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Dental plaque quantitation by light induced fluorescence technology in exclusive Electronic Nicotine Delivery Systems (ENDS) users

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ABSTRACT

Objectives: In comparison to conventional combustible cigarettes, Electronic Nicotine Delivery Systems (ENDS) including both e-cigarettes (ECs) and heated tobacco products (HTPs) significantly reduce exposure to toxic chemical emissions. However, their impact on dental plaque remains unclear. This study measures dental plaque in ENDS (ECs and HTPs) users using quantitative light-induced fluorescence (QLF) technology, comparing them with current, former, and never smokers.

Methods: This cross-sectional study compared dental plaque measurements using QLF technology (Q-ray cam™ Pro) among current smokers (≥10 cigarettes/day), former smokers (quit ≥6 months), never smokers, and exclusive ENDS users (quit ≥6 months). Dental plaque measurements were expressed as ΔR30 (total area of mature dental plaque) and ΔR120 (greater plaque thickness/maturation-calculus). The Simple Oral Hygiene (SOH) score was calculated by the QLF proprietary software. Statistical analyses including ANCOVA was performed by R version (4.2.3) with $p < 0.05$.

Results: A total 30 smokers, 24 former smokers, 29 never smokers, and 53 ENDS users were included. Current smokers had significantly higher ΔR30 and ΔR120 values compared to other groups ($p < 0.001$). ENDS users showed plaque levels similar to never and former smokers ($p > 0.05$) but significantly lower than current smokers ($p < 0.01$). Although ENDS users showed a lower SOH score than smokers, this difference was not statistically significant. Daily toothbrushing and mouthwash usage were significant covariates.

Conclusion: ENDS users exhibited reduced accumulation of dental plaque and calculus compared with current smokers.

Clinical significance: Exclusive ENDS use could less impact dental plaque accumulation compared to cigarette smoking. Further research is needed to confirm these findings and fully understand ENDS impact on dental plaque formation.

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1. Introduction

Dental plaque is a structurally and functionally organized biofilm, consisting of a mass of bacteria, that develops on the tooth surface in a systematic manner with a diverse microbial composition [1]. Dental plaque plays a significant role as one of the main etiological factors for gingivitis and periodontitis [1]. Insufficient toothbrushing and poor oral hygiene are associated with the formation and maturation of dental plaque, leading to the colonization of dysbiotic microbiota. Species present in mature biofilm trigger extensive inflammatory reaction, of which non-specific component is responsible for the destruction of tooth-supporting structures and clinical symptoms of the disease. Regular removal of the plaque is considered to be one of the most effective methods for preventing gum inflammation and its progression into periodontitis [2].

Cigarette smoking is also a significant risk factor for the accumulation of dental plaque, leading to a higher risk of periodontal disease [3,4]. Indeed, multiple studies have provided evidence that smokers tend to have a higher accumulation of supragingival plaque compared to non-smokers and are at a greater risk of experiencing severe periodontitis [5-7].

While abstaining from smoking is likely to improve dental plaque control and reduce the risk of developing periodontal disease, there is a lack of robust evidence supporting this claim. Although further research is needed to better understand the relationship between smoking cessation, dental plaque accumulation, and periodontal health, a recent study has shown that people who regularly smoke have greater dental plaque formation than nonsmokers [8].

Electronic Nicotine Delivery Systems (ENDS) such as e-cigarettes (ECs) and heated tobacco products (HTPs) have emerged as popular yet controversial substitutes for tobacco cigarettes among smokers worldwide [9-12]. In comparison to conventional combustible cigarettes, these products offer a significant reduction in exposure to toxic chemical emissions [13-17]. As a result, they have been suggested for harm reduction in comparison to cigarette smoke [18,19] and as aids for smoking cessation [20]. However, there is limited knowledge about the impact of these combustion-free nicotine delivery technologies on oral health and dental plaque formation. Experimental studies have shown that flavoring agents in ECs may increase microbial adhesion to enamel and promote biofilm formation [21].

To the best of our knowledge, no study has yet addressed the quantitative estimation of dental plaque formation in ENDS users. Therefore, the objective of this study was to measure dental plaque formation using a novel light-induced fluorescence technology in individuals exclusively using ENDS (ECs and HTPs). The test results were then compared with dental plaque measurements of age and sex-matched individuals who are current smokers, former smokers, and nonsmokers, drawing from data obtained in a previous research [8].

2. Methods

2.1. Study population

The study population consisted of four groups: current and former smokers, identified from attendees at a local smoking cessation clinic, and never smokers and ENDS users, recruited from hospital staff, university students, local dental clinics, and via social media.

The four distinct study groups are characterized based on specific criteria:

- Current smokers: Participants who currently smoke at least 10 cigarettes per day, with an exhaled carbon monoxide (eCO) level of ≥ 7 ppm.
- Former smokers: Individuals who were previous smokers but have successfully quit smoking for at least 6 months, and their eCO level is now below 7 ppm.
- Never smokers: People who have never smoked or have only smoked less than 100 cigarettes in their lifetime. Their eCO level is also below 7 ppm to exclude those who might have significant exposure to environmental cigarette smoke.
- ENDS users: Former smokers who have quit smoking for at least 6 months and have switched to using either electronic cigarettes (ECs) or heated tobacco products (HTPs). Their eCO level is below 7 ppm.

The subjects included in the study had to meet specific inclusion and exclusion criteria, as follows:

Inclusion criteria:

- Adult subjects aged between 18 and 50 years old.
- Absence of systemic diseases that could potentially interfere with the measurements.
- Presence of at least 10 natural anterior teeth (from cuspid to cuspid) in both the upper and lower jaws, with no dental prosthetics or crowns.

Exclusion criteria (conditions that could interfere with dental plaque measurements):

- Recent history of oral/dental infection or inflammation within the last 30 days.
- Recent use of antibiotics or anti-inflammatory drugs within the last 15 days.
- Subjects wearing fixed or removable orthodontic appliances or prosthetics, limited to the 12 natural anterior teeth.
- Undergone dental professional cleaning (scaling and polishing) within 6 months prior to screening.
- Pregnancy.

This observational study did not involve the assignment of any medical interventions or discretionary decisions by the investigators. It forms part of a larger project, registered under ID: NCT04649645, which received approval from the local Ethics Review Board (Catania ASP; approval no. 697, dated November 18, 2020). The study adhered to the Principles of Good Clinical Practice (GCP) and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants included in the study.

2.2. Study design

This cross-sectional study aimed to assess and compare dental plaque measurements using light-induced fluorescence technology among four study populations: (1) current smokers; (2) former smokers; (3) never smokers; and (4) exclusive ENDS users (i.e. former smokers who exclusively used either ECs or HTPs).

During the screening phase, potential participants were provided with information about the purpose and objectives of the research. They were checked for eligibility criteria and their nicotine use status (i.e., consumption of tobacco cigarettes, ECs, and HTPs use) and oral hygiene habits (i.e., frequency of toothbrushing, type of toothpaste, etc.) were assessed. Socio-demographic characteristics, such as sex, age, occupation, etc., were also recorded.

Eligible subjects were then invited to attend a study visit for dental plaque measurements. Prior to the visit, they were instructed to:

- Maintain their routine oral hygiene pattern, including toothbrushing, mouth washing, and interdental flossing.
- Avoid scaling and polishing procedures.
- Abstain from smoking for at least 2 h before the study visit.
- Refrain from toothbrushing or flossing for at least 2 h before the study visit.
- Abstain from eating and drinking (except water) for at least 2 h before the study visit.

During the study visit, eligibility criteria were re-checked, and participants were reminded of study restrictions. Exhaled carbon monoxide (eCO) measurement and dental plaque measurements were conducted, and the data were recorded for analysis.

2.3. Exhaled carbon monoxide measurement

The smoking status of the participants was objectively verified using a portable CO monitor (Micro CO; Micro Medical Ltd, UK), which measures exhaled carbon monoxide (eCO) levels. A reading of eCO > 7 ppm indicated that the individual was a smoker. To ensure accurate measurements, subjects were asked not to smoke cigarettes for at least 2 h before the eCO measurements. During the study visit, participants were invited to exhale slowly into a disposable mouthpiece that was attached to the eCO monitor, following the manufacturer's recommendations. The eCO readings were then recorded to determine the smoking status of each participant.

2.4. Dental plaque assessments

Prior to the dental plaque measurements, subjects were asked to rinse their mouth with water, and any food debris was removed by gentle flushing and drying using a triple syringe tip. Excess saliva was removed with dental suction, and cheek retractors (Henry Schein, Gillingham, UK, Double end large, 106–7079) were placed to provide a clear view of the 12 natural anterior teeth.

Dental plaque measurements were performed using the Q-ray cam™ Pro (AIOBIO Co. Ltd., Seoul, Republic of Korea), a high-resolution, handheld, auto-focus QLF™ camera. Images of the anterior teeth were taken with the camera according to the standard QLF digital photography protocol. The camera was positioned as close to the subject's teeth as possible (Fig. 1A and B), black rubber collar attached to the lens was used for standardization of the camera position in relation to the patient's teeth and the minimization of the ambient light level variations. The auto-focus function of the camera was used to focus on the maxillary lateral incisor and canine (focal depth 0.32), and both a white-light and a QLF image were taken automatically and recorded using the Q-Ray™ software (QA v.1.41, Inspektor Research Systems BV, Amsterdam, NL). Q-ray cam™ Pro was used with the following settings: Resolution (image size), full high-definition [1920×1080 pixels]; shutter speed, auto [1/30–1/30,000 s]; aperture, auto [F1.2–360]; sensor object distance, 2.3Mpixel Image Sensor. Images were taken by three different trained investigators.

Fluorescence photographs of the vestibular aspect of the anterior teeth (cuspid to cuspid, upper and lower jaw) were captured in end-to-end position. The images were automatically saved as bitmap images using the QLF proprietary software (QA v.1.41, Inspektor Research Systems BV, Amsterdam, NL). The software analyzed the images and determined the value of ΔR for each pixel on the dental surface. ΔR is a measure of the increase in the red over green fluorescence intensities relative to the tooth surface. Higher ΔR values indicate areas with more active bacterial metabolism within the dental plaque, representing a greater level of dental plaque maturation. An example of white-light and fluorescent images are shown in Fig. 2A and B.

Two key measurements were used in the analysis: ΔR30 and ΔR120 [22]. ΔR30 represents the percentage of the total tooth surface showing an increase in fluorescence intensity of at least 30 % and indicates the total area of mature dental plaque detected. On the other hand, ΔR120 represents an increase in fluorescence intensity of at least 120 % and reveals areas of greater plaque thickness/maturation (e.g., calculus/-tartar) within the total area of mature dental plaque detected.

The QLF proprietary software integrates fluorescence intensity data to calculate the Simple Oral Hygiene (SOH) score, which is synonymous with the Fluorescent Plaque Index (FPI) [22].

This comprehensive assessment of dental plaque using QLF technology allows for a detailed analysis of dental plaque maturation and may provide valuable insights into oral hygiene and gingival inflammation in the study population. The QLF technology and measurements are performed in a standardized manner by trained investigators to ensure the reliability and accuracy of the data obtained.

2.5. Statistics

The distribution of the data was assessed by Kolmogorov-Smirnov test. Clinical data comparisons among study groups were carried out by Chi-square test for categorical data, and Kruskal–Wallis test for continuously skewed data. Multiple regression analyses were performed to assess the interaction of age, gender, frequency of daily domiciliary oral hygiene, frequency mouthwash usage, and frequency of dental floss usage. Comparisons of QLF scores among study groups were performed using ANCOVA with age, gender, frequency of daily domiciliary oral hygiene, and frequency mouthwash usage as covariates, and followed by Tukey's post hoc comparison test. QLF scores, age, and all three oral hygiene parameters showed positive skewness and underwent log-transformation before conducting parametric association testing. The "half the minimum non-zero value" method was employed due to the presence of zero values in the dataset. All analyses were considered significant with a P value$<$ 0.05. R version 4.2.3 (2023–03–15) was utilized for data analysis and generation of graphs.

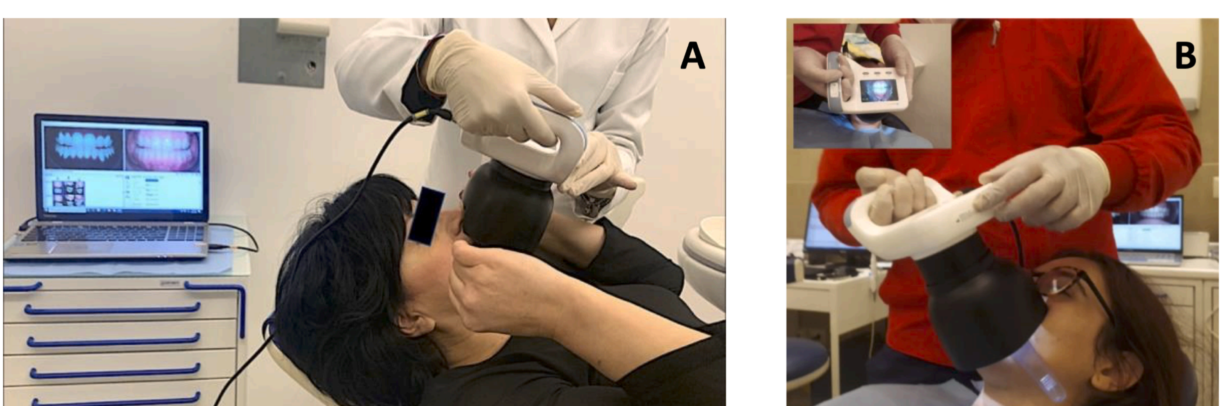

Fig. 1. A,B. Photos of the anterior teeth were taken using the Q-ray cam™ Pro. The camera was positioned as close as possible to the subject's teeth (A). Please note cheek retractors placed to provide a clear view of the 12 natural anterior teeth (B).

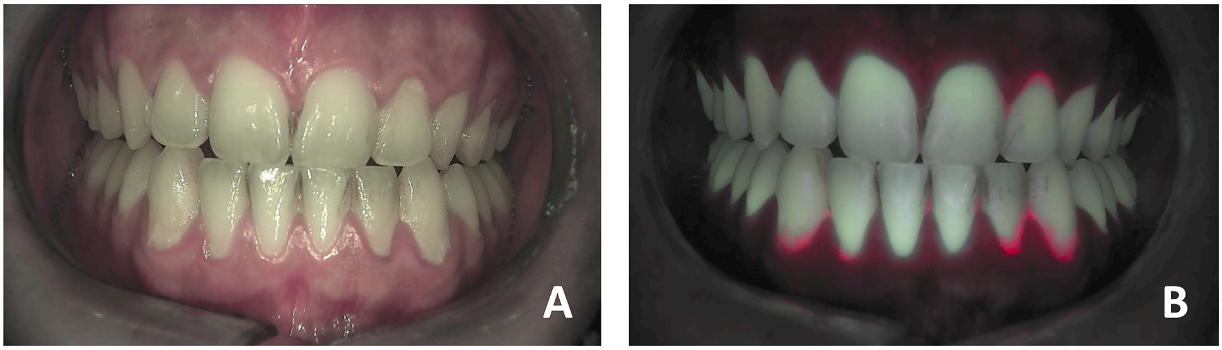

Fig. 2. A,B. Representative white-light (A) and fluorescent (B) images showing the vestibular aspect of the anterior teeth (cuspid to cuspid, upper and lower jaw) in former smokers group. The presence of dental plaque accumulation in the fluorescent image is indicated by red color.

3. Results

3.1. Study participants

A total of 136 subjects (54 Female and 82 Male; median (IQR) age of 33 (25–40) years) were recruited in this study, including 30 smokers, 24 former smokers, 29 never smokers, and 53 ENDS users (Table 1). Significant differences were observed for the “frequency of daily domiciliary oral hygiene” ($p = 0.004$) and “Frequency of mouthwash usage” ($p = 0.0007$) among the study groups. No significant differences were observed for all the other parameters.

3.2. Assessment of interaction effects on QLF parameters

Multiple regression analyses were performed to identify individual variables, including age, gender, Frequency of daily domiciliary oral hygiene, Frequency of mouthwash usage, Frequency of dental floss usage, which may influence the results of QLF measurements. The covariate “Frequency of daily domiciliary oral hygiene” was significantly related to ΔR30 ($p < 0.0001$), ΔR120 ($p < 0.0001$), and Simple Oral Hygiene Score ($p < 0.0001$) scores. The covariate “Frequency of mouthwash usage” was significantly related to ΔR30 ($p = 0.037$), ΔR120 ($p = 0.031$), and Simple Oral Hygiene Score ($p = 0.017$) scores.

Table 1
Clinical characteristics of study groups.

	Current Smokers	Former smokers	Never smokers	ENDS users	*P* value
Subjects n.	30	24	29	53	
Age (yr)	31 (26–40.25)	35.5 (26–49.75)	35 (29–42)	29 (25–41.5)	0.512
Female	10/30 (33.3 %)	10/24 (41.7 %)	13/29 (44.8 %)	21/53 (39.6 %)	0.834
Frequency daily domiciliary oral hygiene	2 (1–3)	2.5 (2–3)	2 (2–3)	2 (1–2.5)	0.004
Frequency mouthwash usage (per week)	0 (0–0)	0 (0–1)	1 (0–2)	0 (0–0.5)	0.0007
Frequency dental floss usage (per week)	1 (0–1.125)	1 (0–1.5)	1 (0–1.5)	1 (0–1.5)	0.417
N. Cig./Day	15 (12–20)	//	//	//	NA
Year smoking	12 (9.5–22)	//	//	//	NA
Pack/years	10 (6.9–15.38)	//	//	//	NA
Year non-smoking	//	2.5 (1.5–10)	//	2.5 (2–3.5)	0.152

Data are summarized as median (interquartile range - IQR). P values were calculated by Kruskal-Wallis test.

The covariate “Frequency of dental floss usage” indicated no relation with QLF measurements. Age and gender did not reveal significant relation with QLF scores.

3.3. Comparisons of QLF parameters among study groups

All comparison and cross-comparison results of QLF parameters were summarized in Tables 2 and 3, respectively. Increased QLF scores were observed with significant differences for all the three parameters, ΔR30 ($p = 0.0002$), ΔR120 ($p = 0.0002$) and SOH ($p = 0.0016$), among study groups. Subsequent multiple comparisons revealed significant differences in ΔR30 scores, showing significance between smokers and former smokers ($p = 0.00005$), between smokers and never smokers ($p = 0.00003$), and between smokers and ENDS users ($p = 0.0088$) (Fig. 3). Similarly, significant variations emerged in ΔR120 scores, indicating differences between smokers and former smokers ($p = 0.0002$), between smokers and never smokers ($p = 0.0003$), and between smokers and ENDS users ($p = 0.0078$) (Fig. 4). Furthermore, multiple comparisons for the SOH score exhibited differences between smokers and former smokers ($p = 0.0018$), and between smokers and never smokers ($p = 0.0016$). Although the ENDS users group shows a lower SOH score than smokers, this difference is not statistically significant (Fig. 5).

3.4. Comparisons of QLF parameters considering the two ENDS subgroups (ECs and HTPs)

We have also conducted further analysis on EC and HTP users separately. For ΔR30, current smokers exhibited significant differences from former smokers ($p < 0.0001$), never smokers ($p < 0.0001$), and HTP users ($p = 0.022$), but not from EC users ($p = 0.108$). Also, no significant differences were observed between EC users and the other groups. Similar findings are reported for ΔR120, with significant differences being observed between current smokers and former smokers ($p < 0.001$), never smokers ($p < 0.001$), and HTP users ($p = 0.017$) and no significant differences between EC users and the other groups.

Table 2
QLF comparisons among smokers, ex-smokers, never smokers, and ENDS (ECs and HTPs) users.

	Current Smokers	Former smokers	Never smokers	ENDS users	*P* value
ΔR30	5.3 ± 4.39	2.13 ± 2.85	2.03 ± 2.31	2.45 ± 1.44	0.0002
ΔR120	2.13 ± 2.60	0.54 ± 1.28	0.52 ± 0.83	0.74 ± 0.79	0.0002
SOH	3.1 ± 1.97	1.5 ± 1.56	1.48 ± 1.50	1.79 ± 1.31	0.0016

Data are summarized as mean± standard deviation (SD). P values were calculated by ANOVA adjusted for age, gender, frequency of daily domiciliary oral hygiene and frequency mouthwash usage. SOH: simple oral hygiene.

Table 3
QLF cross-comparisons among smokers, ex-smokers, never smokers, and ENDS (ECs and HTPs) users.

ΔR30 adj P value *	Current smokers	Former smokers	Never smokers	ENDS users
Current smokers	–	0.00005	0.00003	0.0088
Former smokers	0.00005	–	0.9992	0.1147
Never smokers	0.00003	0.9992	–	0.1175
ENDS users	0.0088	0.1147	0.1175	–
ΔR120 adj P value *	Current smokers	Former smokers	Never smokers	ENDS users
Current smokers	–	0.0002	0.0003	0.0078
Former smokers	0.0002	–	0.9797	0.2395
Never smokers	0.0003	0.9797	–	0.4130
ENDS users	0.0078	0.2395	0.4130	–
SOH adj P value *	Current smokers	Former smokers	Never smokers	ENDS users
Current smokers	–	0.0018	0.0016	0.0533
Former smokers	0.0018	–	0.9987	0.2977
Never smokers	0.0016	0.9987	–	0.3315
ENDS users	0.0533	0.2977	0.3315	–

* Adjusted p values were calculated using Tukey's post hoc comparison test. P-values < 0.05 were considered significant. P value $= 1$ indicates no difference between each pairwise comparison.

4. Discussion

Compared to tobacco cigarettes, ENDS significantly reduce exposure to harmful combustible chemicals [23], potentially lowering the risk of dental plaque accumulation, caries and periodontal disease. However, there is limited knowledge about the impact of aerosol emissions from these devices on dental plaque. This study is the first to investigate dental plaque and calculus in former smokers using ENDS, using light-induced fluorescence technology. The findings revealed that the accumulation of dental plaque (Δ R30) and calculus (Δ R120) in current cigarette smokers was notably worse compared to never smokers and former smokers as well as ENDS users. No significant differences were found among ENDS users, never smokers, and former smokers. This novel finding suggests that the design of ENDS, which operates without combustion, substantially reduces exposure to harmful chemicals that affect oral health by creating favorable conditions for biofilm formation and maturation.

Regular management of dental plaque accumulation is crucial for maintaining optimal oral health. In this study, light-induced fluorescence technology was used to objectively and accurately measure dental plaque and calculus across different study groups. This technology allows for standardized and reproducible quantification of dental plaque and calculus accumulation, providing a more reliable measure compared to traditional clinical indices [8,24].

Current smokers reported worse plaque parameters compared to other study groups, consistent with previous research indicating that smokers tend to accumulate more plaque and are more prone to severe forms of periodontitis [25-27]. These effects are likely due to changes in salivary composition, including enzymes and immunoglobulins, which reduce defensive functions and promote plaque formation [28]. Additionally, smoking has been noted to amplify the harmful effects of periodontal pathogens, particularly red complex microbes, by facilitating their colonization and infection [29].

Our finding that smokers had notably worse plaque parameters compared to exclusive ENDS users is in agreement with previous studies comparing plaque indices between ENDS users and smokers [30-32]. The better plaque parameters observed in exclusive ENDS users compared to current smokers are consistent with what is known about the reduced exposure to harmful combustible chemicals and the lower risk of dental plaque accumulation. Collectively, these studies suggest that ENDS may be a less harmful choice for periodontal health compared

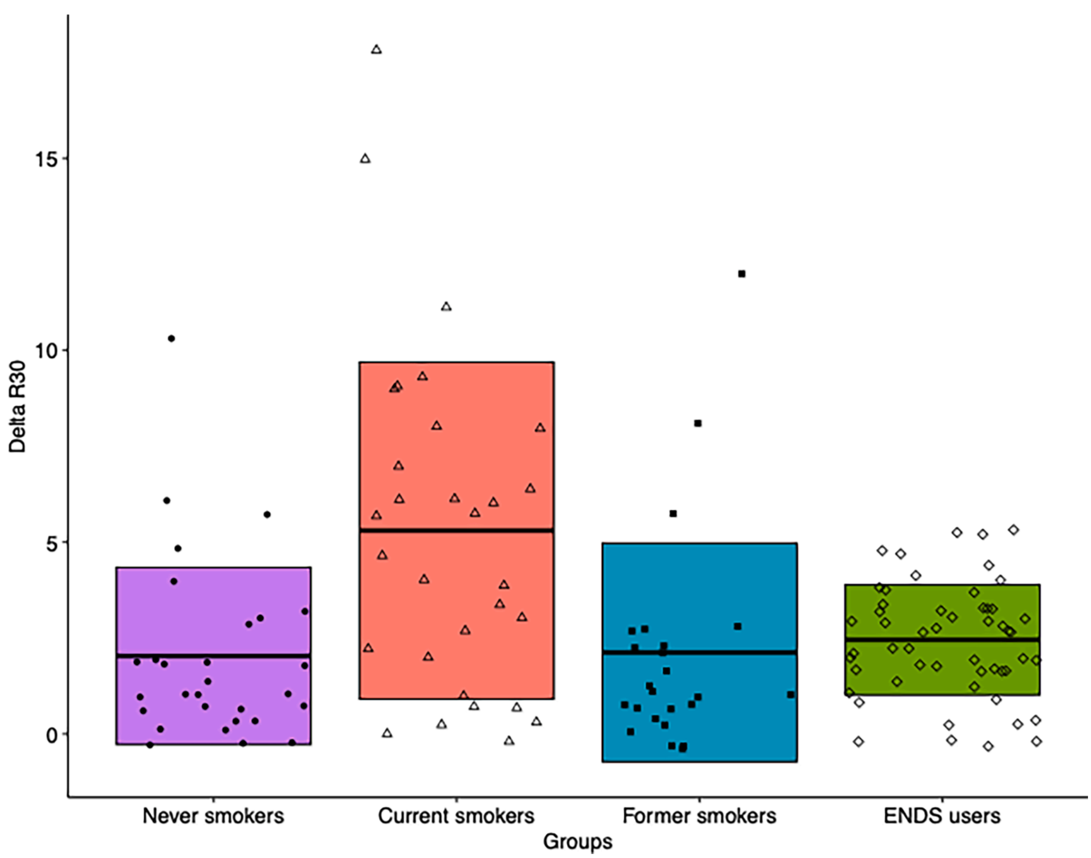

Fig. 3. Comparison of ΔR30 score among smokers, former smokers, never smokers, and ENDS (ECs and HTPs) users groups. Each dot represents the individual values of ΔR30 measurements. Box represent mean ± standard deviation (SD) of ΔR30 score for each study group.

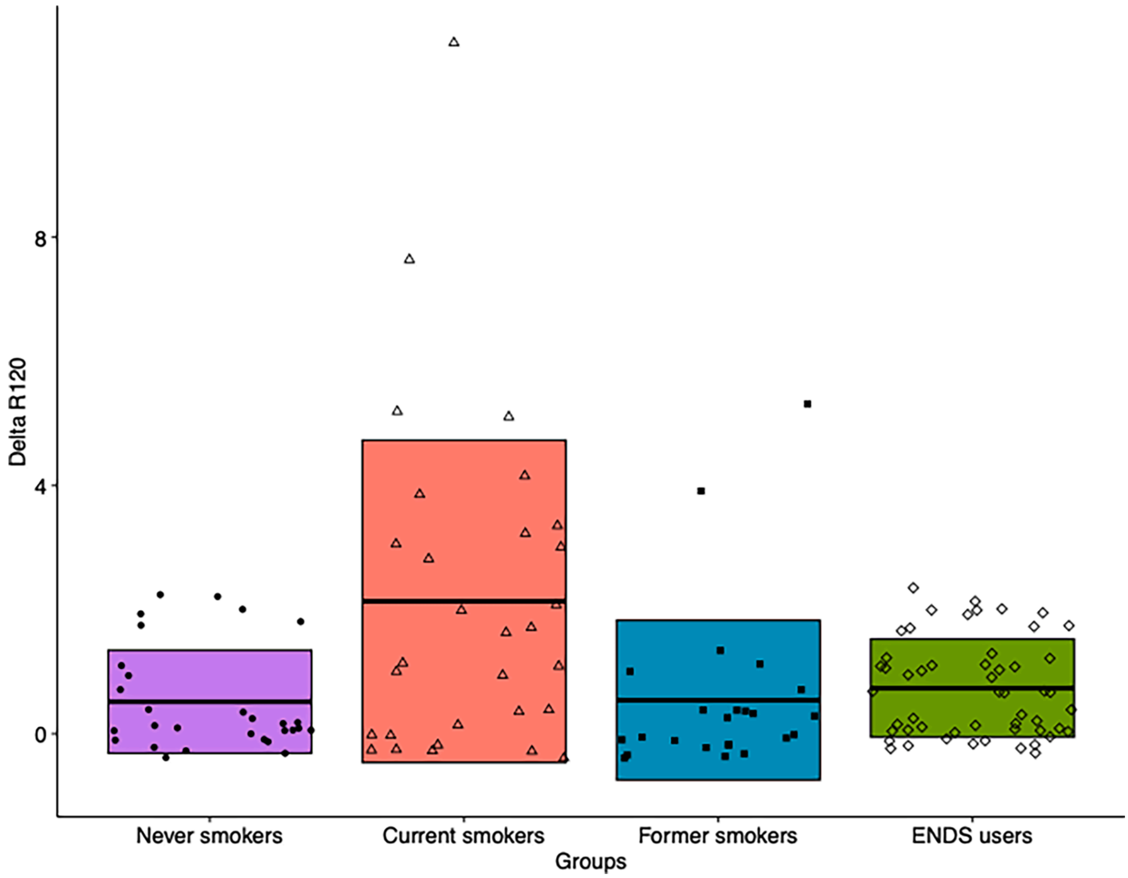

Fig. 4. Comparison of ΔR120 score among smokers, former smokers, never smokers, and ENDS (ECs and HTPs) users groups. Each dot represents the individual values of ΔR120 measurements. Box represent mean ± standard deviation (SD) of ΔR120 score for each study group.

to tobacco cigarettes, as evidenced by recent systematic reviews [33, 34].

When we analyzed EC and HTP users separately, we no longer observed significant differences. This was probably due to the reduced sample size and statistical power, as the study was not powered to identify significant differences among EC vs. HTP users. Therefore, we have maintained the original analysis of combining EC and HTP in the ENDS group to ensure a more robust and representative sample. There is a strong rationale to consider all combustion-free nicotine products combined into a single category because, regardless of their specific nicotine delivery technology (vaporized nicotine solution vs heated tobacco), it is the combusted constituents in tobacco cigarette smoke that play a significant role in the accumulation of supragingival plaque. In the future, much larger and prospective studies will be required to detect the true impact of EC vs. HTP on dental plaque and calculus accumulation. Such a study is currently ongoing [35].

Although we found no significant differences in plaque parameters among ENDS users, never smokers, and former smokers, comparisons of plaque indices between ENDS users and never/former smokers have yielded mixed results [30,31,36]. These differences could be due to variations in study populations, number of years since quitting smoking, and specific ENDS characteristics. Some individuals are more prone to plaque build-up due to genetic susceptibility and dietary factors [37,38], and these factors are rarely accounted for in dental plaque studies. Our population of ENDS users had a relatively long period of smoking abstinence (average, 2.5 years), and this might have contributed to the positive results. Additionally, differences in the frequency of personal dental hygiene may confound results, particularly in small cross-sectional studies. In our study, the frequency of daily tooth-brushing and mouthwash usage significantly affected all dental plaque parameters (ΔR30, ΔR120, Simple Oral Hygiene Score). Nonetheless, since ENDS are intended as tobacco cigarette alternatives, the most relevant comparison should be between ENDS users and smokers.

Some previous studies have evaluated the impact of ENDS on bacterial adhesion and the growth of the oral biofilm [21,39-42]. It is important to note that these studies are not directly comparable to our results, primarily due to the study design, which was mainly in vitro and cannot fully represent the wide variability in human vaping behavior and the differences across various users and devices [21,39,40]. Additionally, the outcomes are often different: while our goal was to evaluate plaque accumulation, some of these studies focused on changes in the composition of the oral microbiome [41,42].

When interpreting the results of the study, some factors and limitations need to be considered. First, the study participants consisted of a relatively younger population, thus these results cannot be extrapolated to the general population. Consequently, additional studies involving more representative age groups are necessary to confirm our findings. Second, dental plaque measurements were performed only on the anterior vestibular surface (from cuspid to cuspid, in both the upper and lower jaw). We limited our assessment to anterior region due to the relatively simpler procedure of capturing a single photograph from the buccal aspect. While the posterior region tend to have more plaque as compared to anterior, a consistent relationship has been demonstrated between the anterior and posterior regions [43]. Therefore, it is unlikely that the interpretation of the study findings would have changed significantly by extending measurements to all dental regions. Third, the cross-sectional design of this study offers limited insights, as it cannot establish causality, determine temporal relationships, and may be susceptible to biases [44]. However, given that exclusive ENDS users in our study have, on average, abstained from smoking for only 2.5 years, the

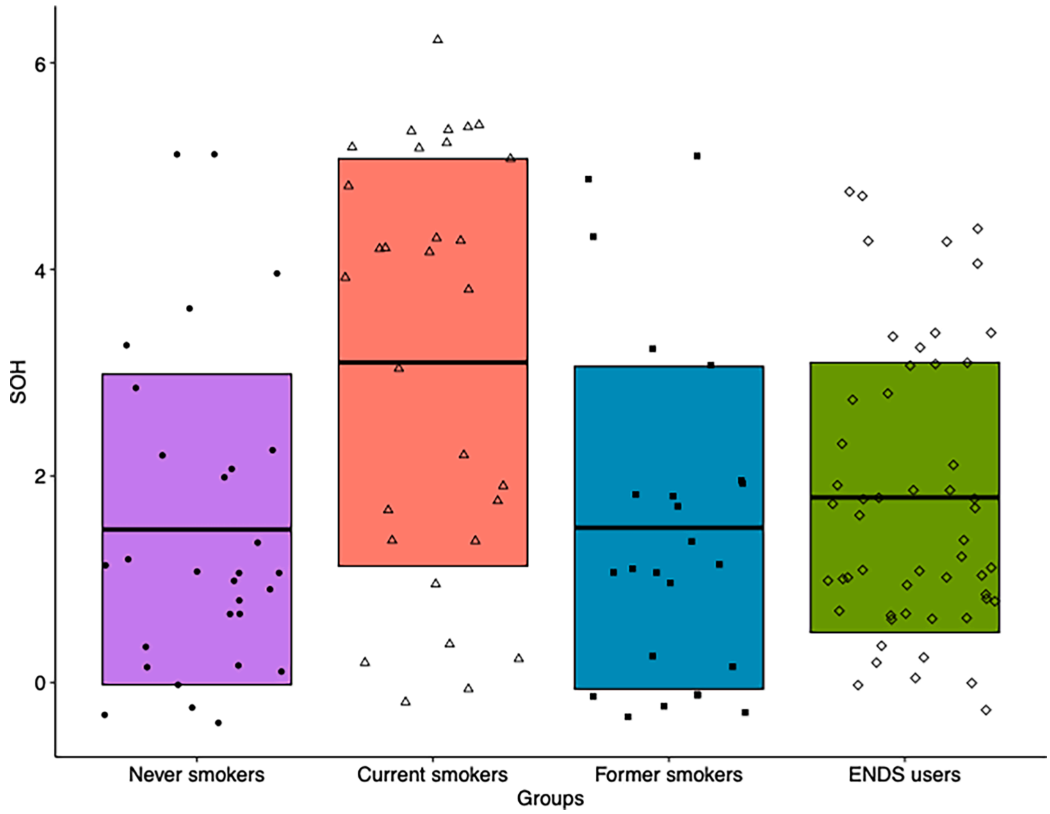

Fig. 5. Comparison of Simple Oral Hygiene (SOH) score among smokers, former smokers, never smokers, and ENDS (ECs and HTPs) users groups. Each dot represents the individual values of SOH measurements. Box represent mean ± standard deviation (SD) of SOH score for each study group.

reported improvement in dental plaque parameters may occur within a relatively short period after transitioning to ENDS following the cessation of tobacco cigarette use. Ideally, large-scale prospective randomized controlled trials would be the best design to investigate the impact of tobacco and nicotine products on dental plaque and calculus [35]. Finally, ENDS usage frequency, and specific product characteristics (e.g. nicotine content, flavorings) could have a different impact on periodontal health [31], but this was not investigated.

5. Conclusion

Considering the limitations of the study, the exclusive use of alternative nicotine delivery systems such as heated tobacco products and e-cigarettes may be associated to reduced accumulation of dental plaque and calculus compared with tobacco smoking. This suggests that combustion-free nicotine delivery technologies are unlikely to contribute to dental plaque development. However, these findings need to be confirmed through large-scale prospective randomized controlled trials that adopt a standardized approach to personal oral hygiene patterns. The use of ENDS may have significant implications for smokers, especially young smokers who perceive bad breath (i.e. halitosis) associated with dental plaque and calculus as an important problem [45]. For these individuals, an oral-centric narrative – emphasizing healthier oral health and improved halitosis - may be a more compelling reason to quit smoking than the fear of future cardiopulmonary diseases or lung cancer.

Ethical statement

This observational study did not involve the assignment of any medical interventions or discretionary decisions by the investigators. It forms part of a larger project, registered under ID: NCT04649645, which received approval from the local Ethics Review Board (Catania ASP; approval no. 697, dated November 18, 2020). The study adhered to the Principles of Good Clinical Practice (GCP) and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants included in the study.

CRediT authorship contribution statement

Giusy Rita Maria La Rosa: Writing – review & editing, Writing – original draft, Validation, Methodology. **Andrea Di Stefano:** Writing – review & editing, Writing – original draft, Methodology. **Deborah Gangi:** Writing – review & editing, Formal analysis. **Rosalia Emma:** Resources, Methodology, Formal analysis. **Valeriu Fala:** Writing – review & editing, Validation, Formal analysis. **Amaliya Amaliya:** Writing – review & editing, Investigation. **Hasan Guney Yilmaz:** Writing – review & editing, Methodology. **Roberto Lo Giudice:** Writing – review & editing, Validation. **Sebastiano Antonio Pacino:** Writing – review & editing, Investigation. **Eugenio Pedullà:** Writing – review & editing, Visualization, Investigation. **Renata Górska:** Writing – review & editing, Formal analysis. **Jan Kowalski:** Writing – review & editing, Investigation. **Riccardo Polosa:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Conceptualization.

Declaration of competing interest

GRMLR is a research fellow at the Department of Clinical and Experimental Medicine of the University of Catania. She declares no conflict of interest.

ADS, DG, RE, VF, AA, HGY, RLG, SAP, EP, RG, JK declare no conflict of interest. RP is full tenured professor of Internal Medicine at the University of Catania (Italy) and Medical Director of the Institute for Internal Medicine and Clinical Immunology at the same University. He has received grants from U-BIOPRED and AIR-PROM, Integral

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