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IL-10 Expression and Pulmonary Fibrosis in COVID-19 and Non-COVID-19: A Cross-sectional Study

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ABSTRACT

Indonesia was declared the country with the highest number of COVID-19 cases in ASEAN, and East Java was the province with the highest confirmed case fatality rate (7.3%) in 2021. In the case of COVID-19, there was a drastic elevation of the anti-inflammatory cytokine IL-10. This study aimed to ensure the role of IL-10 against pulmonary fibrosis in COVID-19 and non-COVID-19 patients. This study is a cross-sectional (non-experimental) study. The study sample consisted of 40 lung tissue samples from COVID-19 and non-COVID-19 patients at Dr. Soetomo General Academic Hospital, Surabaya. The IL-10 antibody reagent (GTX632359, GeneTex) was used to assess IL-10 expression, and hematoxylin and eosin (H&E) were used to determine the degree of pulmonary fibrosis. For Spearman's correlation, the data were analyzed using Statistical Package for the Social Sciences (SPSS). The mean percentage of IL-10 expression in COVID-19 patients (40.07%) was higher than in non-COVID-19 patients (26.60%). However, the mean for the degree of pulmonary fibrosis score in non-COVID-19 patients (4.43) was higher than in COVID-19 patients (3.67). Furthermore, a negative correlation (p=0.000; r=-0.281) was found between the percentage of IL-10 expression and the degree of pulmonary fibrosis. The percentage of IL-10 expression in macrophage cells had a significant negative correlation with the degree of pulmonary fibrosis score.

INTRODUCTION

At the end of 2019, the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) emerged and spread globally from Wuhan, China. SARS-CoV-2 pneumonia was named COVID-19 (Coronavirus Disease 2019), and the disease was declared a global pandemic (Di Gennaro et al., 2020). According to data from the Ministry of Health of the Republic of Indonesia as of May 2021, Indonesia ranks first among ASEAN countries with 1,739,750 confirmed COVID-19 cases. In addition, the case fatality rate (CFR) of COVID-19 in East Java ranks first (7.3%) despite ranking fourth in Indonesia in confirmed cases (Di Gennaro et al., 2020).

COVID-19 has distinct characteristics of acute respiratory distress syndrome (ARDS). One of them is the drastic elevation of Interleukin 10 (IL-10), an anti-inflammatory cytokine that can be expressed by macrophage cells (Akrom et al., 2021). IL-10 may affect fibrosis in ARDS, although its effect cannot be clearly proven (She et al., 2021). IL-10 can potentially be an antifibrotic cytokine for better outcomes in COVID-19 patients if its elevated expression can reduce pulmonary fibrosis. However, if it turns out to be profibrotic, it must be reduced to prevent the COVID-19 patient's condition from worsening.

This study aimed to investigate the correlation between IL-10 expression from macrophage cells and pulmonary fibrosis degree (based on the histopathological appearance) in COVID-19 and non-COVID-19 patients at Dr. Soetomo General Academic Hospital, Surabaya.

Several studies have examined the role of Interleukin-10 (IL-10) in pulmonary fibrosis, particularly in COVID-19 and non-COVID-19 cases. (Chakraborty et al., 2023) found that IL-10 plays a crucial role in inhibiting transforming growth factor-beta 1 (TGF-β1), a cytokine responsible for fibrosis development. Their study demonstrated that IL-10 reduces collagen-I production, which is typically upregulated in fibrotic lung conditions. Similarly, (Nakagome et al., 2012) investigated IL-10 gene delivery in bleomycin-induced pulmonary fibrosis models and found that IL-10 significantly suppressed the activation of TGF-β1 and subsequent fibrosis progression. These findings suggest that IL-10 has a potential antifibrotic effect, particularly in inflammatory lung diseases.

Conversely, some studies suggest that prolonged IL-10 overexpression may contribute to fibrosis rather than prevent it. (Li et al., 2011) reported that excessive IL-10 expression led to an increased recruitment of fibrocytes into lung tissues, thereby exacerbating pulmonary fibrosis. Their study suggested that IL-10-induced

fibrosis is mediated through the interaction between CCL2 and CCR2, which promotes fibrocyte invasion. Additionally, (Sammaritano et al., 2020) proposed that IL-10 plays a dual role in tissue fibrosis, acting as both an antifibrotic and profibrotic cytokine depending on the inflammatory environment. These contrasting findings indicate that the exact role of IL-10 in pulmonary fibrosis remains unclear and warrants further investigation.

Although numerous studies have explored the role of IL-10 in pulmonary fibrosis, there is still no consensus on whether IL-10 acts predominantly as an antifibrotic or profibrotic cytokine. Previous research has focused on experimental animal models or isolated cytokine interactions, with limited studies analyzing IL-10 expression in actual human lung tissue samples from both COVID-19 and non-COVID-19 patients. Moreover, there is a lack of comprehensive studies correlating IL-10 expression with the degree of fibrosis using histopathological data. This study aims to fill this gap by systematically analyzing IL-10 expression and its relationship with pulmonary fibrosis in lung tissue samples from human subjects.

This study introduces a novel approach by directly comparing IL-10 expression in lung tissue samples from COVID-19 and non-COVID-19 patients while simultaneously evaluating the degree of fibrosis. Unlike previous studies that relied on experimental models, this research utilizes human tissue samples, providing clinically relevant insights into the role of IL-10 in pulmonary fibrosis. Furthermore, the study employs a correlation analysis to determine the direct relationship between IL-10 expression levels and fibrosis severity, offering new perspectives on whether IL-10 primarily serves a protective or detrimental role in lung fibrosis.

The findings of this study have significant clinical implications, particularly in the management of post-COVID-19 lung fibrosis. By clarifying the role of IL-10 in pulmonary fibrosis, this research could contribute to the development of targeted therapeutic interventions aimed at modulating IL-10 levels in patients at risk of severe fibrosis. Additionally, the study provides valuable histopathological data that can serve as a reference for future research on cytokine interactions in fibrotic lung diseases. Ultimately, this research may aid in improving treatment strategies for patients suffering from long-term pulmonary complications related to COVID-19 and other chronic respiratory diseases.

METHOD

This non-experimental study utilized a cross-sectional design and was conducted at the Department of Pathology Anatomy, Dr. Soetomo General Academic Hospital, Surabaya, and the Faculty of Medicine, Airlangga University, Surabaya, from August 2021 to June 2022. The population comprised COVID-19 and non-COVID-19 paraffin blocks archived at the hospital, with COVID-19 blocks derived from pathological lung tissue of hospitalized patients and outpatients, while non-COVID-19 blocks were sourced from normal lung tissue of lung tumor patients. The sample was selected based on inclusion and exclusion criteria through consecutive (non-random) sampling; inclusion criteria encompassed lung tissue paraffin blocks suitable for immunohistochemistry, while exclusion criteria included second opinions from outside the hospital. The sample size was calculated using the formula $n=Z2\cdot1a/d22p(1-p)$ $n=2p(1-p)Z2\cdot1a/d2$, resulting in approximately 38 samples. Ethical approval was obtained from the Ethical Committee of Dr. Soetomo General Academic Hospital, with certificate number 0826/LOE/301.4.2/III/2022. Data collection involved staining each sample with IL-10 antibodies and H&E, slicing paraffin blocks into four-micron sections, and evaluating IL-10-positive macrophages in microscopic views, with percentages calculated from five visual fields. Additionally, the degree of fibrosis in H&E-stained slides was assessed using the Ashcroft score. Statistical analyses were performed with SPSS, using a normal distribution test, Independent Samples T-test for normally distributed outcomes, Mann-Whitney U-test for non-normally distributed data, and Spearman's correlation for correlation tests, with significance set at p-value <0.05.

RESULTS AND DISCUSSION

This study collected 40 samples with the sampling period spanning from 2019 to 2021. The sample characteristics of this study from the COVID-19 category are male and female within 26-86 years old, mild to severe COVID status, and pathological anatomy status is non-specific chronic inflammation and/or no signs of malignancy. While, the sample characteristics from the non-COVID-19 category are male and female within 33-72 years old and pathological anatomy status is chronic inflammatory process, non-specific chronic inflammatory process, no signs of malignancy, no malignant cells, granulomatous inflammation consistent with tuberculosis, tuberculous granulomatous inflammation, no visible tumor, non-specific chronic inflammation, and/or squamous cell carcinoma surrounded by normal lung tissue.

The Independent Samples T-test revealed a significant ($p=0.000$; $p<0.05$) difference in IL-10 expression (normally distributed) between groups. In Table 1 the comparative test on the IL-10 expression, the Mann-Whitney U-test revealed a significant ($p=0.000$; $p<0.05$) difference in pulmonary fibrosis degree score (not normally distributed) between groups. In Table 1 showed comparative test on the degree of fibrosis score, Spearman's correlation showed a correlation between variables ($p=0.047$; $p<0.05$). In addition, the value of $r=-0.281$ was also obtained, which stated that this relationship resulted in a negative correlation. In Figure 1 also showed the correlation between IL-10 expression percentage and fibrosis degree score.

Expression of IL-10 in COVID-19 Cases is Higher than in Non-COVID-19 Cases

The results showed that COVID-19 cases had an average percentage of IL-10 expression (40.07%) higher than non-COVID-19 cases (26.60%) at Dr. Soetomo General Academic Hospital, Surabaya. In Figure 2 showed the result of immunohistochemical staining of COVID-19 sample (L.005/20) with monoclonal antibody reagent against IL-10 (x100) and immunohistochemical staining of a non-COVID-19 sample (T.2006/19) with monoclonal antibody reagent against IL-10 (x100).

Role of IL-10 as Antifibrosis Cytokine

This study showed that COVID-19 cases had a lower average score for the degree of fibrosis (3.67) than non-COVID-19 cases (4.43). Furthermore, a negative correlation was discovered between the percentage of IL-10 expression and the degree of pulmonary fibrosis score in COVID-19 and non-COVID-19 cases (r=-0.281; p=0.000). This shows that the higher the percentage of IL-10 expression, the lower the fibrosis degree score. In Figure 3, immunohistochemical staining of the COVID-19 sample (L.005/20) with H&E staining (x100) and immunohistochemical staining of non-COVID-19 sample (T.2006/19) with H&E staining (x100).

Expression of IL-10 in COVID-19 Cases is Higher than in Non-COVID-19 Cases

These results are supported by a study that autopsied and investigated the pathological conditions of two cases who died of COVID-19. The cytokine IL-10 was expressed more strongly by alveolar macrophages using the immunohistochemistry (IHC) method than other cytokines, such as IL-6 and tumor necrosis factor-α (TNF-α), which were only moderately expressed (Sungkar et al., 2021),(Wang et al., 2020). The serum cytokine IL-10 increased by 37% compared to the control (healthy) group. Huang, *et al*. also showed that IL-10 concentration in COVID-19 intensive care unit (ICU) patients was higher than in non-ICU patients. This suggests that SARS-CoV-2 initiates the production of anti-inflammatory cytokines, including IL-10. ICU patients also had higher concentrations of proinflammatory cytokines, which suggests that cytokine storms are related to disease severity (Huang et al., 2020).

IL-10 levels increased during the infection phase of severe COVID-19 cases but not in mild cases. COVID-19 is classified as severe when the patient has one of the following: respiratory distress (respiratory rate >30/min); blood oxygen saturation <93%; arterial PaO_2/FiO_2 <300 mmHg; respiratory failure with mechanical ventilation; shock; or other organ failure requiring transfer to the ICU (Zhao et al., 2020). Increased IL-10 secretion in COVID-19 cases was the distinguishing factor between the pathophysiology and pathogenicity of SARS-CoV-2 and SARS-CoV. According to previous studies, SARS-CoV-2 shares several characteristics with SARS-CoV and MERS-CoV, including an increase in proinflammatory cytokines, such as IL-1β and IFN-γ (Huang et al., 2020). Although an increase in IL-10 in COVID-19 cases has become a hallmark of the pathogenicity of SARS-CoV-2, there has been no clear explanation of the pathogenicity until now (Lu et al., 2021).

Role of IL-10 as Antifibrosis Cytokine

Shamskhou, *et al*. tested the ability of IL-10 to inhibit transforming growth factor-β1 (TGF-β1) using human lung fibroblast cells from healthy individuals and individuals with idiopathic pulmonary fibrosis (IPF). Stimulation with IL-10 or a combination of IL-10 and TGF-β1 can reduce collagen-I expression. Collagen-I production generally increases when fibroblasts are exposed to TGF-β1, a profibrotic cytokine upregulated in IPF. Thus, IL-10 can reduce TGF-β1-mediated production of collagen-I in healthy and IPF lung tissue (Shamskhou et al., 2019).

Nakagome, *et al*. demonstrated that IL-10 could suppress transforming growth factor-β (TGF-β) from alveolar macrophages in bleomycin-injected mice. In addition, administration of the IL-10 gene in vivo has been shown to suppress pulmonary fibrosis even though the cytokine is administered after the chronic phase. The in vitro method showed that high concentrations of IL-10 had a direct effect on TGF-β1 production from macrophages (Nakagome et al., 2006).

Aside from inhibiting TGF-β activity, IL-10 has been shown to reduce the degree of fibrosis by acting as an anti-inflammatory cytokine against an endotoxin. Chronic endotoxin inhalation will cause airway inflammation, persistent airway hyperactivity, and airway remodeling accompanied by subepithelial space thickening. Those impacts can be dampened by IL-10, which is expressed by leukocytes. IL-10, expressed by structural cells, cannot reduce the impact. Adequate control of the acute inflammatory phase can also lead to decreased long-term remodeling. This is because acute inflammation that occurs repeatedly can be another cause of respiratory tract remodeling. Although chronic exposure to endotoxin increases the expression of endogenous IL-10, the protective function of IL-10 against chronic inhalation of endotoxin is still valid. However, the amount of endogenous IL-10 was decreased in patients with chronic respiratory disease. Based on these data, IL-10 may have an important role in maintaining lung architectural homeostasis (Yusuf et al., 2021).

TNF-α is a proinflammatory cytokine that promotes fibrosis by increasing the synthesis of fibronectin, prostaglandins, and TGF-β. Even chronic TNF-α expression causes chronic inflammation and severe fibrosis in transgenic mice lung tissue. TNF-α inhibition by IL-10, as evidenced by Arai, *et al*., may indicate that blocking the effects of TNF-α can inhibit the occurrence of pulmonary fibrosis and the anti-fibrosis role of IL-10 partially works through reducing the expression of TNF-α (Arai et al., 2000). IL-10 may also affect the function of lung fibroblasts directly. This was demonstrated by the fact that IL-10 inhibited the collagen production of human lung fibroblast cells of the WI-38 cell line (WI-38 cells). Given its ability to inhibit TNF-α expression, IL-10 may act as an antifibrotic cytokine in vivo by directly inhibiting collagen gene expression (Arai et al., 2000).

Sun, *et al*. obtained different results, which showed that prolonged excessive IL-10 expression could bring extracellular matrix-producing cells to bronchoalveolar lavage (BAL) and cause pulmonary fibrosis. In addition, IL-10 overexpression also causes fibrocytes to invade lung tissue, as evidenced by the fact that the total number of fibrocytes in mice with IL-10 overexpression is two times higher than in control mice (without IL-10 expression). However, pulmonary fibrosis was significantly reduced when anti-CCL2 antiserum was administered to mice with overexpression of IL-10. It suggests that the interaction mediates IL-10-induced recruitment of fibrocytes between CCL2 and CCR2. This is because of the high upregulation of CCR2 and CCL2 in mice with excess IL-10 expression (Sun et al., 2011).

Other contrast results have also shown that persistent IL-10 overexpression in lung tissue causes a complex phenotype. This is because IL-10 causes inflammation rich in T and B cells, subepithelial fibrosis, and mucous metaplasia, along with its function as an inhibitor of endotoxin-induced inflammation. Thus, IL-10 likely contributes to the pathogenesis of inflammatory, fibrotic, and mucous responses (Lee et al., 2002)(Purwati et al., 2019).

Another study examined the lung inflammatory response in mice injected with silica (crystalline silica). The results proved that the inflammatory response was higher in mice with IL-10 deficiency than in general (wild-type) mice. However, the intensity of fibrotic lesions was significantly higher in wild-type mice than in IL-10-deficient mice. These responses suggest that the profibrotic effect of IL-10 was lung response to silica exposure. Furthermore, IL-10 mediates various pulmonary responses depending on the inflammatory cells involved (macrophages, neutrophils, or lymphocytes) (Huaux et al., 1998)(Han et al., 2020).

These differences provide suggestions regarding the spatiotemporal dependence of the IL-10 response mechanism. IL-10 overexpression over a short time is associated with reduced lung inflammation following LPS impairment. In contrast, overexpression over time increases pulmonary fibrosis through M2 macrophages, lymphocytes, and collagen-producing fibrocytes (Steen et al., 2020),(Saraiva et al., 2019).

The other contrasting results may be due to the inflammatory response. That inflammatory response (the removal of silica particles from the interstitial lung tissue) causes a reduction in fibrosis degree. Therefore, this study obtained an inverse correlation between the strength of the inflammatory response and the intensity of fibrosis in silica-injected mice (Adamson et al., 1992)(Garantziotis et al., 2006).

The limitations of this research are that the control (non-COVID-19) sample is normal lung tissue from lung tumor patients. It is possible that the tumor triggers a desmoplastic reaction that could cause changes in extracellular matrix components and hyperproliferation of components similar to that of chronic fibrosis. In addition, the grading of the tumor itself was not stated in this study, so the desmoplastic effect in non-COVID-19 patients cannot be ascertained. Therefore, there is a possibility that the desmoplastic reaction and the grading of the lung tumor could affect the results of this study. The reason for using non-COVID-19 patients as controls is that there are no lung tissue samples from healthy people at Dr. Soetomo General Academic Hospital, Surabaya. Furthermore, no journal has been identified yet that uses lung tumor patients as controls in research about IL-10 expression and pulmonary fibrosis. As a result, it is not certain how big the difference will be if this study is compared with studies that use healthy people as the control sample.

CONCLUSION

There was a negative correlation between the percentage of IL-10 expression from macrophage cells and the degree of pulmonary fibrosis score. Furthermore, IL-10 expression was higher in COVID-19 than in non-COVID-19 patients, but the degree of pulmonary fibrosis showed the opposite results. Therefore, the elevation of IL-10 expression in COVID-19 patients reduced the degree of pulmonary fibrosis.

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