

Correlation between IL-6 and Age in COVID-19: Insights from a Cross-Sectional Analysis in Malang, Indonesia

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Abstract

Interleukin 6 (IL-6) has been identified as a predictor of severity and mortality in COVID-19. Although morbidity and mortality generally increase with age, it remains uncertain whether there is a correlation between age and IL-6 levels, thereby exacerbating the severity of COVID-19 in patients. The objective of this study was to examine the correlation between age and IL-6 levels in COVID-19 patients. The study was cross-sectional, analyzing laboratory and medical records of 3,171 COVID-19 patients who received treatment in 2021 at Saiful Anwar General Hospital. The Data analyzed using Kruskal-Wallis, Dunnett's Post Hoc, and Spearman Correlation tests. The patients were divided into four age groups: <20 years (1.29%), 20-40 years (18.10%), 40-60 years (43.74%), and >60 years (36.87%). The respective mean levels of IL-6 in each group were 39.44, 192.04, 217.27, and 252.59 pg/ml. A significant difference (p-value = 0.000) was observed in IL-6 levels between the <20 years age group and the other groups. Moreover, a significant positive correlation (p-value = 0.000) was identified between age group and IL-6 levels in COVID-19 patients, indicating higher IL-6 levels in older patients. In conclusion, there were differences in IL-6 levels based on age groups and age was positively correlated with IL-6 levels in COVID-19 patients.

Keywords: Age, Correlation, COVID-19, Interleukin-6, Severity.

Introduction

The COVID-19 pandemic was officially declared a global pandemic in March, 2020, and it has resulted in a staggering number of cases and deaths worldwide. To date, there have been 603 million confirmed cases and 6.48 million deaths reported.¹ Around 20% of individuals infected with COVID-19 experience severe hypoxemia, characterized by a

hyperinflammatory response known as cytokine storm syndrome. This syndrome primarily arises from insufficient immune responses of IFN-I and IFN-III, coupled with elevated levels of proinflammatory cytokines, including IL-1, IL-6, and TNF- α .²⁻³ The dysregulation in cytokine production can be attributed to persistent low-level

inflammation associated with "inflamm-aging" and the presence of underlying comorbidities.³

COVID-19 can affect individuals of all age groups, from infants to the elderly, but older adults tend to experience more severe conditions.⁴ The cytokine storm characterized by a significant release of pro-inflammatory cytokines plays a major role in the pathophysiology of COVID-19 infection among elderly patients.⁵ Aging is associated with increased systemic pro-inflammatory cytokines and decreased systemic anti-inflammatory cytokines. Among these

cytokines, IL-6 is particularly implicated in this process compared to others.^{4,6}

The studies have reported elevated levels of IL-6, IL-1, tumor necrosis factor- α (TNF- α), and C-reactive protein (CRP) in individuals across different age groups.^{5,7} However, there is limited research specifically focused on cytokine profiles, particularly IL-6, in different age groups affected by COVID-19. Therefore, this study aims to investigate the potential correlation between age and IL-6 levels, thereby contributing to our understanding of the severity of COVID-19 in patients.

Materials and Methods

Methods:

Research Design and Subjects

Our study employed a quantitative approach with a cross-sectional design. The data utilized in this research were obtained from the laboratory and medical records of COVID-19 patients at Dr. Saiful Anwar Hospital in Malang, during the year 2021. The total number of participants in our study was 3,176, all of whom were confirmed to have COVID-19. Among these participants, there were 1,747 males (55.01%) and 1,429 females (44.99%). The inclusion criteria for this study involved selecting samples from confirmed COVID-19 patients who received treatment at Dr. Saiful Anwar Hospital in Malang. We excluded five subjects from the study due to the absence of IL-6 level data. To analyze the data, the participants were categorized into four age groups: <20 years, 21-40 years, 40-60 years, and >60 years.

Time and place of the study

This study was conducted at the Central Laboratory Installation of Dr. Saiful Anwar Hospital Malang Hospital. IL-6 data were collected from COVID-19 patient samples examined in the laboratory in 2021. The study was carried out after receiving ethical approval from the Research Ethics Commission of Dr. Saiful Anwar Malang General Hospital under the number 400/011/K.3/302/2021.

Method of analysis

The measurement of IL-6 levels was conducted using the electrochemiluminescence immunoassay (ECLIA) method, specifically utilizing the Cobas

system from Roche Diagnostics in Indianapolis, USA. The analysis principle employed in this method was the sandwich method. Approximately 30 μ L of the sample was incubated with a specific IL-6 monoclonal antibody. Subsequently, the IL-6-specific monoclonal antibody, labeled with microparticles coated with a ruthenium complex and streptavidin, was added, resulting in the formation of a sandwich complex between the antibody and the antigen in the sample. The mixture reaction was then transferred to the measuring chamber, where the microparticles were magnetically captured on the surface of an electrode. Any unbound materials were removed using ProCell. The application of voltage to the electrode induced chemiluminescent emission, which was subsequently measured using a photomultiplier. The results were obtained by referencing a calibration curve that was specific to the particular instrument utilized. The method exhibited a detectable concentration sensitivity of 1.5 pg/ml.

Statistical Analysis

The data collected for this study were analyzed using SPSS version 25 (SPSS Inc., Chicago, USA). To assess the normality of the data, the Kolmogorov-Smirnov statistical test was conducted. Age differences were determined using non-parametric tests, specifically the Kruskal-Wallis test and the Post Hoc Dunnett test. The relationship between age and IL-6 serum levels was evaluated using the Spearman correlation test. A p-value less than 0.05 was considered statistically significant, and a 95% confidence interval was applied to the results.

Results and Discussion

Results:

In 2021, a total of 3,176 individuals tested positive for COVID-19 at Dr. Saiful Anwar Malang Hospital. However, for the purpose of analysis, data was available for 3,171 subjects as five subjects lacked IL-6 level information during their hospitalization. The age range of the participants in this study varied from one year to 93 years, covering a wide spectrum. To facilitate the analysis, the participants were divided into four age groups: <20 years (41/ 3,171), 21-40 years (574/ 3,171), 40-60 years (1,387/ 3,171), and >60 years (1,169/ 3,171) Fig 1.

Table 1 shows the average levels of IL-6 in each age group. In the difference test, a significant difference was found between the subject groups (p-value=0.000). Fig 2 showed that among four groups, the age group of <20 had significantly different IL-6 levels compared to the other groups when analyzed using the post hoc Dunnett test and the age group of 20-40 years had significantly different IL-6 levels compared to the age group of >60. On the Spearman correlation test, we found that there was a positive correlation with a significant result between the age group and IL-6 levels of COVID-19 patients (p-value=0.000).

Table 1. The IL-6 cytokine level of age groups

Age group	Number of Sample (N = 3.171)	Mean (\pm SD), pg/ml	P value
< 20 years old	41	39.44 (\pm 65.70)	0.000
20 – 40 years old	574	192.04(\pm 632.28)	
41 – 60 years old	1.387	217.27(\pm 665.89)	
> 60 years old	1.169	252.58(\pm 688.98)	

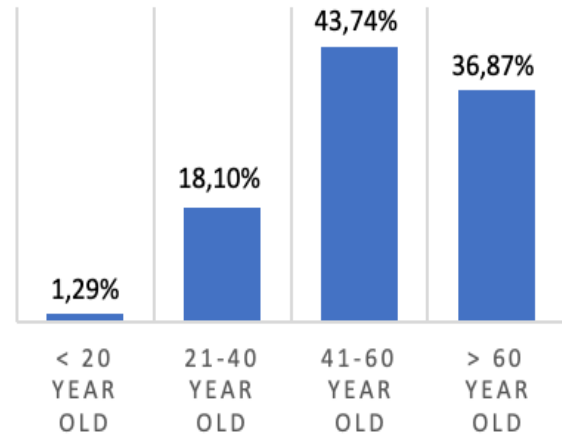


Figure 1. Total Study Subject

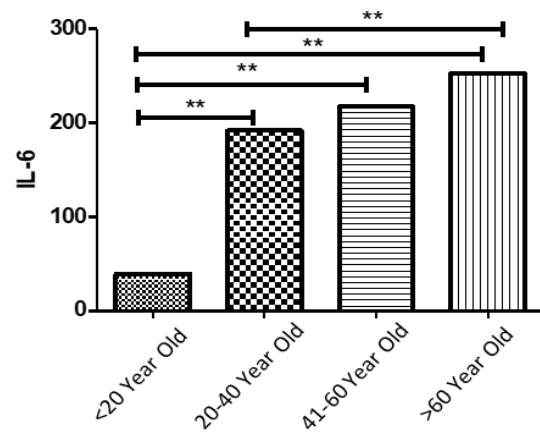


Figure 2. The IL-6 levels of the four age groups

Discussion:

In this study, we performed a cross-sectional analysis of IL-6 obtained in COVID-19-confirmed patient samples based on age group. We discovered a positive correlation between IL-6 levels with the increasing age of the study subjects. Furthermore, we found that young COVID-19 patients had significantly lower IL-6 serum levels compared to the older age group in the study. Several studies have reported that COVID-19 has a different pattern in adults and the elderly compared to children and young adults.⁴ Children and young adults generally have a better prognosis than older patients because their morbidity is lower thus the mortality rate is lower. In older patients or those with comorbidities, COVID-19 can cause severe infections, including pneumonia and Acute Respiratory Distress Syndrome, which is not found in younger individuals.^{7,8}

This finding suggests that immunosenescence may have an impact on the progression of COVID-19 disease. Immunosenescence is a form of progressive immune system modification that causes individuals more susceptible to infection, neoplasia, and other autoimmune manifestations.⁶ This phenomenon is primarily due to long-term antigenic stimulation.⁹ The main changes that cause this immunosenescence process are those related to T cell function. Reduced production of naive and regulatory T lymphocytes, decreased proliferative and functional capacity of effector lymphocytes, increased memory lymphocyte population, fibrotic changes in lymph node architecture, and dysregulation of cytokine production are the results of this process. The reorganization of the immune system contributes to changes in the secretory profile of cytokines, resulting in “inflammation-aging”. Because of dysregulation in the aging process, the abnormal distribution of Th1 and Th2 subtypes occurs, increasing the Th2 relatively compared to Th1. Therefore, the Th2 cytokine proinflammatory and its soluble receptors are higher in older subjects compared to younger ones.^{6,10} Although the findings of this study provide evidence in support of the theory of “inflammation-aging”, additional research is warranted to elucidate the precise role of cytokines in disease prognosis. A study conducted by Mohsin et al. in patients with multiple myeloma (MM) reported no significant association between TNF- α cytokines, age, and prognosis in MM.¹¹ Nonetheless, further investigations are required to comprehensively understand the interplay between cytokines, age, and disease outcomes.

Interleukin-6 is a multifunctional cytokine produced by various cells in the body. Cytokines play a crucial role in regulating immune responses, inflammation, hematopoiesis, metabolism, organ development, and even cancer cell growth and development. Depending on the specific signalling pathway and the type of cells involved, IL-6 can mediate different pathophysiological processes. The activation of the IL-6 pathway leads to the synthesis and release of acute phase proteins by hepatocytes. These

acute phase proteins include CRP, serum amyloid A, fibrinogen, haptoglobin, and α 1-antichymotrypsin. Simultaneously, IL-6 reduces the synthesis of fibronectin, albumin, and transferrin, which are other proteins important for various physiological functions.¹²⁻¹⁴

Interleukin-6 plays a crucial and complex role in inflammation and infection. It has both pro-inflammatory and anti-inflammatory functions, and its dysregulation can contribute to the pathogenesis of various diseases. The study conducted by Mustafa and Ismail demonstrated a positive correlation between the levels of 8-hydroxy-2'-deoxyguanosine, IL-6, and CEA in the bloodstream. They concluded that IL-6, a pro-inflammatory mediator produced by macrophages and epithelial cells within the tumor microenvironment, along with other pro-inflammatory cytokines, contributes to oxidative stress. This oxidative stress plays a significant role in promoting nuclear oxidative DNA damage.¹⁵

Interleukin-6 also plays a significant role in infectious diseases, especially in COVID-19. Various studies have demonstrated a close association between increased IL-6 levels and several clinical symptoms of COVID-19. Moreover, critically ill patients exhibit significantly higher IL-6 levels compared to those with milder disease presentations.¹³ Elevated IL-6 levels have been found to positively correlate with other inflammatory biomarkers, including CRP, lactate dehydrogenase, ferritin, and D-dimer. Additionally, IL-6 levels are associated with the findings of computed tomography scans in COVID-19 patients.¹² There is evidence suggesting that IL-6 serum levels tend to increase with age. Elevated levels of TNF- α , IL-6, and CRP are linked to an increased risk of morbidity and mortality, not only in susceptible populations but also in the elderly who may be considered less vulnerable. Importantly, the dysregulation of cytokines is not significantly influenced by other mortality risks, such as tobacco use, diabetes, hypertension, hypercholesterolemia, and other comorbid conditions.^{3,16}

Conclusion

There were age-related differences in IL-6 levels. The young age group differed significantly from the other three age groups. The older the COVID-19

patient, the higher the IL-6 level was. There was a positive correlation between age groups and IL-6 levels in COVID-19 patients.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Authors sign on ethical consideration's approval.

- Ethical Clearance: The project was approved by the local ethical committee in Universitas Brawijaya, Malang, Indonesia.
- The project was approved by the local ethical committee in Saiful Anwar General Hospital, with the number of 400/011/K.3/302/2021

Authors' Contribution Statement

A. I. contributed to the conception, design, acquisition of data, analysis, interpretation, drafting the manuscript, as well as revision and proofreading. D. K. M. participated in the acquisition of data, analysis, interpretation, and drafting of the manuscript. C. S. S. played a role in the design,

acquisition of data, and analysis. N. S. contributed to the revision and proofreading of the manuscript. A. T. E. was involved in interpretation, drafting the manuscript, and participated in its revision and proofreading. E. W. provided valuable input through revision and proofreading.

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العلاقة بين إنترلوكين-6 والعمر في كوفيد-19: رؤى من تحليل مقطعي في مالانج، إندونيسيا

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الخلاصة

تم تحديد إنترلوكين 6 (IL-6) باعتباره مؤشراً لشدة وفيات فيروس كورونا (COVID-19). على الرغم من أن معدلات الإصابة بالمرض والوفيات تزيد بشكل عام مع تقدم العمر، إلا أنه لا يزال من غير المؤكد ما إذا كان هناك ارتباط بين العمر ومستويات IL-6، مما يؤدي إلى تفاقم شدة كوفيد-19 لدى المرضى. كان الهدف من هذه الدراسة هو فحص العلاقة بين العمر ومستويات IL-6 لدى مرضى كوفيد-19. كانت الدراسة عبارة عن تحليل مقطعي للسجلات المخبرية والطبية لـ 3171 مريضاً من مرضى كوفيد-19 الذين تلقوا العلاج في عام 2021 في مستشفى سيف أنور العام. تم تحليل البيانات باستخدام اختبار كروسكال واليس، واختبار دونيت بوست هوك، واختبار ارتباط سبيرمان. تم تقسيم المرضى إلى أربع فئات عمرية: أقل من 20 عاماً (1.29%)، و20-40 عاماً (18.10%)، و40-60 عاماً (43.74%)، و60 عاماً (36.87%). وكان متوسط مستويات IL-6 في كل مجموعة هو 39.44، 192.04، 217.27، و252.59 بيكوغرام/مل. ولوحظ وجود اختلاف كبير (قيمة $p = 0.000$) في مستويات IL-6 بين الفئة العمرية أقل من 20 عاماً والمجموعات الأخرى. علاوة على ذلك، تم تحديد ارتباط إيجابي كبير (قيمة $p = 0.000$) بين الفئة العمرية ومستويات إنترلوكين-6 لدى مرضى كوفيد-19، مما يشير إلى ارتفاع مستويات إنترلوكين-6 لدى المرضى الأكبر سناً. في الختام، كانت هناك اختلافات في مستويات إنترلوكين-6 على أساس الفئات العمرية، وكان العمر مرتبطاً بشكل إيجابي بمستويات إنترلوكين-6 لدى مرضى كوفيد-19.

الكلمات المفتاحية: العمر، الارتباط، كوفيد-19، إنترلوكين-6، الخطورة