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Original Article

Interleukin-6 (IL-6) as a Marker of Endothelial Dysfunction Confirmed Using Flow Mediated Dilatation in Active Smoker

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ABSTRACT

Background: The pro-inflammatory cytokine interleukin-6 (IL-6) is thought to be the catalyst for endothelial dysfunction caused by smoking. Flow-mediated dilatation (FMD) is a non-invasive method to identify the development of blood vessel endothelial dysfunction at an early stage. However, several studies have not sufficiently explained how smoking and IL-6 interact.
Objective: This study focuses on the role of Interleukin-6 (IL-6) in the process of endothelial dysfunction confirmed using non-invasive modalities such as Flow Mediated Dilatation (FMD) in smokers related to the risk of cardiovascular disease in the future with the hope of providing insight in education to smokers to quit smoking.
Material and Methods: In this study, we gathered 116 male participants, of which 56 were nonsmokers, and 60 were smokers, and we used IL-6 and FMD testing. SPSS for Windows version 23 will be used to analyze the data.
Result: According to the analysis of laboratory tests, the average IL-6 level in light smokers was 67.7±3.2 and in moderate smokers it was 95.1±11.0; with a p-value of 0.009, indicating a significant difference among the groups. The average FMD in the smoking group was 5.4±0.4% (p-value 0.000), whereas the average FMD in the non-smoker samples was 10.9±0.9%. We also looked at the average FMD among light and moderate smokers based on Brinkman Index, which was 5.5±0.4% and 5.0±0.4%, with a p-value of 0.780, indicating no significant difference between the groups
Conclusion: Compared to non-smokers, this study demonstrates a correlation between IL-6 and smoking activity. However, there was no statistically significant difference between the prevalence of endothelial dysfunction and the degree of smoking dependence in this investigation.

1. Introduction

According to the Centers for Disease Control and Prevention (CDC), smoking causes 480,000 deaths in America each year. According to the 2018 Riskesdas, Indonesia's smoking prevalence among those aged 18 and older grew from 7.2% to 9.1%.

Smoking's effects on Interleukin-6 (IL-6) and cytokine-mediated substances such as serum amyloid protein A (SAA) and C-reactive protein (CRP) have been researched and linked to the inflammatory process.[1] A different research discovered that smoking enhanced the production of pro-inflammatory cytokines (IL-1, IL-6, and TNF) in peripheral blood samples. Increased pro-inflammatory cytokines are also known as a risk factor for cardiovascular disease and are linked to the development of atherosclerosis. IL-1, IL-6, and TNF have long been recognized for their critical roles in the immune response associated with inflammation.[2] A non-invasive technique called flow-mediated dilatation (FMD) can assess an atherosclerotic process in blood vessels.[3]

Tumor Necrosis Factor (TNF-), IL-1, and IL-6 are a few pro-inflammatory cytokine indicators well-known to have a role in inflammatory processes. IL-6 was chosen for this investigation because previous studies had not adequately characterized the relationship between IL-6 and smoking. The authors intend to demonstrate that IL-6 is a pro-inflammatory cytokine responsible for the endothelial damage caused by tobacco in active smokers and to link it to smoking duration and amount.

The author believes this study will show how pro-inflammatory mediator levels (IL-6) might be monitored for early detection of the atherosclerotic process in smokers, as demonstrated by non-invasive ultrasound testing, namely FMD. The usage of IL-6 itself must nevertheless consider several additional complicating conditions, including any infections, obesity, diabetes, hypertension, or dyslipidemia the patient may have. With the results of this study, it is hoped that Saiful Anwar Hospital Malang considering the FMD procedure as a tool to detect the presence of endothelial dysfunction and to examine pro-

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inflammatory markers, in this case, IL-6, as a predictor of atherosclerosis.

2. Material and Methods

2.1. Study design and Participation

This study was designed using an analytic observational design with a cross-sectional study aimed to explain the involvement of IL-6 levels as a pro-inflammatory mediator in the mechanisms of endothelial dysfunction in smokers' blood vessels. The study was conducted from November 2022 to June 2023 at the RSSA Central Laboratory and the RSSA Integrated Heart Center Installation. This research has passed ethical clearance by the Health Research Ethics Committee, Universitas Brawijaya. The target population in this research design was active smokers aged 18-65 who lived in Malang.

2.2. Assessment of Inflammatory Markers

A 5 mL blood specimen was obtained via venipuncture at the RSSA Central Laboratory. Examination of IL-6 using the ELISA method.

2.3. FMD Procedure

FMD is measured using ultrasound of the brachial artery by applying an ischemic condition in the distal artery using the pressure created by the sphygmomanometer. Then when the pressure is released, a reactive hyperemia condition appears in the brachial artery, and blood flowing to the distal part of the artery causes a vasodilation condition in the patient. The difference between basal diameter and vessel diameter after reactive hyperemia is defined as FMD.[3]

2.4. Statistical analysis

The examination results will be analyzed using SPSS for Windows version 23.0

3. Result

The study had 116 participants, with a mean age of 33.2±1.1 years for the non-smokers (n=56) and 31.9±1.1 years for the smokers (n=60), respectively, with a p-value of 0.311. Table. 1 displays the characteristics of the study population. Univariate analysis showed no significant difference between the two groups' fasting blood sugar levels (p-value = 0.507).

The initial diameter, peak diameter, and FMD examination were significantly higher in Non-Smoker than in Smokers. (Table. 2)

Laboratory studies revealed significant IL-6 level differences between the two groups. In the smoking group, the mean IL-6 level was 71.8±3.4 ng/L with a p-value of 0.000, whereas it was 41.8±1.0 ng/L in the control group. Additional data in inflammatory markers, including C-Reactive Protein (CRP), confirmed the increase in inflammatory markers in the smoking group. In the smoking group, the mean CRP was 0.13±0.001 with a p-value of 0.040, compared to 0.10±0.001 in the control group. The smoking group underwent follow-up analysis, with a subgroup of light and moderate smokers categorized according to the Brinkman index. The analysis's findings revealed that, on average, light smokers had an IL-6 level of 67.7±3.2, moderate smokers had one of 95.1±11.0, with a p-value of 0.009 (Table 3), indicating a significant difference between the groups. We also looked at the average FMD among light and moderate smokers, which was 5.5±0.4%, 5.0±0.4, with a p-value of 0.780, indicating no significant difference between the groups.

Table 1. Baseline characteristic of the subject sample

	Non-Smoker n (56)	Smoker n (60)	*p* Value
Age (years)	33.2±1.1	31.9±1.1	0.311
Cotinine (ng/mL)	9.0±0.4	32.5±3.1	0.000
MCP1 (pg/mL)	19.9±2.1	85.0±6.6	0.000
FBG mg/dL	94.3±1.7	92.1±1.1	0.507
IMT CCA D	0.06±0.01	0.07±0.01	0.000
IMT ICA D	0.05±0.01	0.08±0.02	0.000
IMT CCA S	0.04±0.01	0.05±0.00	0.000
IMT ICA S	0.04±0.01	0.05±0.00	0.000
Total Cholesterol	179±4	186±5	0.450
Triglycerides	112±11	168±15	0.000
HDL	52±2	45±1	0.002
LDL	125±4	118±4	0.078
CRP	0.10±0.001	0.13±0.001	0.040
WBC	6973±911	7778±1673	0.006

MCP1=Monocyte Chemoattractant Protein 1; FBG=Fasting Blood Glucose; IMT=Intimal Media Thickness; CCA = Common Carotid Artery; ICA = Internal Carotid Artery; HDL= High Density Lipoprotein; LDL= Low Density Lipoprotein; CRP = C Reactive Protein; WBC = White Blood Cell

Table 2. FMD and IL6 examination in Non-Smoker and Smoker Groups.

	Non Smoker n(56)	Smoker n(60)	*p* Value
Initial diameter	0.45±0.01	0.41±0.01	0.000
Peak diameter	0.50±0.01	0.44±0.01	0.000
FMD	10.9±0.9	5.4±0.4	0.000
IL6	41.8±1.0	71,8±3.4	0.000

FMD=Flow Mediated Dilatation; IL6=Interleukin 6

Table. 3 IL-6 Levels between Light and Moderate Smoker Group

	Light n (51)	Moderate n (9)	*p* Value
IL6	67.7±3.2	95.1±11.0	0.009

IL6=Interleukin 6

Table. 4 FMD between Light and Moderate Smoker Groups.

	Light n (51)	Moderate n (9)	*p* Value
FMD	5.5±0.4	5.0±0.4	0.780

FMD=Flow Mediated Dilation

4. Discussion

Based on Riskesdas 2018, 62.9% of male smokers, the majority population in Indonesia, with ages above 15 years old. [4] Therefore, this study's subjects were all men above 15.

In this study, we searched for early indicators of smoking-related vascular endothelial damage, assessed using FMD and elevated IL-6, an inflammatory marker. Numerous variables, including obesity, hypertension, diabetes mellitus, dyslipidemia, and infection, might impact the incidence of endothelial dysfunction. To reduce bias, we rejected patients who had these conditions.

Obesity is associated with vascular endothelial dysfunction caused by reduced nitric oxide (NO) availability due to increased oxidative stress production. The formation of pro-inflammatory cytokines is the primary mechanism by which obesity is associated with reduced NO availability. Under healthy conditions, perivascular adipose tissue (PVAT) secretes factors that influence vasodilation by increasing NO availability. Such a protective effect is lost in PVAT from obese subjects, which leads to decreased vascular NO availability and contributes to endothelin-1/NO imbalance. PVAT plays a direct role in regulating the inflammatory processes associated with the production of TNF-a and IL-6.[5] In this study, research subjects who underwent further analysis were not categorized as obese with an average BMI <25. Hence, obesity was not a confounding factor.[6]

Hypertension is also related to endothelial dysfunction associated with the inflammatory response accompanying an event of hypertension. Another research shows that inflammatory cells are necessary for the development of hypertension. Inflammation that occurs in response to a vasoconstrictor agonist.[7] In this study, we excluded subjects with hypertension so that hypertension was not a confounder in this study.

Dyslipidemia is defined as an increase in pro-atherogenic low-density lipoprotein (LDL) and its oxidative modification, namely oxLDL, an essential factor in endothelial damage, which is the initial step and predictor of atherosclerosis. OxLDL induces several mechanisms of endothelial hardening.[8] In this study, subjects who underwent further analysis were not categorized as having dyslipidemia with an average LDL in the control group of 125 ± 4 and the smoker group with an average of 118 ± 4 (p-value 0.078); therefore, dyslipidemia was not a confounding factor in this study.

Regarding the role of diabetes mellitus in endothelial dysfunction, substantial clinical and experimental evidence indicates that diabetes and insulin resistance cause endothelial dysfunction, which may reduce the anti-atherogenic role in the vascular endothelium. Insulin resistance and endothelial dysfunction appear to precede the development of hyperglycemia in patients with type 2 diabetes.[9] In this study, fasting blood sugar examination obtained average blood sugar levels in the control group 94.3±1.7. While in the group of smokers, 92.1 ± 1.1 (p-value 0.507). So that from these results, the condition of diabetes mellitus was not a confounding factor in this study.

Associated with infection as a factor in vascular endothelial dysfunction, various studies have highlighted that interleukin-6 (IL-6) is an inflammatory cytokine that plays a central role in propagating the inflammatory response responsible for atherosclerosis. The release of IL-6 is stimulated by acute infections, chronic inflammatory conditions, obesity, and physiological stress.[10] In this study, all study subjects were examined but did not find any subjects with complaints of fever. In this study, WBC examination in the control group was 6973±911, while in the group of smokers, 7778±1673 (p-value 0.006). In both groups, the WBC was less than 11.000 $10^3/mm^3$. Therefore, infection was not a confounding factor in this study, so we can ensure that an infection process does not cause an increase in IL-6 in the study group.

Regarding the results of FMD examination in patients associated with the effects of smoking, from this research, it was found that the mean value of FMD in non-smoker samples was 10.9 ± 0.9 %. In contrast, in the smoker group, the average value was 5.4 ± 0.4% (p-value 0.000), regarding a previous study by Maruhashi, Tatsuya, et al. where FMD has a cut-off measurement. FMD above 7.1% is significantly associated with a lower risk of cardiovascular events in patients with coronary artery disease. The FMD cut-off value for normal endothelial function is 7.1%.[11] In this study, the percent FMD in the non-smoker group was still above 7.1%, while in the smokers group, the percent FMD was below 7.1%. So it can be concluded that smoking correlates to endothelial dysfunction, which was evaluated using FMD, and has a higher risk of cardiovascular events associated with coronary artery disease.

In this study, IL-6 levels in the smoking group significantly increased compared to the control group, with an average IL-6 level of 41.8□1.0 ng/L, and in the smoking group, 71,8□3.4 ng/L with a p-value 0.000. These results are consistent with previous studies, which revealed that smokers' average IL-6 level increased substantially (46% compared to non-smokers).[12] Smoking causes an inflammatory process characterized by increased pro-inflammatory markers such as IL-6. We use the Brinkman index to assess the level of dependence on smoking. We also re-analyzed the smokers group by dividing the smokers based on Brinkman Index. Based on the Brinkman index, light smokers have an index of <200, moderate smokers are 200-599, and heavy smokers are >600. Based on the Brinkman Index, there were only two subsets of smoking groups in this study: light smokers and moderate smokers. It was found that the average IL-6 level in light smokers was 67.7±3.2, in moderate smokers, it was 95.1±11.0 with a p-value of 0.009, which showed a significant difference in the groups. This proves that the heavier the dependence on smoking, the higher the inflammatory process that occurs, characterized by an increase in inflammatory markers in the form of IL-6.

Researchers also conducted an analysis that shows the differences in FMD levels in smokers divided into light and moderate smokers based on the Brinkman index. In this study, the average FMD in light smokers was 5.5±0.4%. It was 5.0±0.4 in moderate smokers with a p-value of 0.780. From this study, it was no significant differences among the smoker group. These results differ from previous studies by Celermajer, David S., et al., where FMD is related to smoking-dose dependency. FMD is inversely proportional to the level of smoking dependency.[13] These results are different because there are differences in the distribution of the subset of smokers. In a study by Celermajer, David S., et al., the distribution of smokers based on their dependency was evenly distributed. In this study, 51 were light smokers and only 9 were moderate smokers based on the Brinkman Index.

This study has limitations related to excluding diabetes mellitus using only GDP levels. It would be better if the subject's diabetes mellitus status were determined using HbA1c levels. Then in this study, there is an uneven distribution of smokers based on the degree of smoking severity based on the Brinkman Index, so the evaluation of FMD, which was differentiated based on the level of smoking dependency, could not be carried out properly with results that were different from previous studies.

5. Conclusion

As a conclusion from this study, smoking affects the occurrence of endothelial dysfunction, which was evaluated using a non-invasive method in the form of an FMD examination using Doppler Ultrasonography. Inflammatory processes influenced by smoking were assessed using IL-6, and it was shown a correlation with smoking activity compared to non-smokers. However, the relationship between the incidence of endothelial dysfunction and smoking dependency in this study did not show a significant difference.

6. Declaration

6.1 Ethics Approval and Consent to participate
The subjects in this study are humans, so ethical rules must be followed. This research has passed the ethical due diligence, approved based on the Certificate of Ethical Eligibility No. 400/097/K.3/302/2021 issued by the Health Research Ethics Committee at Dr. Saiful Anwar Malang.

6.2. Consent for publication
Not applicable.

6.3 Availibility of data and materials
Data used in our study were presented in the main text.

6.4 Competing interests
Not applicable.

6.5 Funding Source
Not applicable.

6.6 Authors contributions
Idea/concept: AW. Design: AW. Control/supervision: NK, CTT. Data collection/processing: EF. Analysis/interpretation: EF. Literature review: EF, SW. Writing the article: EF. Critical review: SW, SA, BS, VY. All authors have critically reviewed and approved the final draft and are possible for the content and similarity index of the manuscript.

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