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Effect of Electronic Cigarette and Moderate Intensity Aerobic Physical Exercise to IL-6, IL-15, and FSTL-1 Protein Expression and Fragmentation of Aortic Elastic Lamina of Wistar Rats

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ABSTRACT

E-cigarettes may contribute to aortic stiffness through inflammation and elastic lamina fragmentation. Inflammation triggers IL-6, IL-15, and FSTL-1 production in oxidative cells. Moderate-intensity aerobic exercise also increases these cytokines; however, it confers cardiovascular benefits. This study investigates the effects of e-cigarettes and aerobic exercise on IL-6, IL-15, FSTL-1, and aortic elastic lamina fragmentation in male Wistar rats. Rats were divided into four groups: control, exercise, e-cigarette, and combined e-cigarette plus exercise. After six weeks, aortic tissue was analyzed for cytokine expression using Western blot and for elastic lamina fragmentation using Elastic-Van Gieson staining. E-cigarette exposure significantly increased IL-6 (1.14 ± 0.362) and IL-15 (1.07 ± 0.252) compared to the control group (IL-6: 0.48 ± 0.103, $p = 0.002$; IL-15: 0.49 ± 0.091, $p < 0.001$). Exercise also increased IL-6 (0.72 ± 0.200, $p = 0.017$) and IL-15 (0.69 ± 0.211, $p = 0.046$). Aortic fragmentation was significantly higher in the e-cigarette group (11.00 ± 3.46) compared to the control (4.71 ± 2.69, $p = 0.003$) and combined groups (5.86 ± 2.54, $p = 0.008$). Elastic lamina fragmentation positively correlated with IL-6 ($r = 0.772$, $p = 0.001$) and IL-15 ($r = 0.688$, $p = 0.009$). FSTL-1 expression was inversely correlated with fragmentation in the e-cigarette groups ($r = –0.564$, $p = 0.036$). E-cigarettes promote inflammation and aortic damage, whereas exercise reduces elastic lamina fragmentation. FSTL-1 may have a protective role in limiting aortic damage.

KEYWORDS:
electronic cigarette; moderate intensity aerobic physical exercise; interleukin-6; interleukin-15; FSTL-1; aortic elastic lamina

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INTRODUCTION

The use of electronic cigarettes (e-cigarettes) is currently increasing and is dominated by adolescent users (Cooper, 2022). Reports from the Centers for Disease Control and Prevention (CDC) in 2022 stated that the increase in the use of e-cigarettes among adolescents has increased significantly in 2019–2020 (Cooper, 2022). In 2022, there were 2,140,000 school-aged youth grades 9–12 and 380,000 youth grades 6–8 who actively use e-cigarettes (Cooper, 2022). National Youth Risk Behavior Survey data from the CDC for 2015–2019 showed that 6 out of 10 teenage athletes are users of e-cigarettes (Rapoport et al., 2023).

A systematic review found that e-cigarettes have a detrimental effect on blood vessel function due to the activation of enzymes that produce superoxide and inflammatory factors resulting in endothelial dysfunction (Kennedy et al., 2019). A study by Mayyas et al. showed the highest increase in inflammatory factors was found in e-cigarettes compared to controls, tobacco, and waterpipe cigarettes (Mayyas et al., 2020). Blood vessel stiffness due to e-cigarettes is thought to result from endothelial dysfunction, activation of the sympathetic nervous system against smooth muscle cells in blood vessels (Spoladore et al., 2022). It is to be noted that e-cigarettes are also widely used by athletes because of its nicotine content is used for weight control, relaxation, increasing reaction time, and concentration (Young, Henderson and Couperus, 2020). Currently, animal research evidence regarding aortic stiffness is still not conclusive based on a report from the England Office for Health Improvement and Disparities in 2022 (McNeill et al., 2022). Aortic stiffness can be assessed non-invasively using the pulse wave velocity (PWV) (Mitchell et al., 2010). However, PWV is related to the elastic modulus which is affected by the condition of collagen and elastin fibers (Kohn, Lampi and Reinhart-King, 2015). It is also known that a decrease in the elastic modulus of the elastic lamina can indicate tunica media stiffness, therefore indicating stiffness (Rezvani-Sharif, Tafazzoli-Shadpour and Avolio, 2019).

Aerobic exercise can inhibit aortic stiffness and maintain endothelial function (Pianta et al., 2019; Gao et al., 2021; Kumboyono et al., 2022; Franklin and Eijsvogels, 2023). Exercise induces muscles to release myokines. (Severinsen and Pedersen, 2020). Myokines are cytokines and other peptides that are produced, expressed, and secreted by muscle fibers and have autocrine, paracrine, or endocrine effects (Severinsen and Pedersen, 2020). Interleukin (IL)-6, IL-15 and Follistatin-Like (FSTL)-1 are some of myokines whose levels are affected by exercise (Görgens et al., 2013). Phosphorylation of mitogen-activated protein kinase (MAPK) or adenosine monophosphate protein kinase (AMPK) occurs when skeletal muscles contract, enabling them to become activated forms and induce the expression of IL-6, IL-15, and FSTL-1 (Crane et al., 2015; Görgens, 2015; Hogg, 2018). Increased IL-6, IL-15 and FSTL-1 have the potential to prevent aortic stiffness which is an independent risk factor for cardiovascular events (Cooper et al., 2016; Vasconcelos and Salla, 2018; Jaminon et al., 2019; Lamb et al., 2020; Clayton et al., 2021; Inoue et al., 2022).

IL-15 has been shown to play a dual role as both a pro-inflammatory cytokine and a myokine, making it highly relevant in the context of inflammation, exercise physiology, and vascular health. IL-15 is expressed by various cell types, including endothelial and immune cells, and has been shown to promote the recruitment and activation of T lymphocytes and natural killer (NK) cells, thereby amplifying inflammatory responses in vascular tissue. (Muthumalage et al., 2019; Ali et al., 2022) Studies have reported elevated IL-15 levels in response to electronic cigarette exposure, indicating its involvement in vaping-related inflammation. (Ali et al., 2022) On the other hand, IL-15 is also secreted by skeletal muscle during exercise and has been categorized as a myokine that contributes to lipid oxidation, improved insulin sensitivity, and vascular protection. (Riechman et al., 2004; Vasconcelos and Salla, 2018) These contrasting functions suggest that IL-15 may mediate both detrimental effects of e-cigarette exposure and the beneficial adaptations induced by aerobic exercise. Moreover, its potential role in modulating aortic stiffness and endothelial dysfunction, especially in conditions involving systemic inflammation and lifestyle factors, supports its inclusion as a biomarker of interest in this experimental setting (Quinn et al., 2009; Para, Albu and Porojan, 2021).

Research on e-cigarettes in experimental animals and their effects on the elastic lamina remain limited. IL-6, IL-15, and FSTL-1 are widely associated with the cardiovascular system, but their involvement as inflammatory or myokine biomarkers related to exercise-induced aortic

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stiffness has not been well investigated (Wang et al., 2023). This experiment will examine the effect of e-cigarette exposure and moderate-intensity aerobic exercise on the expression of IL-6, IL-15, and FSTL-1, as well as aortic stiffness by evaluating elastic lamina fragmentation in Wistar rats.

MATERIALS AND METHODS

STUDY DESIGN

This experimental study was conducted in the animal laboratory of the Faculty of Medicine, Padjadjaran University, Bandung, Indonesia from March 2022 to July 2023. This experimental protocol was approved with an ethical serial number 43/UN6/KEP/EC/2022 by the Ethics Committee of the Faculty of Medicine, Padjadjaran University.

ANIMAL CARE

Male Wistar rats (n = 28), 8 weeks old and weighing 220–250 grams, were obtained from PT Biofarma, Bandung, Indonesia, were randomly divided into four groups: A (control), B (moderate-intensity aerobic exercise), C (e-cigarette exposure), and D (e-cigarette exposure plus moderate-intensity aerobic exercise). Rats were housed in groups at room temperature of ± 22–24°C, under a 12:12 h light-dark cycle, and allowed access to food and water ad libitum. All procedures were carried out according to established guidelines for the care and use of laboratory animals (Riechman et al., 2004).

ANIMAL TREATMENT

E-cigarette liquid contained propylene glycol (40%), vegetable glycerin (60%), flavors, and 6 mg of nicotine. The smoking chamber was used for groups C and D. The vape generator carried 3 ml of e-cigarette liquid. After 15 minutes of pre-heating the smoking chamber, e-cigarette exposure occurred. Rats in the treatment groups were exposed to e-cigarette vapor for 30 minutes, five days a week, for six weeks. Pollutant sensors maintained carbon monoxide at 250–300 ppm and carbon dioxide at 700–1200 ppm. The device was cleaned every two days for maintenance. This exposure regimen was modified from El-Bestawy et al., which found that 4 weeks of exposure had already influenced thoracic aorta (El-Bestawy, Sabry and Abdelallahmed, 2023).

A glass aquarium (50 cm long, 20 cm wide, 30 cm high), e-cigarette generator room, generator room cover, fan, one-way valve, adapter, control box, aerator, ashtray, and pollutant sensor comprised the smoking room. The glass aquarium chamber size determined how many rats were possibly exposed to e-cigarettes. The aquarium glass could hold 6–8 rats. The weight of smoke was calculated as follows:

Room volume = 50 cm × 25 cm × 30 cm = 37,500 cm^3 = 0.0375 m^3 = 37.5 liters.
Smoke weight, based on an air density of 1.2 kg/m^3, was calculated as: 0.0375 m^3 × 1.2 kg/m^3 = 0.045 kg = 45,000 mg. Parts per million (ppm) is defined as mg/liter:
Smoke concentration = 45,000 mg/37.5 liters = 1,200 mg/liter = 1,200 ppm

Ventilation at the top of the 30 cm smoking chamber regulates e-cigarette smoke concentration to prevent hypoxia in the rats. This smoking chamber device was granted Intellectual Property Rights from the Ministry of Law and Human Rights on April 5, 2022, with serial number EC00202222499.

Before the exercise protocol, groups B and D underwent adaptation training. Treadmill running was used for 2 weeks of adaptation. Before the exercise treatment, the groups were caged for one week to standardize their lifestyle and diet. The next week, the rats were trained to run on the treadmill daily at a gradually increasing time. Introductory training lasted 5–15 minutes, with a gradual increase in pace up to 20 meters per minute. During the adaptation period, rats were also exposed to e-cigarette smoke. The control group was exposed to e-cigarette smoke alongside the treatment group.

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At a speed of 20 meters per minute, blood lactate levels increased after 20 minutes of exercise (below lactate threshold), and rose 2.5 times from baseline at 25–30 meters/minute. After the adaptation period, rats underwent moderate-intensity aerobic exercise at 20 meters/minute on a treadmill for 30 minutes, 5 days a week, for 6 weeks.

PROTEIN EXPRESSION ASSESSMENT

After six weeks of exposure to e-cigarettes and moderate-intensity aerobic exercise, the rats were euthanized via isoflurane inhalation followed by cervical dislocation. Aortic tissue dissection was performed to measure the protein expression of IL-6, IL-15, and FSTL-1 using the Western Blot method. The process of protein detection with antibodies began with the membrane which was washed with 0.1% PBST solution (3 × 5 minutes), then incubated with 0.25% BSA blocking solution for 30 minutes, followed by washing with 0.1% PBST (3 × 5 minutes). The primary antibody was diluted in 0.20% BSA blocking solution and incubated overnight at 4°C. The membrane was washed with 0.1% PBST solution (3x5 minutes) again. Next, the membrane was incubated with the secondary antibody in 0.20% BSA for 90 minutes, the membrane was again washed with 0.1% PBST solution 3 × 5 minutes. Detection of the target protein began by incubating the membrane with chemiluminescent substrate for 5 minutes. The membrane was detected using the C-Digit tool for 12 minutes. Western blot results were analyzed with Image-J software.

After six weeks of e-cigarettes and moderate-intensity aerobic exercise, the rats were strangled and sacrificed using isoflurane inhalation. Western blotting was used to quantify IL-6, IL-15, and FSTL-1 protein expression in aortic tissue. A 15% separating gel and 5% stacking gel were prepared by following standard Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE) assay protocol technique. The gel from SDS-PAGE was transferred using blotting tools by making a “sandwich” for 30 minutes/200 milliampere. The transferred membrane was stained with Ponceau solution to verify protein transfer. The membrane was rinsed with 0.1% PBST solution (3 × 5 minutes) before incubating with 0.25% BSA blocking solution for 30 minutes and washing again. BSA 0.20% was used to dissolve primary antibody and incubate overnight at 4°C and then the membrane washed again. After 90 minutes of secondary antibody incubation in 0.20% BSA, the membrane was washed again. The membrane was then treated with chemiluminescence substrate for 5 minutes to identify target protein. Twelve minutes were spent detecting the membrane using C-Digit. Western blot images were analyzed using ImageJ software.

HISTOPATHOLOGY ASSESSMENT

Histopathological evaluation of the descending thoracic aorta was performed using an Olympus CX31 microscope equipped with a 5-megapixel IndoMicro HDMI digital camera directly connected to a computer. Two anatomical pathology specialists independently assessed the specimens in a blinded manner, without knowledge of the treatment group assignments. Each assessor recorded their findings in a standardized assessment table.

Elastic lamina fragmentation was quantified by counting the number of fractures in four different quadrants at 40× magnification using Elastic-Van Gieson (EVG) staining. The results from both pathologists were compared for consistency. If the assessments were found to be significantly different, further review was conducted; otherwise, the average of both readings was used for subsequent statistical analysis.

STATISTICAL ANALYSIS

The data were statistically analyzed using IBM SPSS Statistics version 26, with a 95% confidence level ($p < 0.05$). Independent t-tests or Mann–Whitney U tests were used to compare two treatment groups. Pearson or Spearman correlation tests were performed to assess relationships between variables. Simple linear regression and coefficient of determination analyses were conducted to evaluate the effect of elastic lamina fragmentation on IL-6, IL-15, and FSTL-1 protein expression.

RESULTS

PROTEIN EXPRESSION OF IL-6, IL-15 AND FSTL-1

The expression levels of IL-6, IL-15, and FSTL-1 for all groups are presented by Figure 1. The highest IL-6 and IL-15 level were observed in group C, followed by group D, B, and A. The highest FSTL-1 was found in group D, followed by group B, C and A. IL-6 and IL-15 expression levels were significantly higher in group B and C compared to group A. Meanwhile, there is no significant difference for FSTL-1 expression between group B and C compared to group A. Western blot results for IL-6, IL-15 and FSTL-1 can be found in Figure 2.

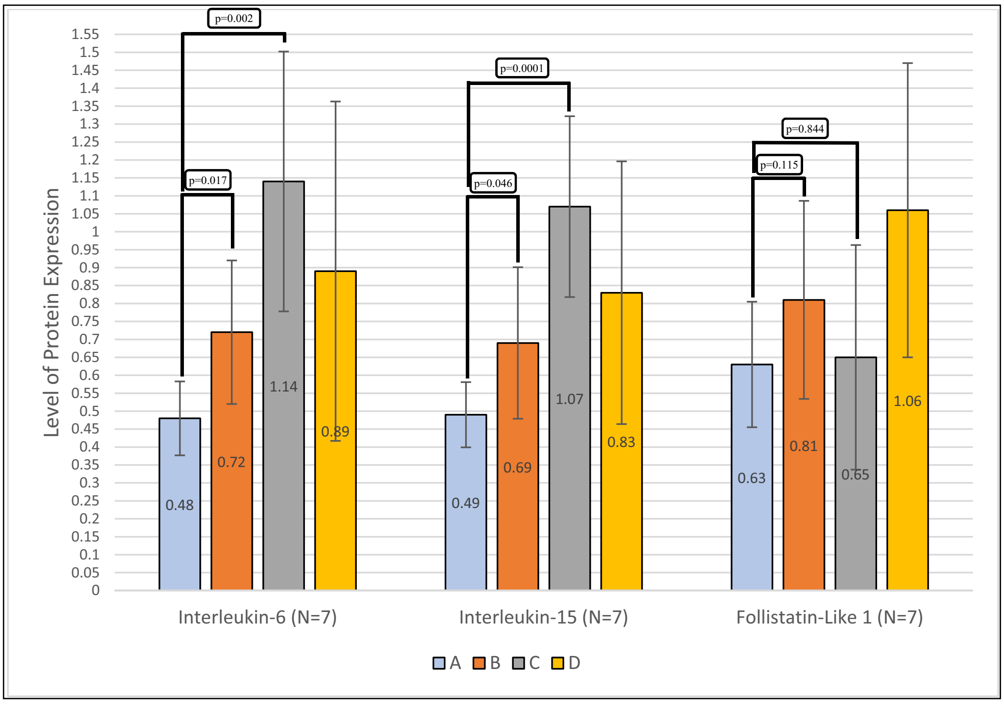

Figure 1 IL-6, IL-15 and FSTL-1 Expression for All Groups with Difference between group A VS B, and A vs C. A: control group; B: moderate intensity aerobic exercise; C: e-cigarette exposure; D: e-cigarettes and moderate intensity aerobic exercise.

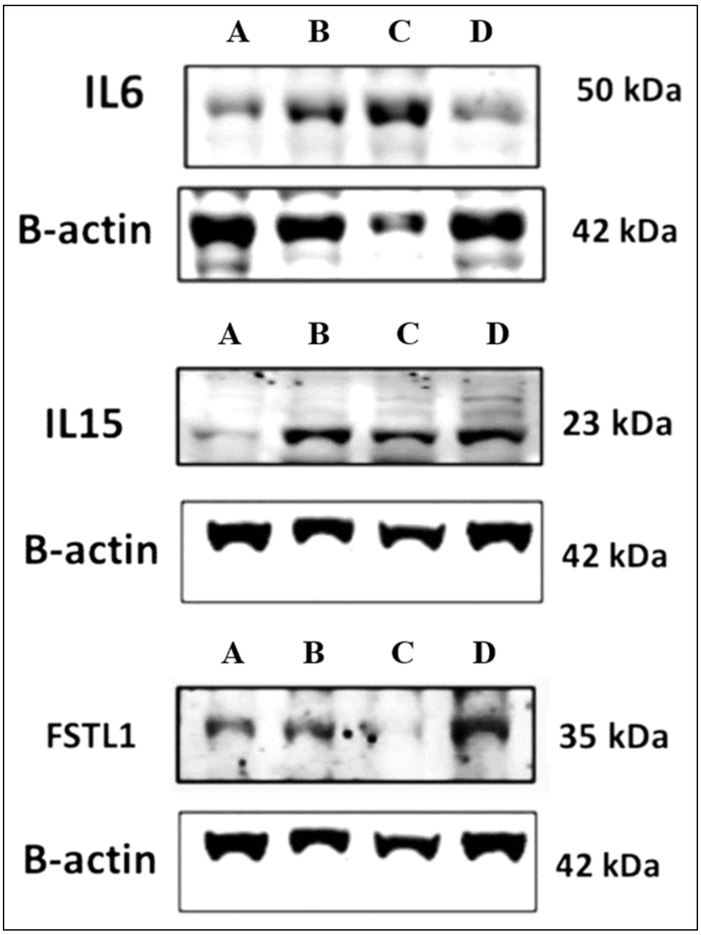

Figure 2 IL-6, IL-15 and FSTL-1 western blot results.

A: control group; B: moderate intensity aerobic exercise;
C: e-cigarette exposure;
D: e-cigarettes and moderate intensity aerobic exercise.

ELASTIC LAMINA FRAGMENTATION

Elastic lamina fragmentation results for all groups are presented in Table 1.

Table 1 Elastic Lamina Fragmentation for All Groups with Difference between group A VS C, and C VS D.

GROUP	N	ELASTIC LAMINA FRAGMENTATION	P VALUE	
		MEAN ± SD	A VS C	C VS D
A	7	4.71 ± 2.69	0.003	0.008
B	7	4.86 ± 2.41		
C	7	11.00 ± 3.46		
D	7	5.86 ± 2.54		

A: control group; B: moderate intensity aerobic exercise; C: e-cigarette exposure; D: e-cigarettes and moderate intensity aerobic exercise.

Figure 3 shows elastic lamina fragmentation with Elastic Van Gieson (EVG) staining. The highest degree of fragmentation was observed in group C, followed by D, B, and A. Elastic lamina fragmentation was significantly increased in group C compared to group A, and in group C compared to group D.

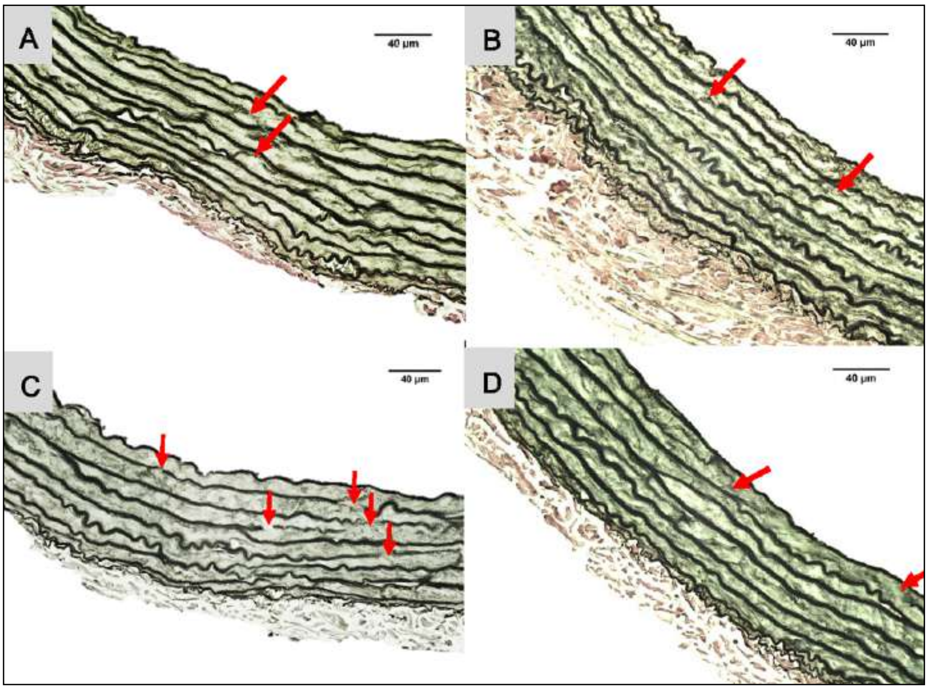

Figure 3 Elastic lamina fragmentation with Elastic-Van Gieson (EVG) staining (red arrow). **(A)** Control group, **(B)** Moderate intensity aerobic exercise group, **(C)** E-cigarettes group showed increased elastic lamina fragmentation **(D)** E-cigarettes and moderate intensity aerobic exercise group shows lower elastic lamina fragmentation than group C (red arrow).

There is a strong correlation between elastic lamina fragmentation and IL-6 and IL-15 expression in groups A and C (Table 2).

Table 2 Correlation between Elastic Lamina Fragmentation and IL-6, IL-15, FSTL-1 Expression on Control Group (A) and E-cigarette Exposure Group (C).

VARIABLE	IL-6		IL-15		FSTL-1	
	r	P VALUE	r	P VALUE	r	P VALUE
Elastic Lamina Fragmentation	0.772	0.001*	0.688	0.009**	0.059	0.843**

**Spearman's rho test*. ***Pearson test*. r = coefficient of correlation.

However, no significant correlation was found with FSTL-1. Additionally, no significant correlation was observed between elastic lamina fragmentation and IL-6 or IL-15 in groups C and D; however, a significant correlation was found with FSTL-1. Linear regression and coefficient of determination analyses showed that IL-6, IL-15, and FSTL-1 each had a strong correlation with elastic lamina fragmentation, accounting for 35.7%, 44.6%, and 31.8% of the variance, respectively (Table 3).

Table 3 Correlation between Elastic Lamina Fragmentation and IL-6, IL-15, FSTL-1 Protein Expression on E-Cigarette Group (C) and E-cigarette with Moderate-Intensity Aerobic Exercise Group (D).

VARIABLE	IL-6		IL-15		FSTL-1	
	r	P VALUE	r	P VALUE	r	P VALUE
Elastic Lamina Fragmentation	–0.015	0.958	0.125	0.670	–0.564	0.036

r: Coefficient of correlation.

Linear regression and coefficient of determination tests showed that each IL-6, IL-15, and FSTL-1 had a strong correlation and have accounting for 35.7%, 44.6%, and 31.8% on elastic lamina fragmentation (Tables 4–6).

Table 4 Simple Linear Regression with Coefficient of Determination Result for Evaluating the Effect of IL-6 Expression on Elastic Lamina Fragmentation on Control Group (A) and E-cigarette Exposure Group (C).

β: linear regression test value, r: Coefficient of correlation, r^2: coefficient of determination, SE: Standard of error.

VARIABLE	IL-6 PROTEIN EXPRESSION						
	β	SE	P VALUE	r	r^2	ADJUSTED r^2	SE
Constant	2.827	2.183	0.220	0.598	0.357	0.304	3.68637
IL-6	6.192	2.398	0.024				

Table 5 Simple Linear Regression with Coefficient of Determination Result for Evaluating the Effect of IL-15 Expression on Elastic Lamina Fragmentation on Control Group (A) and E-cigarette Exposure Group (C).

β: linear regression test value, r: Coefficient of correlation, r^2: coefficient of determination, SE: Standard of error.

VARIABLE	IL-15 PROTEIN EXPRESSION						
	β	SE	P VALUE	r	r^2	ADJUSTED r^2	SE
Constant	1.391	2.273	0.552	0.668	0.446	0.400	3.42296
IL-15	8.288	2.667	0.009				

Table 6 Simple Linear Regression with Coefficient of Determination Result for Evaluating the Effect of FSTL-1 Expression on Elastic Lamina Fragmentation on Control Group (A) and E-cigarette Exposure Group (C).

β: linear regression test value, r: Coefficient of correlation, r^2: coefficient of determination, SE: Standard of error.

VARIABLE	FSTL-1 PROTEIN EXPRESSION						
	β	SE	P VALUE	r	r^2	ADJUSTED r^2	SE
Constant	13.098	2.172	0.000	0.564	0.318	0.262	3.39936
FSTL-1	–5.443	2.299	0.036				

DISCUSSION

E-cigarette exposure increased aortic IL-6 expression compared to the control group. Our result is consistent with previous studies supporting IL-6 as a proinflammatory cytokine (Singh et al., 2019; Gellatly et al., 2020). IL-15 expression also increased with e-cigarette exposure, which is in line with previous studies identifying this cytokine as proinflammatory (Muthumalage et al., 2019; Ali et al., 2022). This study may serve as a reference regarding e-cigarette-induced IL-6 and particularly IL-15 expression, given the limited research on IL-15 compared to other myokines.

FSTL-1 did not increase with e-cigarette exposure in our study, which is supported by Kim et al. who suggested that the degree of organ dysfunction determines the level of FSTL-1 expression (Kim et al., 2023). Kon et al. also showed that FSTL-1 expression is tissue-specific (Kon, Tanimura and Yoshizato, 2022). Thus, our findings could serve as a reference for future experiments, as the elevation of IL-6 and IL-15 was not accompanied by FSTL-1, likely due to its regulation by the degree of dysfunction and tissue specificity.

The increase in IL-6 and IL-15 after moderate-intensity aerobic exercise also supports their role not only as pro-inflammatory cytokines but also as myokines secreted by skeletal muscle in this experiment. This finding is also consistent with previous studies that evaluated expression levels in serum samples (Steensberg et al., 2001; Riechman et al., 2004; Keller et al., 2005). Our finding, based on aortic expression, could serve as a new reference for further research.

Our experiment showed no increase in FSTL-1 after moderate-intensity aerobic exercise. This could be explained by previous studies that found differential expression across various tissue types (Inoue et al., 2022). Inoue et al. showed that while eight weeks of exercise increased

FSTL-1 expression in serum and soleus muscle, this was not observed in the tibialis anterior muscle and myocardium (Inoue et al., 2022). Thus, mapping FSTL-1 expression across different tissue types is necessary, and our finding from aortic tissue could contribute as a reference for future studies.

Elastic lamina fragmentation was significantly increased after e-cigarette exposure. This effect was previously evaluated only in the context of nicotine exposure to the aorta or based on PWV measurements (Wagenhäuser et al., 2018; Iannarelli et al., 2021). Our study demonstrated a direct effect of e-cigarette exposure on aortic histopathology. Meanwhile, the group exposed to both e-cigarettes and exercise had lower elastic lamina fragmentation, demonstrating the protective effect of exercise against e-cigarette-induced damage. This had previously been assessed only through PWV measurements (Park, Miyachi and Tanaka, 2014). Furthermore, our study supports the role of IL-6 and IL-15 in elastic lamina fragmentation induced by e-cigarette exposure. These cytokines, upregulated by smoking, influence extracellular matrix composition, cause endothelial dysfunction, promote smooth muscle cell proliferation, and ultimately degrade the elastic lamina (Garcia-Arcos et al., 2016; Cai and Wang, 2017). FSTL-1 showed no association with elastic lamina fragmentation in our study, possibly because its expression is regulated by the degree of dysfunction, as suggested by Li et al (Li et al., 2016). Thus, this result may serve as a reference for future experiments exploring the role of tissue damage in regulating FSTL-1 expression.

Our experiment showed no difference in IL-6 and IL-15 expression between the e-cigarette only group and the e-cigarette plus exercise group. This may be because these cytokines can function both as pro-inflammatory agents due to e-cigarette exposure and as anti-inflammatory agents during exercise. In other words, the anti-inflammatory effects of exercise may protect the aortic elastic lamina from the pro-inflammatory effects of e-cigarette exposure. Thus, the protective effect of exercise—evidenced by lower elastic lamina fragmentation—may be mediated by other factors, possibly including FSTL-1. Ogura et al. previously described the role of FSTL-1 in preventing apoptosis in ischemic myocardium (Ogura et al., 2012). In our study, exercise-induced FSTL-1 expression appeared to mitigate the destructive effects of e-cigarette exposure.

The findings of this study provide compelling evidence concerning the impact of electronic cigarettes and moderate-intensity aerobic exercise on the expression of IL-6, IL-15, and FSTL-1, as well as the fragmentation of the elastic lamina in the descending thoracic aorta of Wistar rats. Exposure to electronic cigarettes within a controlled smoking chamber was observed to significantly elevate the expression of IL-6 and IL-15 proteins within the aortic vascular tissue. Notably, this upregulation in cytokine expression coincided with the occurrence of aortic elastic lamina fragmentation, revealing a robust positive correlation between structural deterioration and the heightened presence of IL-6 and IL-15 proteins.

Furthermore, this study unveiled a pivotal element in the intricate interplay between moderate-intensity aerobic exercise and the preservation of aortic elastic lamina integrity in the context of electronic cigarette exposure. Notably, moderate-intensity aerobic exercise was found to enhance the expression of IL-6 and IL-15 proteins within the aortic vascular tissue, with these myokines exhibiting an anti-inflammatory effect during exercise. Moreover, in conjunction with the contribution of FSTL-1 induced by exercise, these myokines demonstrated a mitigating effect on aortic elastic lamina fragmentation resulting from electronic cigarette exposure.

Western blot results showed a marked increase in FSTL-1 band intensity in the exercise group, indicating upregulation of this myokine at the protein level. This supports its proposed role as a vascular-protective factor, potentially counteracting the harmful effects of e-cigarette-induced inflammation and fragmentation. The absence of a corresponding increase in FSTL-1 in the e-cigarette-only group, despite elevated IL-6 and IL-15, further suggests that FSTL-1 induction is exercise-dependent and may mediate part of the exercise-induced protective effect.

Importantly, this investigation provides valuable insights into the pathophysiology of elastic lamina fragmentation and the influence of exercise on this process. These insights may not be readily attainable through cell culture analyses or human experimentation. Thus, this research serves as a noteworthy addition to the scientific literature, offering a visual representation of these intricate phenomena for reference and further exploration.

While the sample size per group was relatively small, it was sufficient to reveal statistically significant differences in IL-6, IL-15, FSTL-1 expression, and elastic lamina fragmentation. The consistency of these findings, in conjunction with correlation and regression analyses, suggests adequate power for detecting meaningful biological changes. Moreover, ethical guidelines in animal research encourage minimal use of animals when valid results can be obtained (Naderi et al., 2012).

There are several limitations in this study. Oxidative stress levels were not measured; thus, their extent in each treatment group remains unknown. Nitric oxide levels, an indicator of endothelial dysfunction, were also not assessed. Additionally, IL-6, IL-15, and FSTL-1 levels were not measured in blood or skeletal muscle for comparative analysis.

CONCLUSIONS

E-cigarette exposure increased aortic elastic lamina fragmentation in Wistar rats, accompanied by elevated IL-6 and IL-15 expression. In contrast, moderate-intensity aerobic exercise reduced elastic lamina fragmentation and was associated with increased FSTL-1 expression. This suggests that FSTL-1 may play a compensatory or protective role in mitigating vascular injury, particularly in the context of exercise. These findings highlight the dual role of cytokines and myokines in vascular remodeling and the potential therapeutic relevance of exercise-induced FSTL-1 expression.

DATA ACCESSIBILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

DECLARATION OF ARTIFICIAL INTELLIGENCE USE

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

ETHICS AND CONSENT

Ethics Review Approval: All procedures were reviewed and preapproved by the Ethics Committee of Faculty of Medicine Padjadjaran University, identification number: 43/UN6/KEP/EC/2022, approval date: January 14th, 2022.

Adherence to the 3Rs Principle: The research followed the “Replacement, Reduction, and Refinement” principles to minimize harm to animals.

Animal Welfare: This article provides details on the housing conditions, care, and pain management for the animals, ensuring that the impact on the animals is minimized during the experiment.

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COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

The authors confirm contribution to the paper as follows: study conception and design: Damay VA, Setiawan S, Lesmana R, Akbar MR, Lukito AA; data collection: Damay VA, Setiawan S, Lesmana R, Akbar MR, Lukito AA, Tarawan VM, Martha JW, Nugroho J; analysis and interpretation of results: Damay VA, Setiawan S, Lesmana R, Akbar MR, Lukito AA, Tarawan VM, Martha JW, Nugroho J, Sugiharto S; draft manuscript preparation: Damay VA, Setiawan S, Lesmana R, Akbar MR, Lukito AA. All authors reviewed the results and approved the final version of the manuscript.

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