

ELEVATED INTERLEUKIN-6 AND COTININE LEVELS FOLLOWING ELECTRONIC CIGARETTE EXPOSURE: STUDY IN VIVO

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ABSTRACT

Background: Periodontitis is a chronic inflammatory disease affecting the supporting tissues of the teeth. Animal models are commonly used to study its pathogenesis and the effects of various exposures. E-cigarette vapor contains nicotine and other harmful substances that may induce inflammation. Cotinine and interleukin-6 (IL-6) serve as biomarkers of nicotine exposure and inflammatory response. **Purpose:** This study aimed to evaluate cotinine and IL-6 levels in Wistar rats exposed to e-cigarette vapor and to assess the relationship between nicotine exposure and systemic inflammation in periodontitis. **Methods:** Twelve Wistar rats were divided into treatment and control groups. The treatment group was exposed to e-cigarette vapor for 35 days (30 puffs/day), while the control group received no exposure. Cotinine and IL-6 levels were measured using Enzyme-Linked Immunosorbent Assay (ELISA). Independent t-tests and Pearson correlation analyses were performed. **Results :** The treatment group exposed to e-cigarette vapor showed higher IL-6 and cotinine levels compared to the control group. The mean for IL-6 was 27,5 pg/mL (treatment group) and 13,8 pg/mL (control group), while for cotinine it was 227,6 ng/mL (treatment group) and 175,4 ng/mL (control group). A positive correlation was observed ($r = 0.515$; $p = 0.086$), although this did not reach statistical significance. **Conclusion:** E-cigarette vapor exposure increased cotinine and IL-6 levels in Wistar rats, indicating enhanced systemic inflammation. However, these findings should be interpreted with caution due to the small sample size and animal model. Further studies involving larger samples and human subjects are needed to confirm these results and determine their clinical relevance.

Keywords: Cotinine, Electronic_Cigarette, Interleukin-6, Periodontitis

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INTRODUCTION

Periodontitis is a chronic inflammatory disease that affects the supporting structures of the teeth, including the alveolar bone, cementum, gingiva, and periodontal ligament. It is characterized by tissue destruction, bone loss, and loss of tooth attachment¹ While local factors such as plaque and poor oral hygiene play a major role, systemic and environmental factors also contribute to disease progression².

In recent years, the use of electronic cigarettes (e-cigarettes) has increased, especially among adolescents and young adults. National survey data in Indonesia (Risksdas, 2018) reported prevalence rates of 10.6% among individuals aged 10–14, 10.5% among those aged 15–19, and 7% among those aged 20–24.³ Although often considered safer than conventional cigarettes, e-cigarettes still contain nicotine and other harmful substances that can induce oxidative stress and trigger inflammatory responses^{6–8}.

Nicotine is metabolized into cotinine, a stable biomarker widely used to measure systemic nicotine exposure.⁴ Elevated cotinine levels are associated with increased production of interleukin-6 (IL-6), a pro-inflammatory cytokine that plays an important role in tissue destruction and bone resorption in periodontitis.^{5,7,9} While the rationale for investigating IL-6 and cotinine as biomarkers is well established, there remains a critical gap in understanding how e-cigarette exposure contributes to systemic inflammation and periodontal disease progression.

Most existing evidence is derived from human observational studies, which are limited by confounding factors and the inability to control exposure precisely. Therefore, a controlled experimental model is needed to better understand the underlying biological mechanisms. Animal models provide a suitable approach, as they allow standardized exposure conditions and more accurate measurement of biological responses.

Based on this gap, the present study used Wistar rats to evaluate the effects of e-cigarette vapor exposure on cotinine and IL-6 levels. This approach aims to clarify the mechanistic link between nicotine exposure and systemic inflammation relevant to periodontitis under controlled experimental conditions.

MATERIALS AND METHODS

This study used twelve healthy male Wistar rats without anatomical defects. Animals underwent a one-week acclimatization period followed by five weeks of treatment. The treatment group was exposed to e-cigarette vapor using a commercially available device (VSP Arroz Pocket Rockid) equipped with a cartridge containing e-liquid with a nicotine concentration of 9 mg/mL. Exposure was standardized at 30 puffs per day for 35 consecutive days, while the control group received no exposure. Following the ethically required euthanasia of all animals on day 35, the Enzyme-Linked Immunosorbent Assay (ELISA) method was used to detect the levels of cotinine and Interleukin-6 (IL-6), which are inflammatory indicators. The ELISA procedures for measuring cotinine and IL-6 were performed using commercially available kits according to the manufacturers' instructions. To ensure assay validity, calibration curves were generated for each run using known standards, and only assays with acceptable linearity ($R^2 \geq 0.99$) were included. Internal quality controls at low and high concentrations were also measured to confirm accuracy and consistency. Data were first tested for normality and homogeneity. If the data were normally distributed and homogeneous, an independent t-test was used to determine whether there were differences in cotinine and IL-6 levels between the control and treatment groups. Additionally, Pearson correlation analysis was performed to examine the relationship between cotinine and IL-6 levels and to assess whether a positive association existed between the two variables.

RESULTS

The normality of the data was tested using the Kolmogorov–Smirnov test with Monte Carlo significance values. The results showed that IL-6 ($p = 0.405$) and cotinine ($p = 0.204$) were normally distributed ($p > 0.05$). These findings indicate that the data met the assumptions required for parametric testing. Higher inter-test subject variability in cotinine levels is indicated by this.

Table 1. The Results of the Normality Test

Variable	Sig. (2-tailed)	Information
IL 6	0.405	> 0.05
Cotinine	0.204	> 0.05

Following the confirmation of normality, group comparisons were conducted using independent t-tests. IL-6 levels were significantly higher in the treatment group compared to the control group, with $p < 0.001$. Cotinine levels showed not statistically significant difference between groups, with $p = 0.073$.

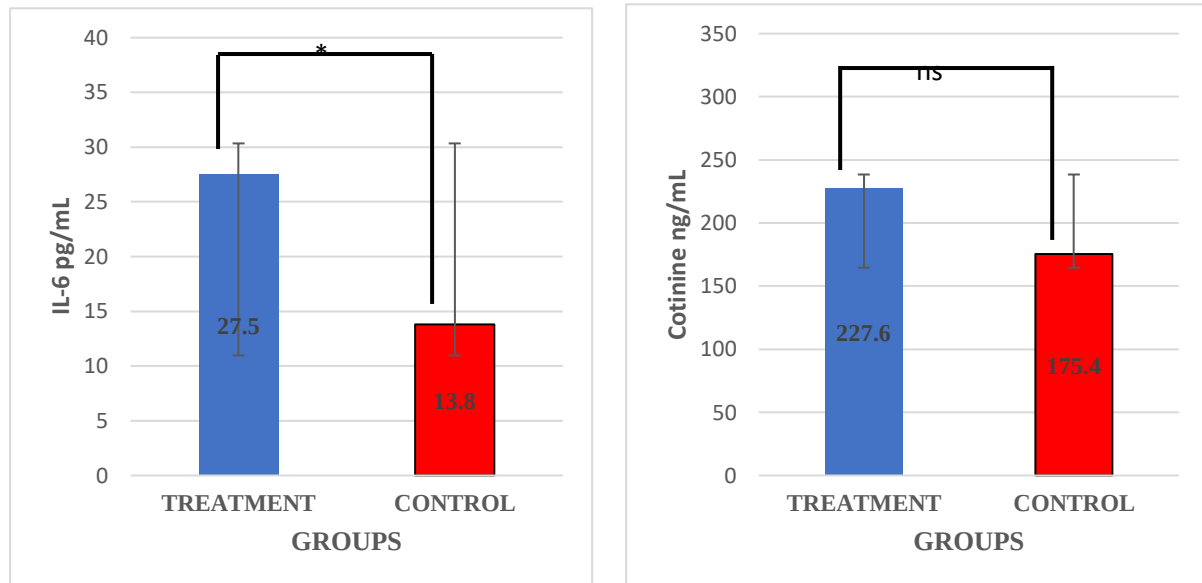
Table 2. The Results of Independent T-Tests

Variable	Groups	Mean $\pm$ SD	Sig. (2-tailed)	Information
IL 6	Treatment	27,5 $\pm$ 4,01	0.000	< 0.05
	Control	13,8 $\pm$ 3,03		
Cotinine	Treatment	227,6 $\pm$ 51,35	0.073	> 0.05
	Control	175,42 $\pm$ 37,69		

According to Pearson correlation analysis, cotinine and IL-6 levels were positively correlated ($r = 0.515$ and $p = 0.086$). While the correlation value shows a potential for elevated cotinine levels to influence the rise in IL-6, the p-value is more than 0.05. Bar charts in Figure 1 illustrate the mean levels of interleukin-6 (IL-6) (pg/mL) and cotinine (ng/mL) in control and treatment groups. The treatment group exposed to e-cigarette vapor showed higher IL-6 and cotinine levels compared to the control group. The mean for IL-6 was 27,5 pg/mL (treatment group) and 13,8 pg/mL (control group), while for cotinine it was 227,6 ng/mL (treatment group) and 175,4 ng/mL (control group). Error bars represent standard deviation (SD). Asterisks (*) indicate statistically significant differences between groups ($p < 0.05$).

Table 3. The Results of the Pearson Correlation Test

Variable	Korelasi Pearson (r)	Sig. (2-tailed)	Information
IL 6 and Cotinine	0.515	0.086	> 0.05



Note: * indicates a significant difference and ns indicates not statistically significant

Figure 1. Comparison of IL-6 and Cotinine Levels Between Control and Treatment Groups.

DISCUSSION

The use of electronic cigarettes (e-cigarettes) has increased significantly in recent years, particularly among adolescents and young adults. Although often promoted as a safer alternative to conventional cigarettes, accumulating evidence indicates that e-cigarette aerosols contain harmful substances, including nicotine, formaldehyde, heavy metals, and fine particulate matter. These compounds can increase mucosal permeability, induce oxidative stress, and activate inflammatory signaling pathways, thereby contributing to systemic inflammatory responses.^{6-7,10}

In the oral environment, repeated exposure to e-cigarette vapor may disrupt the balance of the oral microbiota and promote immune activation. This process involves the activation of macrophages, dendritic cells, and epithelial cells, which subsequently release pro-inflammatory cytokines. Among these mediators, interleukin-6 (IL-6) plays a central role due to its pleiotropic effects in inflammation and immune regulation. IL-6 contributes to osteoclast differentiation, B-cell activation, and acute-phase protein production, all of which are implicated in periodontal tissue destruction.^{11,12,13}

The present study demonstrated that cotinine levels were higher in the treatment group compared to the control group; however, this difference did not reach statistical significance ($p = 0.073$). Despite the lack of statistical significance, the observed increase suggests that systemic absorption of nicotine likely occurred following exposure to e-cigarette vapor. Cotinine, as the primary metabolite of nicotine, is widely recognized as a reliable biomarker for assessing nicotine exposure due to its longer half-life and greater stability in biological fluids.¹

In addition, IL-6 levels were significantly increased in the treatment group compared to controls ($p < 0.001$), suggesting the presence of a systemic inflammatory response following exposure. This finding supports the hypothesis that e-cigarette vapor can stimulate inflammatory pathways. However, it is important to emphasize that IL-6 is a non-specific inflammatory marker, and its elevation does not directly indicate periodontal tissue destruction in the absence of clinical or histological periodontal assessment.^{11,12,13}

Despite the significant increase in both cotinine and IL-6 levels, Pearson correlation analysis revealed a moderate positive correlation ($r = 0.515$) that did not reach statistical significance ($p = 0.086$). This result indicates that although there is a trend suggesting a potential association between nicotine exposure and inflammatory response, the relationship cannot be considered statistically significant. Therefore, caution is required in interpreting this finding, and it should not be overgeneralized as evidence of a causal relationship.

From a biological perspective, the association between nicotine exposure and increased IL-6 production is plausible. Nicotine interacts with nicotinic acetylcholine receptors (nAChRs) on immune cells, potentially modulating cytokine production and inflammatory responses. Furthermore, oxidative stress induced by e-cigarette

aerosols may amplify inflammatory signaling pathways. However, in the present study, these mechanisms were not directly investigated, and thus remain theoretical.¹⁴

The findings of this study are consistent with previous reports demonstrating increased IL-6 expression following e-cigarette exposure. Wu et al. reported elevated IL-6 levels in airway epithelial cells after exposure to e-liquid, while Charde et al. observed increased IL-6 and alterations in oral microbiota among e-cigarette users. Goldenberg demonstrated that nicotine exposure enhances inflammatory mediators in lung tissue, and Hasan reported an association between elevated salivary cotinine levels and poor gingival status.⁶⁻⁷

However, not all studies report consistent results. Some investigations have shown minimal or non-significant changes in inflammatory biomarkers following e-cigarette exposure. These discrepancies may be attributed to differences in nicotine concentration, duration of exposure, experimental design, and biological models used. Such variability highlights the complexity of inflammatory responses and underscores the importance of cautious interpretation and further research.

Importantly, this study did not assess direct periodontal parameters such as alveolar bone loss, probing depth, or clinical attachment loss. Therefore, it cannot be concluded that e-cigarette exposure contributes to the progression of periodontitis. The findings are limited to demonstrating increased systemic inflammation and nicotine exposure in an animal model.¹¹⁻¹²

Several limitations should be acknowledged. The relatively small sample size (n = 12) may limit statistical power, particularly in detecting correlations. The use of an animal model restricts direct extrapolation to human populations. Additionally, the absence of direct periodontal measurements limits the clinical relevance of the findings. From a methodological standpoint, although ELISA was used to measure cotinine and IL-6 levels, detailed information regarding assay validation and replication reliability was limited.

Future studies should include larger sample sizes, incorporate human subjects, and perform direct periodontal assessments to enhance clinical relevance. In addition, molecular analyses are needed to clarify the underlying biological mechanisms. Standardization of exposure protocols and improved reporting of laboratory validation procedures will further strengthen the quality and reproducibility of future research.

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