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Original Article

Correlation Between Smoking and II-1 Level and Arterial Stiffness as Measured By Cavi in the Young Adult Population Without Other Cardiovascular Risk Factors

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ABSTRACT

Background: Recent studies have shown that arterial stiffness is a strong predictor of cardiovascular events and all-cause mortality, with CAVI (Cardio-Ankle Vascular Index) as a non-invasive arterial stiffness testing method in daily practice.
Objective: This study was conducted to examine the relationship between smoking -as a risk factor for arterial stiffness- and CAVI values, as well as levels of IL-1β (Interleukin 1β) as a cytokine that plays a role in the pathophysiology of arterial stiffness.
Methods: Eighty-four participants, including smokers and non-smokers without other cardiovascular risk factors, were included in the study. Demographic data, medical history, and smoking behavior were taken using a questionnaire, then IL-1β and CAVI levels were examined.
Result: The mean level of IL-1β in smoking subjects was significantly higher (15.09 ± 0.48) than in non-smoking subjects (5.53 ± 0.79; p=0.001). CAVI values in smoking subjects were also significantly higher (8.0 ± 0.06) than in non-smoking subjects (6.9 ± 0.02; p=0.001). Further analysis showed a strong positive correlation between smoking and IL-1β levels (r=+0.776; p=0.001) and CAVI values (r=+0.759; p=0.001).
Conclusion: This study shows that smoking significantly correlates with IL-1β levels and CAVI values. The greater number of cigarettes used per day and the longer duration of smoking, there was a positive correlation between IL-1β levels and arterial stiffness as measured by CAVI.

1. Introduction

The results Global Adult Tobacco Survey (GATS) launched by the Indonesian Ministry of Health showed there is an increase in the number of adult smokers by 8.8 million people, from 60.3 million people in 2011 to 69.1 million people in 2021. have described the association of smoking as a risk factor for cardiovascular disease.[4,5] It is stated that cigarettes are proatherogenic, playing a role in the early stages of atherosclerosis, especially in endothelial cells. Cigarettes are not only a source of reactive oxygen species (ROS) but also induce the formation of endogenous ROS and cause a decrease in the bioavailability of Nitric Oxide (NO), which reduces the vasodilatory, antiplatelet, and anti-inflammatory effects of the endothelium.[6] Oxidative stress will also damage endothelial integrity, increase platelet reactivity, increase Low-Density Lipoprotein (LDL), increase expression of pro-inflammatory cytokines such as IL-1β, IL-6, Tumor Necrosis Factor (TNF-α), and increase smooth muscle proliferation. These structural changes in the arterial wall eventually result in arterial stiffness.

Arterial stiffness is a strong predictor of cardiovascular events and all-cause mortality, also found in individuals without symptoms or overt cardiovascular disease.[7] Many methods have been developed to determine the degree of narrowing or blockage of coronary and peripheral arteries, such as CT angiography, MR angiography, and angiography with contrast. However, these techniques are invasive, complex, and expensive for the patient. Therefore, a more straightforward and cheaper non-invasive technique was developed to assess the degree of arterial stiffness.

The Cardio-Ankle Vascular Index (CAVI) is a new index of overall arterial stiffness. There have been several previous studies on smoking and arterial stiffness.[10-15] However, the study examined arterial stiffness about many confounding factors such as diabetes, hypertension,

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and hyperlipidemia. In our study, we wanted to specifically examine the association between arterial stiffness and smoking without confounding factors by recruiting smokers and nonsmokers who had no underlying disease that might influence arterial stiffness.

2. Methods

This study used an analytical observational design with a cross-sectional approach. The study population aged 20-40 had no known cardiovascular risk factors. The research sample was taken by purposive sampling on the population that met the inclusion and exclusion criteria and agreed to be involved in the study. This study had 84 participants, consisting of 11 non-smokers and 73 smokers. The variables studied in this study were smoking behavior (length of smoking, number of cigarettes per day), levels of IL-1β, and the level of arterial stiffness as measured by CAVI.

Smoking behavior was measured by a questionnaire adapted from the GATS (Global Adult Tobacco Survey) questionnaire from WH) 2020. Smoking behavior included smoking duration and the number of cigarettes smoked in 1 day. The subject filled out the questionnaire and was confirmed by the family.

A 5 mL blood specimen was obtained by venipuncture at the RSSA Central Laboratory. The specimens were measured for lipid profile and blood sugar levels. IL-1β . test using a blood serum sample from the subject. Using the Human IL-1β (Interleukin 1 Beta) ELISA Kit from Elabscience®; Catalog No : E-EL-H0149. The measurement procedure is by the procedure manual from the manufacturer. CAVI was measured using a CAVI device (Vasera; Fukuda Denshi, Tokyo, Japan).

2.1. Statistical Analysis

The examination results will be analyzed using SPSS for Windows version 23.0. Descriptive analysis aims to determine the characteristics of the sample owned. This analysis obtained the mean value, standard deviation, and prevalence of essential characteristics (age, BMI, number of cigarettes per day, duration of smoking, GDS, lipid profile, and CAVI values). Differences between variables were tested with the Mann-Whitney test. Then done normality and homogeneity test, Aims to determine the distribution of average data or not by using the Kolmogorov-Smirnov normality test. Then, a correlation test was conducted between the duration and the amount of smoking with IL1β levels and CAVI values, measured using the Spearman correlation test. It is considered significant if $p < 0.05$.

3. Results

Table 1. Essential characteristics of research subjects

Characteristic	N=84		p
	Non-smoker (N=11) (13.1%)	Smoker (N=73) (86.9%)	
Age (year)	33.18 + 2.04	30.10 + 0.68	0.138
LDL (mg/dl)	88.45 + 10.16	97.71 + 2.95	0.126
HDL (mg/dl)	54.91 + 4.78	51.86 + 1.59	0.414
FBG (mg/dl)	92.27 + 3.67	91.88 + 1.16	0.537
Total cholesterol (mg/dl)	166.36 + 12.26	164.75 + 4.83	0.837
Triglyceride (mg/dl)	130 + 22.85	145.68 + 17.83	0.811
BMI	20.1 + 0.52	19.43 + 0.19	0.375

*note: LDL: Low-Density Lipoprotein; HDL: High-Density Lipoprotein; FBG: Fasting Blood Glucose; BMI: Body Mass Index

After going through a purposive sampling based on inclusion and exclusion criteria, in this study conducted at RSUD dr Saiful Anwar Malang, 84 research subjects were collected. The research subjects were then given informed consent regarding the study, their blood samples were taken, and CAVI measurements were taken. Characteristics of research subjects can be seen in Table 1.

Measurement of CAVI was done using the device mentioned above. The level of IL-1β and CAVI value describe in Table 2. The mean difference between variables was measured by the Mann-Whitney test, significant when $p < 0.05$.

Table 2. Value of CAVI and IL-1β level of research subjects

Parameters	N=84		p
	Non-smoker (N=11) (13.1%)	Smoker (N=73) (86.9%)	
CAVI	6.9 + 0.02	8.0 + 0.06	0.001
CAVI > 9	0	5/73 (6.84%)	0.000
CAVI < 9	11 (100%)	68/73 (93.16%)	0.001
IL-1ß level (pg/ml)	5.53 + 0.79	15.09 + 0.48	0.001

*note: CAVI: Cardio-Ankle Vascular Index; IL-1ß: Interleukin-1 beta

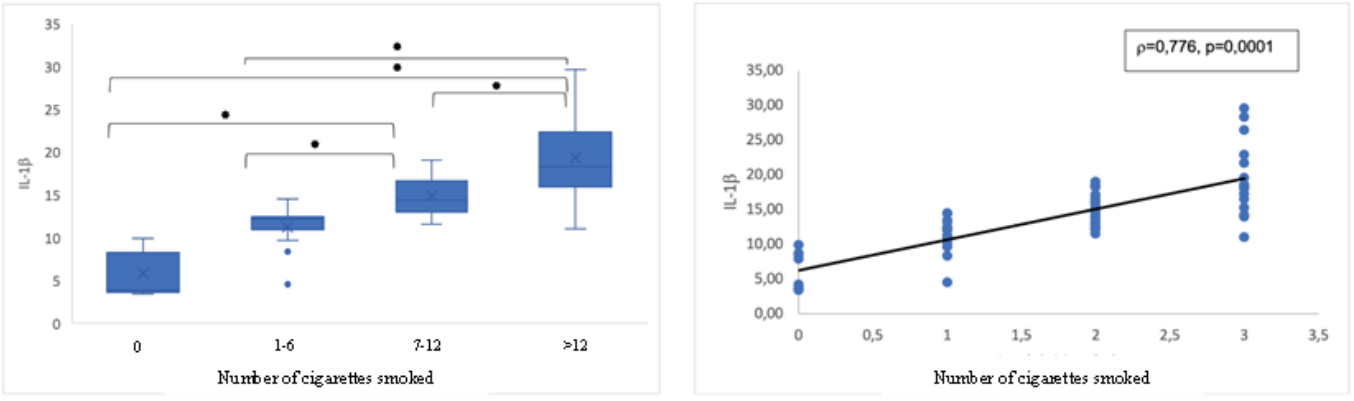
From the table above, it can be seen that the characteristics that are significantly different in the two different smoking groups are CAVI values and IL-1β levels. The mean value of CAVI in smoking subjects was significantly higher (8.0 + 0.06) than in non-smoking subjects (6.9 + 0.02; p=0.001). The mean level of IL-1β in smoking subjects was also significantly higher (15.09 + 0.48) than in non-smoking subjects (5.53 + 0.79; p=0.001). Other characteristics such as age, LDL, HDL, fasting blood sugar, total cholesterol, triglycerides, and BMI did not show significant differences in the smoking group compared to non-smokers.

3.1 Correlation of Number and Duration of Smoking with Levels of IL-1β

The smoking group was divided into four (4) groups according to the number of cigarettes smoked, namely group 1 (not smoking; smoking 0 cigarettes/day), group 2 (smoking 1-6 cigarettes/day), group 3 (smoking 7-12 cigarettes/day) and group 4 (smoking more than 12 cigarettes/day). We started by comparing the IL-1b levels of all analyzed groups using the Kruskal-Wallis test (p=0.001). Then the comparative analysis of IL-1β levels per group using repeated Mann-Whitney test showed significant differences in group 1 with groups 3 and 4, group 2 with groups 3 and 4, and groups 3 and 4 (p=0.000). The summary results of these tests can be seen in Figure 1.

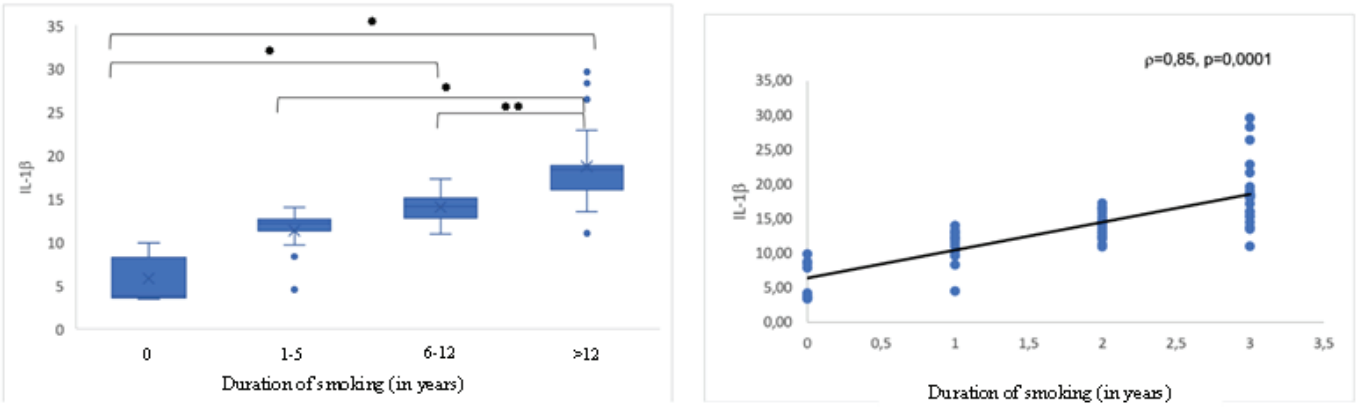
Using Spearman's Rank Test, the correlation test was used to see the relationship between the number of smoking and IL-1β levels. These results indicate that there is a significant relationship between the amount of smoking and increased levels of IL-1β (correlation coefficient = +0.776; p = 0.001).

The smoking group was also divided into four (4) groups according to the length of smoking, namely Group 1 (no smoking; smoking 0 years); Group 2 (prolonged smoking 1-5 years); Group 3 (prolonged smoking 6-12 years) and group 4 (long smoking more than ten years). We started by comparing the IL-1b levels of all analyzed groups using the Kruskal-Wallis test (p=0.001). Then the comparative analysis of IL-1b levels per group using repeated Mann-Whitney test showed a significant difference, which was obtained in group 1 with groups 3 and 4 (p=0.000); and group 2 with group 4 (p=0.000); and group 3 with group 4 (p=0.004). The summary results of these tests can be seen in Figure 2.



a) b)

Figure 1. a) Differences in IL-1β levels based on the number of cigarettes smoked (* indicates a significant difference between groups (p=0.000).



a) b)

Figure 2. a) Differences in IL-1b levels based on the duration of smoking (* sign indicates a significant difference between groups p=0.000; ** p=0.004). b) Spearman's correlation results between IL-1b levels and smoking duration

Using Spearman's Rank Test, the correlation test was used to see the relationship between smoking duration and IL-1b levels. These results indicate that there is a significant relationship between the length of smoking and increased levels of IL-1b (correlation coefficient=+0.850; p=0.001)

3.2. Correlation of Number and Duration of Smoking with CAVI Value

We also used the Kruskal-Wallis test to compare the CAVI values of all groups (p=0.001). Then the comparative analysis of CAVI values per group using repeated Mann-Whitney test showed significant differences in groups 1 with groups 3 and 4 (p = 0.000) and 2 with groups 3 and 4 (p = 0.000). The summary results of the test can be seen in Figure 3. Using Spearman's Rank Test, the correlation test is used to see the relationship between the number of smoking and the level of CAVI. These results indicate a significant relationship between the amount of smoking and the increase in CAVI value (correlation coefficient = +0.883; p = 0.001).

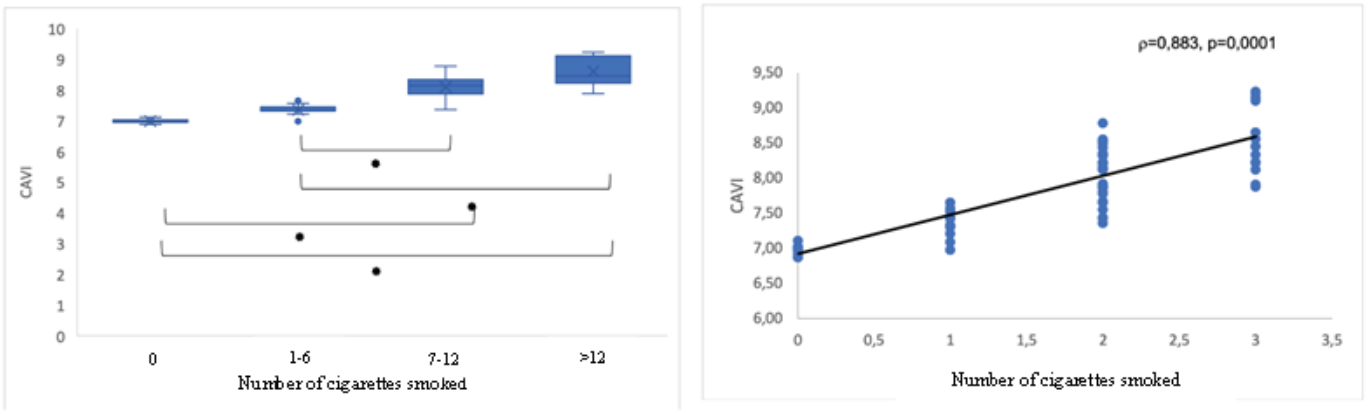
We also compared the CAVI value of each group for the duration of smoking using the Kruskal-Wallis test. The results of the analysis showed that there were significant differences in group 1 with groups 3 and 4 (p=0.000) and group 2 with groups 3 (p=0.019) and 4 (p=0.000). The correlation between smoking duration and increasing CAVI value also had a significant relationship (r=+0.769; p=0.001). A summary can be seen in Figure 4.

3.3. Correlation of Degree of Smoking (Brikman Index) with IL-1β and CAVI Values

According to the Brikman Index, the degree of smoking results from multiplying the length of smoking by the average number of daily cigarettes. The results of comparing IL-1β levels and CAVI values to the Brinkman index (p=0.001) are shown in Figure 5. The Spearman's rank test showed a significant relationship between the Brinkman index and increased levels of IL-1b (r=+0.776; p=0.001) and CAVI value (r=+0.759; p=0.001).

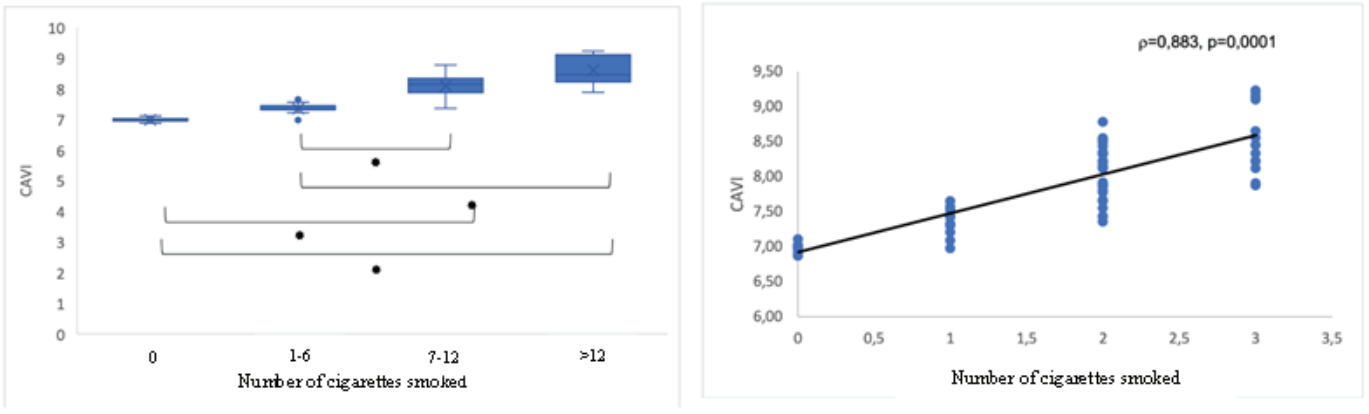
3.4. Correlation between IL-1b levels and CAVI values

The correlation test to determine the relationship between IL-1b levels and CAVI values was carried out by Spearman analysis. In this test, it was found that there was a significant relationship between the increase in IL-1b levels (ρ=-0.848; p=0.001) and the CAVI value.



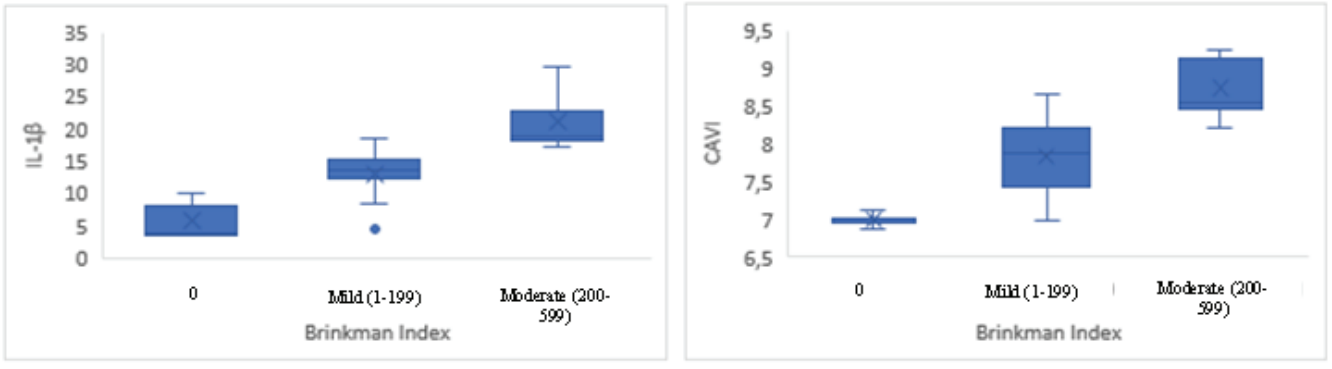
a) b)

Figure 3. a) Differences in CAVI values based on the number of cigarettes smoked (* indicates a significant difference between groups (p=0.000). b) Spearman's correlation results between CAVI values and the number of smoking



a) b)

Figure 4. a) Differences in CAVI values based on the duration of smoking (* sign indicates a significant difference between groups p=0.000; **p=0.019). b) Spearman's correlation result between CAVI value and smoking duration



a) b)

Figure 5. (a) Differences in IL-1b levels based on the Brinkman index (b) Differences in CAVI levels based on the Brinkman index

4. Discussion

From this study's characteristic data, the subjects' age data were obtained with a mean age of 33.18 + 2.04 years for non-smokers and 30.10 + 0.68 years for smokers. No other studies on smoking and arterial stiffness have specifically sampled the young adult population. A 2007 Japanese study examined CAVI values in a working population with a mean age of 48.5±8.6 years for nonsmokers and 48.7±8.1 years for smokers.[19] Another study by Thitiwuthikiat et al. used non-smokers with an average age of 38.4±11.8 years and 41.1±10.7 years for smokers. Age is one of the factors that can increase the degree of arterial stiffness. Theoretically, the older a person is, the stiffer the arteries and the higher the CAVI value. In our study, we intentionally recruited young adults. The results of the Mann-Whitney showed that there was no significant difference in the mean age of smokers and non-smokers of our subjects. This shows that the age variable does not affect the significance of the difference in CAVI values in smokers and non-smokers in this study.

The results of Mann Whitney in this study also showed that from the two groups of smokers and non-smokers, there was a significant difference in the average levels of IL-1β which were examined from the subjects' peripheral blood serum.

4.1 Correlation of smoking with levels of IL-1β

Our results show a strong positive correlation between the number and duration of smoking and IL-1β levels. In our study, mean IL-1β levels were significantly higher than IL-1β levels in the non-smoker's group (15.09 + 0.48 pg/mL vs. 5.53 + 0.79 pg/mL: $p<0.05$). This result is to the results of several previous studies. Syam et al. showed that the mean levels of IL-1β in COPD patients who smoked were higher than in non-smokers (35.08 pg/mL vs 8.85 pg/mL; $p<0.05$). Another study reported that IL-1β levels were 1.7 times higher in smokers than in non-smokers. The mean level of IL-1β in the serum of individual smokers was 11.48 pg/mL, while that of non-smokers was 7.59 pg/mL ($p<0.05$).[68] In adult individuals, normal levels of IL-1β in the blood range from 0.5 to 12 pg/mL.

In this study, we observed that IL-1β levels in the serum of smoking subjects were significantly higher than that of non-smokers. We suspect these cytokines originate in the lung, most likely from epithelial cells and leukocytes. Our assumption is based on the results of previous studies that exposure to secondhand smoke increases levels of IL-1β and TNF-α in bronchial lavage fluid, and levels of IL-1β and TNF- in circulating mononuclear cells in smokers were higher than in nonsmokers.[20]

The role of the IL-1β activation pathway in the pathophysiological effects of smoking has been described by Churg in a study using mice in which the IL-1β receptor had been removed. In particular, Churg et al.[70] showed that blocking the IL-1β receptor prevented the increase in the number of inflammatory cells in bronchial lavage fluid due to exposure to cigarette smoke. Other studies have shown that cigarette smoke condensate or benzo(a)pyrene can induce activation of the IL-1β gene promoter in human lung epithelial cells that have a specific polymorphism in the -31T/C gene.[22]

4.2 The Role of IL-1β in the Pathophysiology of Atherosclerosis and Arterial Stiffness

The results of our study also showed a positive correlation between IL-1β levels and CAVI values as a marker of arterial stiffness. A wealth of evidence has demonstrated the critical role of IL-1β in atherosclerosis. First, IL-1β protein and mRNA levels in atherosclerotic patients were significantly increased compared to normal individuals; levels are also positively correlated with disease severity. In addition, IL-1β gene family variations are also associated with coronary heart disease. Other evidence suggests that the association between increased atherosclerotic susceptibility and the presence of clonal hematopoiesis in peripheral blood cells is regulated at least in part by the NLRP3/IL-1 β pathway.[16]

Our study subjects were young adults with no risk factors other than smoking. Our analysis showed that the number and duration of smoking were strongly positively correlated with circulating levels of IL-1β in subjects and the level of arterial stiffness represented by higher CAVI values. In the light smoker group (Brinkman index 1-199), IL-1β levels and CAVI values significantly differed from those in the non-smokers group. This shows that there has been an inflammatory process in the light smoker's body and an increase in arterial stiffness due to atherosclerosis.[22]

Studies have been conducted in vivo to determine the role of IL-1β in atherosclerosis. These studies involve loss of function or increase in function of IL-1β genetically induced, manipulation of IL-1RA, and pharmacological inhibition of IL-1β. Exposure to IL-1 in the peri adventitial porcine arteries exacerbated intimal thickening in these arteries, suggesting the involvement of IL-1β in arterial hyperplasia. Moreover, inhibition of IL-1β limits the response to this injury. In hyperlipidemic mice, IL-1β induces the formation of atherogenic lesions, and impaired IL-1β signaling limits the process of atherogenesis. IL-1RA deficiency in mice increases arterial inflammation and aneurysm formation. Hemizygous IL-1RA deficiency limits the early atherosclerotic process and reduces macrophage content in hyperlipidemic mice. Another experiment in hyperlipidemic mice lacking the IL-1 β receptor demonstrated impaired expansive remodeling during lesion formation caused by reduced expression of MMP-3 (stromelysin). Activated platelets can express IL-1α and complex microparticles containing functional IL-1β, suggesting a correlation between this cytokine and atherothrombosis.[23]

IL-1β is also known to play an essential role in atheroma growth. In in vitro experiments with experimental atherosclerosis, it has been shown that selective neutralization of IL-1β induces monocytes in the plasma to shift to a lower inflammatory state, in which case monocytes produce higher plasma levels of IL-10 and reduce the size of atherosclerotic plaques in the absence of atherosclerotic plaques—compensatory arterial remodeling.[24]

It was previously discussed that the increase in arterial stiffness is also the result of an increase in smooth muscle cell thickness. IL-1β has multiple effects on human vascular smooth muscle cells, which are closely involved in atherogenesis. Many studies have investigated this. IL-1β can induce autocrine production of platelet-derived growth factors that stimulate vascular smooth muscle cell proliferation. IL-1β is also known to induce the expression of its gene in many cell types, including endothelial cells and smooth muscle cells. This characteristic strong amplification loop can enhance the proliferative effect of vascular smooth muscle cells.[21] The increased number of smooth muscle cells in the tunica intima is an essential sign of arterial stiffness.

4.3. Smoking Correlation with CAVI Value

Several previous studies have shown that age affects CAVI scores. Age is one of the factors that can increase the degree of arterial stiffness. Theoretically, the older a person is the stiffer the arteries, and the higher the CAVI value. Arterial stiffness is thought to be associated with arteriosclerosis. However, it is ethically challenging to measure arteriosclerosis by invasive methods. CAVI measures arterial stiffness in a noninvasive and convenient way, and these advantages make CAVI the right choice for assessing arterial stiffness caused by smoking.

Theoretically, the CAVI score reflects arterial stiffness. Soft and flexible arteries give low CAVI scores, whereas sclerotic, less flexible ones produce high CAVI scores. Increased arterial stiffness is generally associated with increased BMI and age, particularly in persons 60 or older. One of the strengths of our study is that potential confounding variables such as BMI and age between smokers and non-smokers were not significantly different. In this study, the participants' average BMI was within the normal range. The results of our analysis showed that there was no significant difference in the mean age of smokers and non-smokers of our subjects. This indicates that the age variable did not significantly affect the difference in CAVI scores between smokers and non-smokers in this study, which means that the CAVI results in our study more accurately reflect the arterial stiffness associated with smoking.[25]

In our study, the mean CAVI value of smokers (8.0 ± 0.06) was significantly higher than non-smokers (6.9 ± 0.02). This result is from previous research. A 2007 Japanese study examined CAVI values in the working population with the result that the mean CAVI scores in smokers were significantly higher than in nonsmokers66. Another study by Thitiwuthikiat et al. showed that the average CAVI value in smokers was significantly higher than in non-smokers (7.88 ± 1.26 vs 7.17 ± 0.94; $p < 0.001$).[19] According to the recommendations from the manufacturers of the CAVI measuring equipment we use, a CAVI value <8.0 is normal, whereas a CAVI 8 and <9 are considered borderline, whereas a CAVI >9 suggests a presumptive diagnosis of arteriosclerosis. Not all studies support this recommendation. For example, reports of mean CAVI in groups at high risk for cardiovascular disease found mean CAVI values <9. The study reported the mean CAVI values in the CVD risk-free group and the CVD high-risk group. For the CVD risk-free group aged 40-49 years, the mean CAVI score for males was 7.59±0.70, and for females, 7.29±0.66. For the high-risk group for CVD in the same age range, the average CAVI score for males was 7.79±0.85, and for females was 7.58±0.9184. In addition, another study reported that the CAVI value 8 was the best cut-off value that correlated with the presence of coronary artery disease. Thus, the CAVI score of smokers in our study, which was higher than that of nonsmokers, indicates a higher risk of cardiovascular disease.

We also perform analysis based on the Brinkman Index. According to the Brinkman Index, the degree of smoking results from multiplying the length of smoking by the average number of daily cigarettes. This index describes the cumulative severity of smoking. The longer a person smokes and the more cigarettes smoked daily, the more severe the smoking will be. Showed Spearman's rank test showed a significant relationship between the Brinkman Index and the increase in CAVI values in the smoker's group. This finding is consistent with a previous report that found that the arterial stiffness index of baPWV in chronic smokers was significantly higher than that of non-smokers. Of chronic smokers vs. nonsmokers was also significantly increased in the long-term measurement.[25] The average CAVI value in moderate smokers in this study was >8, indicating arterial stiffness in this group.

In the population in this study, young adults without other cardiovascular risk factors, all participants did not show symptoms related to arterial disease, such as angina complaints in CAD or intermittent claudication typical of peripheral arterial disease. Abnormal CAVI can be considered an early parameter of arterial stiffness due to arteriosclerosis. We suggest that CAVI is a helpful index as a screening tool for smoking-induced overall arterial stiffness. Many harmful chemicals in cigarette smoke can contribute to arterial stiffness, especially nicotine and free radicals that damage blood vessel endothelial cells. Endothelial dysfunction causes several problems that lead to atherosclerosis.

The results of this study can also give rise to new ideas that, besides being used for screening, CAVI examination can also be used as a marker to make lifestyle modifications and control risk factors. Our study showed that in individuals without symptoms but with risk factors for smoking, the CAVI value was high. Quitting smoking is an absolute choice for the individual.

Our study has several limitations. First, the design of this study was cross-sectional. A prospective cohort study is needed to assess causal relationships accurately. Then the number of samples in our study is relatively small, which may affect the results and subgroup analysis. Another limitation is that all study subjects were male, so our findings cannot be applied to the female population. There may be differences in IL-1β values and CAVI values between men and women due to differences in lifestyle or other underlying diseases. Therefore it is necessary to conduct studies including female subjects to determine any differences between the sexes.

5. Conclusion

This study concluded that: There is a strong positive relationship between smoking and IL-1β levels and CAVI values in the young adult population without other cardiovascular risk factors. From our results, it is reasonable to consider using CAVI as a screening tool for arterial stiffness in a smoking population at risk for future cardiovascular events.

6. Declarations

6.1 Ethics Approval and Consent to participate
This study has passed a test of ethical excellence certified based on the Letter of Ethical Excellence No. 400/097/K.3/302/2021 issued by the Universitas Brawijaya Health Research Ethics Committee.

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: AGP, CTT. Design: AGP, CTT. Control/supervision: CTT, NK, DS, SA. Data collection/processing: AGP, CTT. Analysis/interpretation: AGP, CTT. Literature review: AGP, DS, CTT. Writing the article: AGP, CTT. Critical review: CTT, NK, DS, SA. All authors have critically reviewed and approved the final draft and are possible for the content and similarity index of the manuscript.

6.7 Acknowledgements
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