cases, COPD is idiopathic (ICOPD) and the main molecules involved in the pathogenesis of ICOPD are yet to be clarified.

Based on the fact that COPD is a pro-inflammatory disease [2, 3], it appears that the immune system plays key roles in the pathogenesis of the disorder. Cytokines are the important arms of the immune system which perform their functions in network manner [8-10]. Thus, it has been hypothesized that cytokines may be the important molecules which participate in the ICOPD pathogenesis.

Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) are the main innate immunity cytokines which are produced in the first min-

utes after inductions of innate immune cells [9, 11]. TNF-α plays key roles in the pathogenesis of several human pro-inflammatory diseases such as infection shocks [12], biliary cirrhosis [13], liver cirrhosis [14] and asthma [1]. Additionally, IL-6 which has similar functions also plays key roles in the development of some immune cells such as plasma cells, the main producing cells of antibodies such as IgE [9]. Moreover, transforming growth factor-beta (TGF-β) is a cytokine with dual functions including progression of Th17 lineage and development of T regulatory lymphocytes [8]. Based on the various ranges of TNF-α, IL-6, and TGF-β functions and also due to the fact that cytokines play their roles in a network dependent manner, it may be hypothesized that the cytokines may participate in the pathogenesis of ICOPD. Although the situation of TNF-α, IL-6, and TGF-β serum levels have been evaluated in some ethnics previously, Iranian population are naïve and the relation between ICOPD and TNF-α, IL-6, and TGF-β serum levels need to be explored in this population. Thus, this project was aimed to assess the cytokine serum levels in the Iranian ICOPD patients.

Material and methods

Subjects

In this cross-sectional study, 70 non-smoker ICOPD patients and 70 sex and age matched controls were evaluated in Rafsanjan, Kerman, Iran. The patients with COVID-19, influenza and Mycobacterium tuberculosis infections, plural infusion, primary lung hypertension, embolic lung alcohol consumptions, administrating immunosuppressive drugs, tumors and immunologic and hematologic disorders were excluded from the study. The COPD patients with certain inducers were excluded from the study because they were not considered ICOPD. The existences of ICOPD were confirmed by an expert specialist physician based on the clinical parameters and spirometry results (Irrevocable FEV1/FVC<0.7). The severity of ICOPD was also determined using Medical Research Council (MRC) breathlessness scale [15]. Briefly, The MRC scale is a questionnaire that is used to evaluate perceived breathlessness and consists of five statements. Accordingly, based on the questionnaire the patients are divided into 5 grades [15]. The study was approved by the Ethical Committee of Rafsanjan University of Medical Sciences (IR.RUMS.1396.117, 2017-10-24) and all participants (ICOPD patients and controls) gave written informed consent. Five mL of blood samples were collected in anti-coagulant free tubes to collate serum, and subsequently kept at -20°C.

Cytokine assay

TNF-α, IL-6, and TGF-β serum levels were explored using the commercial kits from Karmania Pars Gene Company, Kerman, Iran and based on the manufacture guidelines. Briefly, 50 µL from the samples and standards were added to the ELISA plate and incubated for 1 hour. Then the plates were washed by the washing buffer and the detection antibody and HRP-AVIDIN were added, respectively. After incubation for 1 hour, the plates were washed, and the substrate was added. After 15 minutes, the reaction was stopped by the stopping solution, and the optical density (OD) was detected using an ELISA reader (RAYTOO, China) at 450 nm.

Statistical analysis

Analysis of data was performed by parametric independent test for determination of TNF-α, IL-6, and TGF-β serum level differences between ICOPD and control groups and also between male and female ICOPD patients. To evaluate the correlation between age, duration of ICOPD and of TNF-α, IL-6, and TGF-β serum levels, Pearson correlation test was used and the significant values was considered for the cases with *P*-value less than 0.05.

Results

Data analysis revealed that IL-6 serum levels significantly decreased in the ICOPD patients (40.16 ± 3.25 pg/mL) when compared to controls (139.89 ± 12.84 pg/mL, $p<0.001$). Serum levels of TGF-β also significantly decreased in the ICOPD patients (145.73 ± 19.53 pg/mL) in comparison to controls (664.26 ± 39.33 pg/mL, $p<0.001$). However, TNF-α serum levels were 169.74 ± 14.84 pg/mL in ICOPD patients and 176.11 ± 22.75 pg/mL in controls in which the difference was not significant (P=0.815). **Figure 1** presents the serum levels of TNF-α, IL-6, and TGF-β in ICOPD patients versus controls.

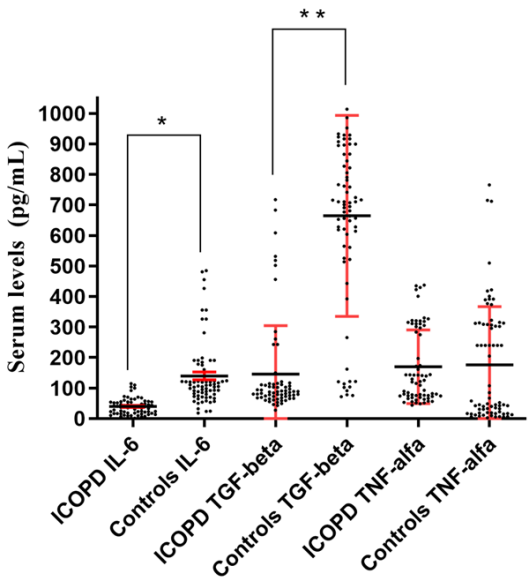

Figure 1. Serum levels of IL-6, TNF-α and TGF-β the ICOPD patients in comparison to controls. Serum levels of IL-6 and TGF-β significantly decreased in the ICOPD patients in comparison to controls, however, serum levels of TNF-α did not alter in ICOPD patients. *P<0.001. **P<0.001.

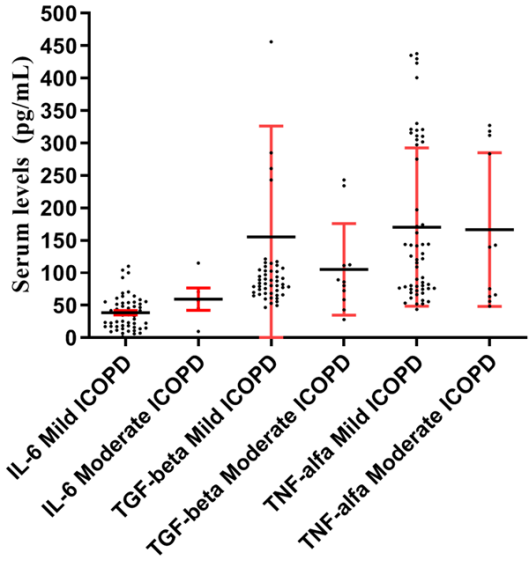

Figure 2. Serum levels of IL-6, TNF-α and TGF-β in the moderate and mild ICOPD patients. Serum levels of IL-6, TNF-α and TGF-β did not alter in moderate when compared to mild ICOPD patients.

The results also demonstrated that 13 and 57 patients suffered from moderate and mild ICOPD which their serum levels of TNF-α (P= 0.343), IL-6 (P=0.200), and TGF-β (P=0.922) were not significantly different (**Figure 2**).

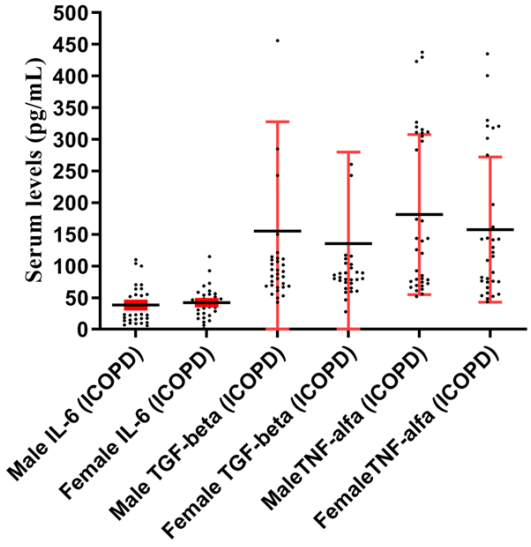

Figure 3. Serum levels of IL-6, TNF-α and TGF-β in the male and female ICOPD patients. Serum levels of IL-6, TNF-α and TGF-β did not alter in females when compared to male ICOPD patients.

As it is demonstrated in **Figure 3**, serum levels of TNF-α (P=0.426), IL-6 (P=0.572), and TGF-β (P=0.617) in male ICOPD patients (41.96 ± 4.02, 181.31 ± 21.69 and 155.30 ± 29.60 pg/mL, respectively) were not significantly altered when compared to female ICOPD patients (38.24 ± 5.21, 157.44 ± 20.27 and 135.56 ± 25.52 pg/mL, respectively).

Statistical analysis also demonstrated the poor correlations among age with TNF-α serum levels (r=0.241, P=0.050) and age with duration of ICOPD (r=0.277, P=0.022). As it is illustrated in **Table 1**, there were no significant correlations between other variables.

Discussion

Results of the current study demonstrated that IL-6 and TGF-β were lower in the ICOPD patients than controls. As mentioned previously, TGF-β can down-regulate the immune responses via increased development of T regulatory lymphocytes [8]. Our previous investigations on the viral infections revealed that TGF-β plays key roles in the induction of chronic forms of the disorders [16]. Our current results demonstrated that TGF-β is down-regulated in the ICOPD patients. Due to the fact that TGF-β plays anti-inflammatory roles when work in the cytokine network lonely [17], authors concluded that down-regulation of the anti-inflammato-

Table 1. Correlation between age, IL-6, TNF-α, TGF-β and duration of the disease in the COPD patients

		Duration	Age	IL-6	TGF-β	TNF-α
Duration	Pearson Correlation	1	0.277*	-0.088	-0.049	-0.117
	P value	-	0.022	0.480	0.693	0.349
Age	Pearson Correlation	0.277*	1	0.165	-0.114	0.241
	P value	0.022	-	0.193	0.368	0.050**

*Table illustrates a poor positive correlation between age and duration of COPD. **There was a poor positive correlation between age and TNF-α serum levels.

ry molecule can be a main cause of chronic inflammation in the ICOPD patients. In another word, decreased expression of TGF-β can be associated with lower regulation of inflammatory responses of immune cells, hence the inflammation can be induced in the ICOPD patients. TGF-β also participates in the tissue remodeling and repairing [8]. Accordingly, it may be hypothesized that down-regulation of TGF-β may be associated with increased inflammation and decreased in tissue repairing of the lung tissue and respiratory tract during CODP which is reported previously [18].

The results revealed that IL-6 also decreased in the ICOPD patients. Thus, it appears that the pro-inflammatory responses in the ICOPD patients are independent of IL-6. It has been confirmed that IL-6 in association with IL-2 and TGF-β participate in the development of Th17 lineage, which is a pro-inflammatory lymphocyte participates in the autoimmune disease pathogenesis [19]. Therefore, based on the results, it appears that the development of Th17 lineage may be disrupted in the Iranian ICOPD and the inflammation in the patients is related to other T helper (Th) subsets, including Th1. Although previous investigations revealed that Th17 and its related cytokines play key roles in the pathogenesis of COPD [20], based on our results, it may be concluded that ICOPD may suffer from different mechanisms, independent of Th17. Accordingly, it has been documented that different compartments of the lower airways in the COPD patients show various levels of TGF-β and some compartment revealed selective reduction in TGF-β [21]. Additionally, a study by Guan and colleagues on the animal models revealed that, although serum levels of IL-17A, the pro-inflammatory cytokine, are up-regulated in the COPD animal models, TGF-β serum levels were decreased [22]. The authors have concluded that the alteration in the TGF-β1/IL-17A balance may play key roles in the pathogenesis of COPD [22]. Another study by Michaeloudes et al., showed that TGF-β plays critical roles in the reduction of mitochondrial oxidant levels, the main molecules participate in the pathogenesis of COPD [23]. However, it has been demonstrated that TGF-β play key roles in development of M2 macrophages which are the main cause of deterioration of cigarette smoke induced COPD pathogenesis [24]. Higher levels of TGF-β in the cigarette smoke induced COPD patients have also been reported by Chiang and colleagues [25]. Another study on the cigarette smoke induced COPD patients demonstrated that TGF-β serum levels were significantly increased when compared to healthy controls [26].

Together, due to the fact that our patients were non-smoker ICOPD patients, it may be hypothesized that decreased expression of TGF-β in the non-smoker ICOPD, but not in the cigarette smoke-induced COPD, is a risk factor for the development of the diseases. Additionally, the previous investigations were on non-human animal models, further studies on human models need to be considered to evaluate the roles played by TGF-β in the pathogenesis of ICOPD.

Moreover, several investigations reported increased expression of pro-inflammatory cytokines, such as TNF-α, IL-8, and interferon-gamma (IFN-γ), in the cases who suffer from COPD [27].

Altogether, it appears that Iranian ICOPD suffer from the inflammation independent of the Th17 pathway and probably Th1 is the main source of the inflammation. Thus, the authors suggest evaluating the Th-related molecules, including transcription factors and cytokines, in the future studies on the ICOPD patients. Additionally, due to the significant roles played by genetic variations on the expression of the cytokines, it is worthy to evaluate the gene

polymorphisms of IL-6, TGF-β, and TNF-α in the population in comparison to healthy controls and COPD with well-known inducers.

Conclusion

Due to the results, it may be hypothesized that the following reasons can be considered for the controversy: 1. The participants in this study were non-smoker ICOPD, who were different from other investigations, 2. The most studies were on the animal models, 3. The ethnics of our patients were different from other investigations, 4. Some of the studies were performed on the small sample size.

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Disclosure of conflict of interest

None.

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