LETTER to the EDITOR **Editorial Process:** Submission:02/09/2026 Acceptance:05/31/2026 Published:06/22/2026

Methodological and Interpretive Considerations in "The Impact of Electronic and Conventional Cigarette Use towards Saliva Profile and Oral Microbiota in Adolescents"

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Dear Editor

We read with great interest the article by Kurniawan et al. [1] on the impact of electronic and conventional cigarette use on saliva profile and oral microbiota in adolescents. The study provides timely data on salivary characteristics and selected oral microbiota among users of electronic and conventional cigarettes compared with non-smokers. However, several methodological and interpretive aspects warrant clarification to strengthen the validity and applicability of the findings.

First, there is a critical discrepancy between the age range of participants and the terminology employed. The study enrolled individuals aged 17–25 years yet describes the entire sample as "adolescents" throughout the title, abstract, and main text. According to widely adopted global health frameworks, adolescence typically encompasses ages 10–19 years, while individuals aged 20–24 years are more accurately classified as young adults or "young people"[2]. Applying the term "adolescents" to a population that includes many participants aged 21–25 years may mislead readers and complicate the inclusion of this study in systematic reviews focused strictly on adolescent populations. The manuscript would be strengthened by explicitly acknowledging that the sample comprises late adolescents and young adults. Where feasible, presenting age-stratified analyses (e.g., ≤19 versus ≥20 years) or clearly discussing the absence of such stratification as a limitation would enhance transparency [1, 2].

Second, there is an inconsistency between the abstract and the detailed methods regarding the statistical approach. The abstract states that chi-square and t-tests were used to analyze the data. In contrast, the methods section and Table 2 footnotes indicate that categorical variables were analyzed with chi-square tests, while continuous variables underwent independent t-tests and one-way ANOVA. Additionally, the Kruskal-Wallis test was applied in at least one instance for comparisons of salivary flow rate across five groups. For continuous outcomes such as salivary pH and flow rate, ANOVA or appropriate non-parametric analogues are preferable to multiple unadjusted t-tests when comparing several exposure groups. Clarifying in the abstract that one-way ANOVA and Kruskal-Wallis tests where the primary analytical tools for multi-group comparisons would improve transparency and ensure better alignment between the summary and the analytical strategy described in the methods and results sections [1].

Third, although the study reports statistically significant differences in salivary pH and flow rate between exposure groups with dual users showing lower pH and all smoker subgroups exhibiting reduced flow rates compared with non-smokers the analyses appear to be essentially univariable. Table 1 reveals notable differences in age and sex distributions across the five groups. Salivary pH and flow rate are known to vary with age, sex, hydration status, physical activity, and other behavioral and physiological factors [3, 4]. Without adjustment for such potential confounders, the estimated associations between tobacco product use and salivary outcomes may be biased. Employing multivariable regression models (e.g., linear or generalized linear models) that adjust at minimum for age and sex, and ideally for other relevant covariates such as oral hygiene behaviors, alcohol consumption, and systemic conditions, would yield more robust and clinically interpretable effect estimates [1, 3, 4].

Fourth, the exposure definitions could be more comprehensively characterized. "Smoker" status is defined as use of electronic and/or conventional cigarettes for a minimum of one year, yet the article provides limited detail on frequency and intensity of use (e.g., daily versus occasional use, cigarettes per day, e-liquid consumption per day, nicotine concentration). Prior research indicates that salivary properties and oral microbiota can vary with intensity and pattern of e-cigarette use and smoking [3, 5]. Collapsing a broad range of exposure intensities into wide categories such as "e-cigarette smokers" or "dual users" may obscure potential dose-response relationships and hinder cross-study comparisons. Even basic descriptive data on frequency and approximate consumption levels would help contextualize the findings and facilitate their integration into the existing literature [1, 3, 5].

Fifth, the microbiota analyses include only four groups (non-smokers and three smoker subgroups) because participants who smoked conventional cigarettes exclusively did not meet the random sampling criteria for microbiota assessment, as the authors acknowledge in the limitations. Nonetheless, some narrative conclusions are framed in terms of "smokers" versus non-smokers. Given that the microbiota results exclude exclusive conventional smokers, generalizing these findings to all smoking phenotypes should be approached cautiously. More explicitly distinguishing between results derived from the full five-group salivary analyses and those based

on the reduced four-group microbiota analyses would clarify the scope and limitations of the conclusions [1].

Most importantly, there is a critical internal inconsistency between the numerical results and the final summary statement. Table 2 demonstrates that non-smokers have the highest mean salivary pH (7.24 ± 0.39) and the highest mean salivary flow rate (0.62 ± 0.35 mL/min), with lower values observed in all smoking groups and statistically significant overall differences for both variables. However, the final paragraph of the discussion incorrectly states that smokers "exhibit higher salivary pH and lower flow rate compared to non-smokers." This statement directly contradicts the data presented in Table 2, where smokers consistently show lower pH values than non-smokers. Correcting this fundamental error is essential to prevent misinterpretation by readers and ensure accurate citation in future research [1].

Finally, the discussion could more clearly distinguish statistical significance from clinical relevance. While the observed differences in salivary pH and flow rate are statistically significant, and emerging evidence suggests that e-cigarette use can alter oral physicochemical properties and microbiological profiles in ways that may increase disease risk [3-5], the manuscript would benefit from explicit reporting of effect sizes (mean differences with 95% confidence intervals) and discussion of whether the magnitude of pH and flow rate changes is clinically meaningful for caries risk, periodontal disease, or other oral outcomes[1, 4].

In summary, addressing these methodological concerns particularly correcting the critical error regarding pH direction, clarifying age terminology, aligning statistical reporting, adjusting for confounders, and better characterizing exposure would substantially strengthen this valuable contribution and facilitate its integration into future systematic reviews on the oral health consequences of e-cigarette and dual use among young people.

References

1. Kurniawan AV, Amtha R, Gunardi I, Heriandi A, Sari EF. The impact of electronic and conventional cigarette use towards saliva profile and oral microbiota in adolescents. Asian Pac J Cancer Prev. 2025;26(1):309-18. https://doi.org/10.31557/apjcp.2025.26.1.309.
2. Roberts J, Sanci L, Haller D. Global adolescent health: Is there a role for general practice? Br J Gen Pract. 2012;62(604):608-10. https://doi.org/10.3399/bjgp12X658458.
3. Cichońska D, Kusiak A, Kochańska B, Ochocińska J, Świetlik D. Influence of electronic cigarettes on selected physicochemical properties of saliva. Int J Environ Res Public Health. 2022;19(6). https://doi.org/10.3390/ijerph19063314.
4. Kubala E, Strzelecka P, Grzegocka M, Lietz-Kijak D, Gronwald H, Skomro P, et al. A review of selected studies that determine the physical and chemical properties of saliva in the field of dental treatment. Biomed Res Int. 2018;2018:6572381. https://doi.org/10.1155/2018/6572381.
5. Isik Andrikopoulos G, Farsalinos K, Poulas K. Electronic nicotine delivery systems (ends) and their relevance in oral health. Toxics. 2019;7(4). https://doi.org/10.3390/toxics7040061.

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Reply to the letter to the editor: Methodological and Interpretive Considerations in "The Impact of Electronic and Conventional Cigarette Use towards Saliva Profile and Oral Microbiota in Adolescents"

Dear Editor

We sincerely appreciate the thoughtful and constructive critique offered by HaghighiKian and Khosravi Mashizi regarding our recently published study. We would like to address each point raised in a systematic and transparent manner.

First: Age Terminology "Adolescents" versus "Young Adults"

We sincerely appreciate the reviewer's thoughtful comment regarding the age classification used in our study. We acknowledge that, according to the WHO framework, adolescence is typically defined as the period between 10–19 years. However, we would like to clarify that the terminology used in this manuscript aligns with the official age classifications and legal frameworks established in Indonesia, where the study was conducted.

In Indonesia, the definition of "Remaja" (adolescents/youth) is broader and encompasses individuals up to the age of 24 or 25, depending on the specific regulatory body:

Ministry of Health of the Republic of Indonesia (Kemenkes RI)

According to the Regulation of the Minister of Health No. 2009, the "Remaja" (adolescent) group is defined as individuals in the age range of 17 to 25 years.

We have attached a screenshot of the criteria from Indonesian Constitutional Law regarding this age group (page 5).

1. https://www.mkri.id/public/filepermohonan/Perbaikan%20Permohonan_3314_3042_Perbaikan%20Permohonan%20Registrasi%20107%20PUU%20XXI%202023%20(via%20email).pdf

4. Bahwa berdasarkan Departemen Kesehatan Republik Indonesia Tahun 2009 terkait kriteria umur yang ditentukan ada 9 (sembilan) kategori yaitu:

a)	Masa balita	= 0 – 5 th
b)	Masa kanak-kanak	= 5 – 11 th
c)	Masa remaja awal	= 12 – 16 th
d)	Masa remaja akhir	= 17 – 25 th
e)	Masa dewasa awal	= 26 – 35 th
f)	Masa dewasa akhir	= 36 – 45 th
g)	Masa lansia awal	= 46 – 55 th
h)	Masa lansia akhir	= 56 – 65 th
i)	Masa manula	= > 65 th

2. We also attached the screenshot from magazine TEMPO in Indonesia about the age group. https://www.tempo.co/gaya-hidup/kategori-umur-balita-

tentang Kesejahteraan Lanjut Usia karya Lukman Nul Hakim (2020), klasifikasi usia menurut Kemenkes (2009) adalah sebagai berikut.

- Masa balita: 0-5 tahun.
- Masa kanak-kanak: 5-11 tahun.
- Masa remaja awal: 12-16 tahun.
- Masa remaja akhir: 17-25 tahun.
- Masa dewasa awal: 26-35 tahun.
- Masa dewasa akhir: 36-45 tahun.
- Masa lansia awal: 46-55 tahun.
- Masa lansia akhir: 56-65 tahun.
- Masa manusia lanjut usia (manula): lebih dari 65 tahun.

Second: Inconsistency in Statistical Methods Between the Abstract and Methods Section

We sincerely thank the reviewer for identifying the inconsistencies between the abstract and the methods section. We apologize for this lack of clarity.

Regarding the statement of statistical tests in the abstract, we would like to offer the following clarifications:

1. Independent t-test vs. One-Way ANOVA

We agree with the reviewer's observation. In our initial analysis, the t-test was used for specific pairwise comparisons (two groups). However, we recognize that, for comparing multiple exposure groups (more than two), one-way ANOVA is the more robust and appropriate method to prevent the inflation of Type I error. Conceptually, there is a direct "genetic" link between the t-test and ANOVA. In statistics, ANOVA is essentially an evolution of the t-test designed to handle more complex scenarios.

Both tests function by comparing the signal (the difference) against the noise (the random variation in the data).

* t-test: Compares the difference between two means divided by the standard error.

t = Difference Between Means / Variation Within Groups

* ANOVA: Compares the variance between multiple groups divided by the variance within those groups.

F = Variance Between Groups / Variance Within Groups

The Mathematical Relationship ($t^2 = F$)

The clearest evidence that ANOVA is mathematically related to the t-test is that, when both tests are applied to exactly two groups, the square of the t-value equals the F-value:

$t^2 = F$

In this sense, ANOVA may be viewed as a "squared" extension of the t-test. Squaring the values ensures that they are always positive, allowing the formula to accommodate multiple groups simultaneously without differences canceling each other out.

2. Kruskal-Wallis and the Chi-Square Distribution

For the analysis of salivary flow rates, where the data did not meet the assumptions for parametric testing, the Kruskal-Wallis test was employed. While this test analyzes the mean ranks of the groups (rather than the raw means), statistical significance is determined based on the chi-square distribution principle. The H-statistic follows a chi-square distribution to evaluate whether the observed differences in mean ranks among the five groups are statistically significant.

Regarding the incompleteness noted in the abstract, none of the reviewers' comments specifically requested the inclusion of multiple statistical analyses in the abstract.

Third: Absence of Multivariable Adjustment for Potential Confounders

We appreciate the reviewer's insightful suggestion regarding the use of multivariable regression to adjust for potential confounders such as age and sex. We would like to offer the following perspective on our analytical approach:

1. Nature of the Study and Random Sampling

This study was designed as an epidemiological investigation using a random sampling approach to capture a "snapshot" of the population. In such real-world settings, variations in demographic distributions (such as age and sex) across different exposure groups occur naturally. Our primary objective was to compare the observed population groups.

2. Focus on Descriptive Association

The core aim of this study was not to identify independent predictors or specific causal pathways underlying salivary changes, but rather to determine whether significant differences exist among various tobacco-user categories compared with non-smokers.

3. Foundation for Future Research

We agree that salivary parameters are influenced by a complex interplay of factors. However, we view our findings as a crucial foundational step. By highlighting these statistically significant differences, our study provides the necessary empirical basis for future research employing more targeted and controlled designs, or large-scale multivariable modeling, to isolate the specific

effects of each covariate. This can be explored further in future studies.

Fourth: Insufficient Characterisation of Exposure Intensity and Frequency

We sincerely appreciate the reviewer's feedback regarding the importance of consumption frequency and intensity in characterizing tobacco and e-cigarette exposure.

1. Clarification of Study Objective

We acknowledge that specific details, such as daily versus occasional use, number of cigarettes per day, e-liquid volume, and nicotine concentration, were not collected from the participants in this study. The primary objective of our research was to investigate the broad physiological differences in salivary pH and flow rate based on the mode of exposure (conventional, electronic, or dual use), rather than the dose-dependent intensity of the products.

2. Assumption and Research Design

The study was not designed to analyze dose-response relationships. Instead, our categorization focused on the presence of a sustained habit (minimum of one year) to identify general shifts in salivary health across different types of university-aged adolescent users. While we agree that collapsing different intensities into broad categories such as "e-cigarette smokers" or "dual users" limits the granularity of the data, we believe these broad categories still provide valuable baseline information regarding the distinct impacts of different delivery systems on oral physiology. Future studies may further investigate these assumptions using more detailed exposure measurements.

Fifth: Exclusion of Conventional Cigarette Smokers from Microbiota Analysis

We appreciate this insightful observation. We have addressed this point explicitly in the Study Limitations section of the manuscript.

Sixth: Critical Internal Inconsistency in the Final Summary Statement

We sincerely apologise for this typographical and interpretive error, which is inconsistent with the data reported in Figure 2 of the manuscript. On page 316, the manuscript states:

"The study's findings indicate that smokers, including e-cigarette smokers, e-cigarette and former conventional cigarette smokers, dual users, and conventional cigarette smokers, exhibit higher salivary pH and lower flow rate compared to non-smokers."

This statement should read "lower salivary pH" rather than "higher salivary pH."

Seventh: Distinction Between Statistical Significance and Clinical Relevance

We appreciate the reviewer's valuable suggestion to discuss the clinical meaningfulness of our findings. We agree that, while statistical significance (p-values) indicates the reliability of the results, the magnitude of the effect is crucial for clinical interpretation.

Reporting of Effect Sizes

We recognize that reporting effect sizes enhances the transparency and interpretability of the data. While effect sizes were not explicitly detailed in the initial manuscript, they can be derived using Cohen's d formula, based on the sample sizes, means, and standard deviations provided in our results. Should readers require further calculations regarding these indices, we would be pleased to provide the full calculations through the corresponding author.