# Suppression of Gingival Inflammation in Smokers, but not in Vapers during an Experimental Gingivitis: A Pilot Study

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**Abstract**

Smokers display less gingival inflammation and gingival bleeding than non-smokers due to the altered inflammatory response. Vape or e-cigarette has been said to be less harmful than combustible cigarette, but no information regarding the gingival inflammation in vapers.

This pilot study aimed to evaluate gingival response in vapers, smokers and non-smokers. We recruited fifteen participants consisted of non-smokers (n=5), smokers (n=5), and vapers (n=5). Participants were instructed not to clean teeth in lower jaw throughout the duration of the experimental gingivitis phase (21 day).

The primary outcome measures of gingival inflammation were Gingival Index (GI) and Angulated Bleeding Index (AngBI) during experimental gingivitis period. Plaque Index (PII) and salivary cotinine levels were also determined. Despite the similar amount of bacterial plaque accumulation in 3 study groups, smokers showed reduced inflammation and bleeding while in vapers with nicotine vapour and non-smokers there were obvious increases of clinical features of inflammation as gingival response to bacterial challenge, suggesting that the use of e-cigarettes with nicotine vapour did not mask the clinical features of inflammation.

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## Introduction

Periodontal inflammation is a response to accumulations of bacterial plaque in the gingival sulcus and manifesting in periodontium as gingivitis and periodontitis.[1] Clinical appearance of gingivitis is redness, swelling or bleeding on gentle provocation of the gingival sulcus, whereas periodontitis is characterized by increased probing depth, loss of attachment and alveolar bone destruction.[2] Several factors affect the disease progression, but smoking is considered to be an important independent risk factors for the onset and severity of periodontal disease, since the risk to develop destructive periodontal disease is 5- to 20-fold increase in smokers compared to non-smokers.[3]

Moreover, smokers appear to be liable to suffer from severe periodontal disease, even after adjusting for oral hygiene levels.[4] Nevertheless, smokers demonstrated less gingival inflammation and less gingival bleeding as a result of disturbed inflammatory response.[5] An altered gingival inflammatory response to supragingival plaque in smokers had been demonstrated by several authors.[6-9] Clinically, smokers presented reduced signs of inflammation compared to nonsmokers.[10] Meenawat et al (2015) suggested that this can be due to the temporary gingival vasoconstriction and decreased vascular density induced by nicotine.[11] The clinical appearance of reduced signs of inflammation in smokers suggests a suppressed inflammatory response.[12-14] Different gingival response in smokers during inflammation, particularly reduced gingival bleeding on probing, may hide the clinical markers often used by dentists to monitor periodontal health, thus make it difficult to define a good clinical diagnosis and prompt treatment.

Gingivitis is defined as “an inflammatory lesion resulting from interactions between the dental plaque biofilm and the host's immune-inflammatory response, which remains

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contained within the gingiva and does not extend to the periodontal attachment (cementum, periodontal ligament and alveolar bone). Such inflammation remains confined to the gingiva and does not extend beyond the mucogingival junction and is reversible by reducing levels of dental plaque at and apical to the gingival margin".[15] Despite similar levels of dental plaque in smokers and nonsmokers during experimental gingivitis, it was demonstrated that smokers presented less gingival inflammation.[16-18] In a classic experimental gingivitis model proposed by Loe et al (1965) gingivitis was induced by abstaining from any oral hygiene measures for a period of 21 days.[19] More recently, there is a modified method of experimental gingivitis using an intra-oral stent covering the selected area from cleaning during toothbrushing. This modified version has been shown to be feasible to use in examining the gingival response and host–bacteria interactions in nonsmokers and current smokers during experimental gingivitis.[20]

The vast majority of smokers are unwilling to ask for help from formal treatment for smoking cessation and the most of them attempts to quit without assistance, despite the perception that stopping smoking will have favorable impacts on systemic as well as oral health and teeth appearance.[21] Eighty percent of smokers live in low and middle income countries and 46% live in just three countries – China, India and Indonesia.[22,23] Consequently, the need for novel and more efficient approaches is required. Options for smokers changed with the arrival of the modern electronic cigarette which was patented in 2004. E-cigarettes are thought to be less harmful compared to traditional or combustible cigarette products.[24] E-cigarette as an alternative tobacco product provide nicotine for inhalation in a vapour or aerosol form generated by heating, not combustion, of solution called as e-liquid or e-juice containing water, nicotine, propyl-ene glycol and vegetable glycerine.[25] Despite the large number of manufacturers and users, their safety and use as a substitute for tobacco smoking have been surrounded by medical and public controversy. However, a recent report by Public Health England concluded that e-cigarettes are likely to be much safer than smoking.[26] In addition, a systematic review provided evidence that alternative tobacco and nicotine products have effectiveness in smoking reduction and cessation, emphasizing their role in the tobacco harm reduction approach.[27]

The effect of e-cigarette use on the gingival inflammation in experimental gingivitis has not yet been investigated. A pilot study by Wadia et al (2016) examined the effects of vaping with nicotine vapour, on the gingiva and inflammatory biomarkers.[28] The author found significant increase in gingival inflammation when smokers switch from smoking to e-cigarette use, opposing the previous studies suggesting that nicotine induces gingival vasoconstriction leading to reduction in bleeding.[29]

To the best of the authors' knowledge, experimental gingivitis has not been studied yet in e-cigarette users or vapers. In the present study, we sought to identify how vapers response to experimental gingivitis assessed from gingival inflammation and bleeding upon probing.

## Materials and methods

This pilot study was designed to analyze gingival response following experimental gingivitis model proposed by Löe et al (1965).[19] The study protocol was submitted to and approved by the Ethical Committee of Medical Faculty Universitas Padjadjaran, Bandung, Indonesia (896/UN6.KEP/EC/2020). The protocol was registered at UMIN clinical trial registry (UMIN000044214). All subjects gave their informed consents.

In order to be enrolled, subjects had to meet the following inclusion criteria:

(1) 18 to 45 years of age,
(2) for the smokers' group, use of a minimum of 10 cigarettes per day for at least 2 years,
(3) for the vapers' group, use of minimum 5 ml nicotine-containing e-liquid per day for at least 2 years,
(4) for non-smokers' group, no previous history of smoking nor vaping,
(5) a minimum of 10 teeth in the lower jaw,
(6) no pocket probing depth ≥ 4 mm,
(7) willingness to comply with all study requirements and signing informed consent.

The exclusion criteria were :

(1) Dual user of both combustible tobacco product and vape,
(2) suffering from systemic diseases,
(3) being pregnant or giving breastfeeding,
(4) having a history of drug abuse,

(5) using nonsteroidal or steroidal anti-inflammatory drugs, contraceptives, analgesic or antibiotics within 6 weeks before the study,

(6) having untreated tooth decay, crowns or orthodontic appliances in the lower jaw.

Fifteen subjects were recruited including 5 non-smokers, 5 smokers and 5 vapers in Periodontology Clinic, Dental Hospital, Universitas Padjadjaran, Bandung, Indonesia. Before baseline, participants received detailed oral hygiene instructions, thorough scaling and dental prophylaxis at initial visit. Acrylic stents were custom-made to cover an area ranging from the lower central incisor to the second molar (Figure 1). Experimental gingivitis phase started at day- 0. All of the subjects were asked to refrain from any oral hygiene measures of all teeth in the lower jaw for a period of 21 days, while continuing cleaning of the teeth in the upper jaw as usual. Subjects were instructed to always cover the teeth in the lower jaw when brushing throughout the du-ration of the experimental gingivitis phase (21 days), in order to avoid brushing of teeth in the lower jaw.

Plaque accumulation was assessed by Plaque Index (PII), gingival inflammation was scored by Gingival Index (GI ) (Löe 1967) and the bleeding component of the GI was assessed by Angulated Bleeding Index (Van der Weijden et al, 1994) recorded at the start of experimental gingivitis (D0) as well as on day-14 (D14), and day-21 (D21) of the experimental gingivitis period.[30,31] A controlled lateral force of 0.25 N was applied with a Hawe click-probe (Kerr Dental, Orange, CA, USA) held at an angle of approximately 60º to the long axis of the tooth and in contact with the sulcular soft tissue, stretching the gingiva. One calibrated examiner performed all measurements under the same conditions (JG). Reproducibility of scores was assessed in 10 volunteers where examinations were repeated after 8 h, showed an intraclass correlation coefficient of 0.91 and 0.85 for plaque and gingivitis measurements, respectively. Clinical parameters were assessed at the mesial, mid, and distal surfaces from the buccal and lingual aspect of all the teeth in lower jaw except the third molars. Participants received a thorough oral prophylaxis and restarted their habitual oral hygiene procedures after the experimental period.

Saliva were obtained in two visits, in D0 and D21 of experimental gingivitis and prepared for salivary cotinine measurements. Subjects were instructed not to eat or drink 60 minutes before sampling and to rinse mouth thoroughly with water 10 minutes before sample is collected. Whole saliva was collected by unstimulated passive drool. Subjects were asked to tilt the head forward, allowing the saliva to pool on the floor of the mouth, then the saliva was passed through the SalivaBio Collection Aid (SCA) into a polypropylene vial. The samples were collected in clean plastic containers, frozen im-mediately, and transported to the analytical laboratory (Laboratory of Pathology Clinic, Hasan Sadikin Hospital, Bandung) where they were assayed by a laboratorist without knowledge of the exposure status of the subjects. Cotinine levels were determined by high sensitivity salivary cotinine quantitative enzyme immunoassay kit (Salimetrics).

Data Analysis

Descriptive statistics and data analyses were carried out by a statistician, who was blinded for the group allocation, with SPSS version 20.0 (SPSS, IBM, New York, NY, USA). To test the normality of the data, Shapiro–Wilk test was employed, and all data were normally distributed. Demographic characteristics between study groups were analyzed by means of one-way analysis of variance (ANOVA) and Chi-Square where appropriate. Mean values for PII, GI and salivary cotinine level were calculated per in-dividual. Tukey's post hoc for multiple comparisons were subsequently employed to measure specific differences between pairs. p-values $<0.05$ were considered statistically significant.

## Results

All participants completed the pre- and experimental gingivitis study. The mean age of the 15 participants was 29.4 years without significant differences between groups and ranged between 19 and 38 years. The study population consisted of 10 males and 5 females which was unbalanced distributed over the 3 study groups (Table 1).

The mean (±SD) current consumption of the smokers was 13.6 (±3.6) cigarettes/day and the mean (±SD) of smoking duration was 11 (±4.1) years. As former smokers, the mean (±SD) previous consumption of the vapers was 22.8 (±3.5) cigarettes/day with mean (±SD) smoking

duration was 7.8 (±7.2) years before they switched. The mean (±SD) current consumption of e-liquid was 6.4 (±3.5) ml/day, while the mean (±SD) nicotine content as written in the labels was 7.0 (±3.0) mg/ml and the mean (±SD) of vaping duration was 4.4 (±1.7) years.

The pre-experimental period in which the participants received oral hygiene instructions, scaling and prophylaxis resulted in comparable low PII and GI values at D-0. During the 14 days and the third week of oral hygiene abstention, PII increased significantly in all 3 groups with no significant difference could be assessed in plaque accumulation of all groups (Table 2).

With regard to gingival inflammation, GI increased significantly in both non-smoker and vaper group, whereas no increase of gingival inflammation could be assessed in the smoker group (Table 2). Statistical comparison of the 3 groups showed that GI increase during the 14 days experimental period as well as during the third week was lesser in smoker group compared to non-smokers and vapers. Comparison of the 3 groups showed that during the whole 14 days experimental period as well as the third week, both non-smoker and vaper group showed more gingival bleeding than smoker group.

Tukey's post hoc analysis revealed that there were no significant differences in PII between non-smokers, smokers and vapers after 21 days of oral hygiene abstention. Nevertheless, GI in smokers was different from non-smokers and vapers, gingival inflammation in non-smokers and vapers were significantly greater compared to smokers as well as AngBI in smokers was different from non-smokers and vapers, demonstrating that gingival bleeding in smokers were significantly lesser than non-smokers and vapers.

Salivary cotinine levels assessed at baseline and D-21 confirmed the nicotine intake of the smoker- and vaper participants. At baseline, mean salivary cotinine levels in non-smokers, smokers and vapers were 4.16 ng/mL, 314.19 ng/mL, and 158.48 ng/mL, respectively, while at D-21, the value were 4.37 ng/mL, 293.7 ng/mL and 180.21 ng/mL, respectively (Table 3). Tukey's post hoc analysis indicated that salivary cotinine levels in smokers and vapers were significantly greater than non-smokers.

Trial design and procedures were described in Trial Flow Chart (Figure 1). In order to avoid tooth cleaning in the allocated sites, customized-acrylic stent was used and placed in lower jaw each time the subjects brushed their teeth (Figure 2). Clinical features of gingival bleeding upon gentle probing were captured from nonsmoker, vaper and smoker in Figure 3, Figure 4, and Figure 5, respectively. It was obvious that nonsmoker and vaper showed greater bleeding on probing and clinical appearance of inflammation compared to smoker after 21 day-period of experimental gingivitis.

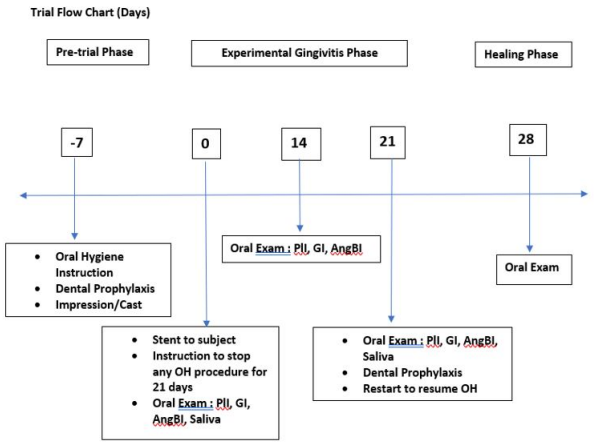


**Figure 1.** Trial Flow Chart.

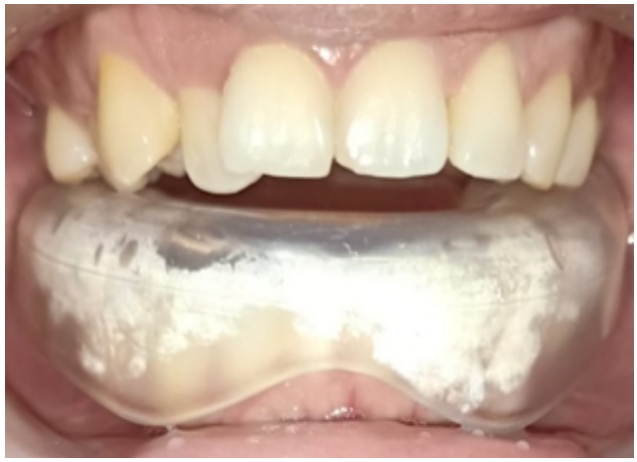

**Figure 2.** Custom-made acrylic stents to cover an area in lower jaw to prevent cleaning during tooth brushing.

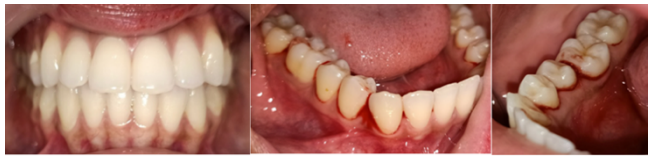

**Figure 3.** Bleeding on probing of non-smoker subject at D-21.

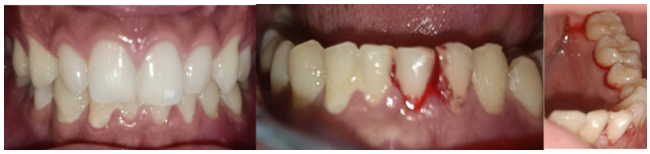

**Figure 4.** Bleeding on probing of vaper subject at D-21.

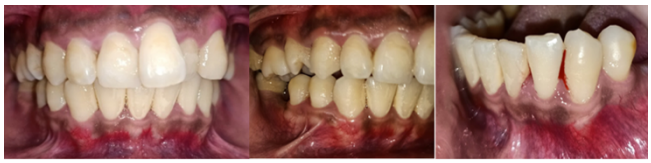

**Figure 5.** Bleeding on probing of smoker subject at D-21.

| | | Experimental group | | |
|---|---|---|---|---|
| Variable | NS | S | V | p-value |
| Age (years±SD) | 29.8 (4.9) | 28.8 (6.7) | 29.6 (7.5) | p>0.05 |
| Gender (M/F) | 0/5 | 5/0 | 5/0 | |
| Cigarette/day (sticks±SD) | | 13.6 (3.6) | 22.8 (33.5)$^{+}$ | p>0.05 |
| Duration of smoking (years±SD) | | 11 (4.1) | 7.8 (7.2)$^{+}$ | p>0.05 |
| E-liquid/day (mL±SD) | | | 6.4 (3.5) | |
| Nicotine in e-liquid (mg/mL±SD) | | | 7.0 (3.0) | |
| Duration of vaping (years±SD) | | | 4.4 (2.6) | |

**Table 1.** Demographic characteristic of study groups.
NS = nonsmokers, S = smokers, V = vapers, ($^{+}$) = amount and duration of previous smoking habit in vapers.

| Variable | Time in days | NS (N=5) | Within NS group Difference | S (N=5) | Within S group Difference | V (N=5) | Within V group Difference |
|---|---|---|---|---|---|---|---|
| PII | 0 | 1.67±0.14 | | 1.85±0.15 | | 1.90±0.16 | |
| | | | 0.32±0.10 | | 0.26±0.13 | | 0.35±0.21 |
| | 14 | 1.99±0.07 | | 2.11±0.02 | | 2.25±0.15 | |
| Increase | | | 0.14±0.08 | | 0.18±0.16 | | 0.10±0.14 |
| | 21 | 2.13±0.85 | | 2.29±0.17 | | 2.35±0.12 | |
| | 0-21 | 0.46±0.16 | | 0.44±0.14 | | 0.45±0.09 | |
| GI | 0 | 1.22±0.13 | | 1.28±0.17 | | 1.54±0.10 | |
| | | | 0.16±0.17 | | 0.17±0.27 | | 0.21±0.16 |
| | 14 | 1.38±0.25 | | 1.45±0.22 | | 1.75±0.11 | |
| Increase | | | 0.33±0.15 | | -0.03±0.19 | | 0.08±0.08 |
| | 21 | 1.71±0.12 | | 1.42±0.17 | | 1.83±0.06 | |
| | 0-21 | 0.49±0.07 | | 0.14±0.17 | | 0.29±0.11 | |
| AngBI | 0 | 0.43±0.27 | | 0.62±0.34 | | 1.31±0.21 | |
| | | | 0.33±0.40 | | 0.36±0.55 | | 0.49±0.34 |
| | 14 | 0.76±0.54 | | 0.98±0.49 | | 1.80±0.32 | |
| Increase | | | 0.73±0.26 | | -0.13±0.55 | | 0.25±0.07 |
| | 21 | 1.49±0.35 | | 0.85±0.45 | | 2.05±0.29 | |
| | 0-21 | 1.06±0.19 | | 0.23±0.55 | | 0.74±0.29 | |

**Table 2.** Mean values (SD) of the clinical parameters during 21 days of experimental gingivitis.
NS = nonsmoker, S = smoker, V = vaper.

| Variable | Time in days | NS (N=5) | Within NS group Difference | S (N=5) | Within S group Difference | V (N=5) | Within S group Difference |
|---|---|---|---|---|---|---|---|
| Cotinine | 0 | 4.16±2.1 | 0.21±1.15 | 314.18±263.2 | -20.48 ±132.32 | 158.48±127.1 | 21.72 ±107.03 |
| | 21 | 4.37±2.7 | | 293.70±228.0 | | 180.20±139.6 | |

**Tabel 3.** Salivary cotinine levels (ng/mL).
NS = nonsmoker, S = smoker, V = vaper.

## Discussion

The present study was conducted to assess whether differences in gingival inflammation and bleeding tendency in non-smokers, vapers and smokers were present during experimental gingivitis. We demonstrated that during the 21-day experimental gingivitis, despite the similar rate of plaque accumulation in the three study groups, the percentage of sites with inflammation and bleeding on probing increased significantly in vapers and non-smokers, while smokers failed to display 'a normal' inflammation and bleeding upon provocation, reflecting a suppressed gingival defense mechanism.

To the best of our knowledge this is the first study to show gingival response in subjects using alternative tobacco product, i.e. e-cigarettes during an experimental gingivitis. Peruzzo et al (2016) in an experimental gingivitis study found that both smokers and nonsmokers developed gingival inflammation after supragingival plaque accumulation, but smokers had less pronounced bleeding.[20] It was also shown that defense reaction to a given plaque challenge is reduced and not maintained in smokers.[32] That is why smoking is considered to have a masking effect due to pertubed or impaired clinical manifestation of inflammation in smokers, despite the presence of the disease. In the present study, we observed that non-smokers showed gingival redness, swelling and bleeding, as expected when a subject refrain from normal oral hygiene procedures. It is in agreement with results from experimental gingivitis by Loe et al (1965) that after 2-3 week period of oral hygiene measure abstention, plaque accumulates and subject demonstrates a concomitant increase in gingival bleeding as gingivitis develops.[19] It is interesting to note that vapers, also demonstrated gingival inflammation and bleeding upon probing similar to nonsmokers, despite the use of nicotine vapour.

Previous studies claimed that smoking may lead to vasoconstriction of gingival microcirculation resulting in reduced blood flow in gingival tissues. This effect was attributed to the actions of nicotine-stimulated adrenaline and noradrenaline on a1-adrenergic receptors.[33,34] In vitro, production of inflammatory mediators i.e. interleukin-1 (IL-1) and IL-8 in activated macrophages has been shown to be suppressed

by nicotine and this theory was supported in animal models.[35,36] Nevertheless, in the present study, both cigarette and e-cigarette provide a source of nicotine and subjects who used e-cigarette with nicotine vapour showed similar gingival response to plaque accumulation as non-smoker did. Nicotine intake can be estimated from concentrations of cotinine in biologic fluids i.e. plasma, urine, and saliva of cigarette smokers. The nicotine used by smoker and vaper subjects were confirmed by salivary cotinine levels, a metabolite of nicotine. Cotinine and nicotine-N-oxide are inactive metabolites of nicotine. The half-life of nicotine following inhalation or parenteral administration is approximately 2 hours, and the half-life of its metabolite, cotinine, is about 19 hours. Therefore, the systemic distribution of nicotine in smokers can be estimated from the presence of nicotine metabolite in the saliva.[37] Heavy passive smokers can have cotinine concentration levels ≥10 ng/mL, while passive smokers usually have concentrations in saliva below 5 ng/mL. Levels between 10 and 100 ng/mL may result from infrequent active smoking or regular active smoking with low nicotine intake. Regular active smoking or nicotine intake may result in levels >100 ng/mL.[38] Based on these categorizations of tobacco exposure assessed from cotinine level, the smoker and vaper subjects participated in the present study were confirmed as regular active smokers/vapers.

Furthermore, Morozumi et al (2004) and Nair (2003) observed in smokers who quit, in three to five days following smoking cessation, gingival blood flow, bleeding on probing and gingival crevicular fluid flow increase, which may reflect the repletion of gingival normal condition.[39,40] The recovery in gingival microcirculation to normal in the early stages of smoking cessation, may contribute to improvement of gingival tissue health. These results provide further evidence that tobacco smoking affects the inflammatory response and that these changes are reversible on quitting.[41] In the present study, we found that vapers have clinical features of gingival response to bacterial plaque accumulation similar to non-smokers or when smokers quit. These results are in agreement with Silva (2021) suggesting that cigarette smoke has more influence on the microcirculation than just nicotine alone.[42] In addition, Holliday et al (2019) conducted a systematic review on the effect of nicotine on human gingival, periodontal ligament and oral epithelial cells. He concluded that according to findings from in vitro studies, nicotine, at levels found in tobacco smokers, nicotine replacement therapy users and e-cigarette users, despite high level of salivary nicotine in smokeless tobacco users, is not likely to be cytotoxic to human gingival and periodontal cells. It is presumed that instead of nicotine, some other substances or properties in smoking have the detrimental effects on periodontal health.[43]

E-cigarettes or vapes are categorized as Electronic Nicotine Delivery Systems (ENDS). Nicotine is delivered to the users in aerosol or vapour contributing to the chemical part of the addiction and at the same time they offer sensory and motor stimuli resembling smoking, but without the occurrence of the tobacco burning process.[44] There are also assortments of nicotine contents of e-liquid designed to help switcher tapering off with the level of nicotine. This combustion-free process in delivering nicotine is thought to be the key difference since it is considered tar-free. Displacing combustible tobacco products with non-combustion products that deliver nicotine with a lower toxic and risk profile is key to tobacco harm reduction, and may promote the cessation of cigarette smoking.[45-47] Franco et al (2016) and Muqawwi (2021) evaluated the micro-nucleated cells (MN) from buccal swab of smokers versus e-cigarette users. They found that the oral cavity cells of e-cigarette smokers showed MN values similar to those of healthy controls, indicating the safety of e-cigarettes. The authors suggested e-cigarette as an aid to smoking cessation. [48,49]

In the present study, the similarity of clinical features of gingival response to bacterial plaque accumulation in vapers (who was former smokers) and nonsmokers might be due to reversed gingival vasculatures to normal condition suggesting a similar direction to that which occurs when smokers quit. George et al (2019) demonstrated in a prospective, randomized-controlled trial that within 1 month of switching from combustible cigarette to electronic cigarettes, there were significant improvement in endothelial function and vascular stiffness regardless the use of nicotine in e-liquid. The results were in line with the present study that improvement appears to be unrelated to the abstinence from nicotine but rather from other

toxic material produced by combustion in tobacco cigarettes.[50]

Nevertheless, the present result must be interpreted with extreme caution since this is a pilot study. The recruitment of subjects was restricted due to Covid19 outbreaks in Indonesia before the commencement of the study. The sample size was also limited (5 subjects each group) but since the unit of analysis is not subject per se, but tooth sites, the statistical analysis was able to be employed. The present study also did not observe the changes when smokers switch to e-cigarettes but in the time point when they already use e-cigarettes for at least 2 years. Further investigation is required to understand the impact of e-cigarette and nicotine itself on longer term of vascular function.

## Conclusions

Within the limitation of the present study, we concluded that gingival response to plaque accumulation as measured by gingival inflammation and bleeding on probing during experimental gingivitis are similar in vapers and nonsmokers, suggesting that the use of e-cigarettes with nicotine vapour did not mask the clinical features of inflammation, while smokers showed a reduced response despite similar plaque accumulation. We encourage that complete tobacco cessation is the best outcome for smokers, and any efforts to make reduced-risk products available need to be part of a comprehensive tobacco control strategy aimed at reducing or minimizing tobacco use through cessation and prevention.

Institutional Review Board Statement: The study was conducted in accordance with the Decla-ration of Helsinki, and approved by the Institutional Review Board Ethical Committee of Medical Faculty Universitas Padjadjaran, Bandung, Indonesia (896/UN6.KEP/EC/2020, issued on 30th September 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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## Declaration of Interest

The authors report no conflict of interest.

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