RESEARCH ARTICLE

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The Impact of Electronic and Conventional Cigarette Use towards Saliva Profile and Oral Microbiota in Adolescents

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Abstract

Objective: To compare the salivary profiles of smokers (e-cigarette smokers, e-cigarette and former conventional cigarette smokers, dual users, and conventional cigarette smokers) and non-smokers in adolescents, focusing on acidity level, flow rate, viscosity, as well as the quantity of *Streptococcus mutans, Porphyromonas gingivalis*, and *Candida albicans*. **Methods:** This analytical observational study, with a cross-sectional design, involves collecting saliva samples from five groups through the draining method. Saliva viscosity was assessed visually, while saliva flow rate was monitored over a ten-minute period. Quantification of bacterial presence was performed using qPCR, and salivary acidity was determined using a digital pH meter. Chi square and t-test were used to analyze the data. **Result:** The total of 390 subjects (smokers n=195; non-smokers n=195), aged 17-25 years, participated in the study. Dual smokers exhibited a notable decrease in salivary pH (p=0.039) compared to non-smokers. Furthermore, significant reductions in flow rate (p<0.001) were observed across the five groups, however, no significant differences were found in saliva viscosity (p=0.070). When considering the four groups excluding conventional cigarette smokers, significant differences were observed in the quantity of *Porphyromonas gingivalis* (p=0.010) and *Candida albicans* (p=0.005). Conversely, the prevalence of *Streptococcus mutans* did not exhibit a significant difference (p=0.635). **Conclusion:** The study demonstrates that salivary pH, flow rate, and quantity of *P. gingivalis* as well as *C. albicans* are significantly different among the five groups.

Keywords: Salivary profile- electronic cigarette- Porphyromonas gingivalis- Candida albicans- Streptococcus mutans

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Introduction

In the past few years, there has been a notable increase in the popularity of e-cigarette (electronic cigarette) use in Indonesia. The prevalence of vaping in Indonesia has surged tenfold, rising from 0.3% in 2011 to 3% in 2021 according to Global Adult Tobacco Survey (GATS) [1]. Notably, the prevalence of vaping in DKI Jakarta has reached 5.9%, surpassing the national prevalence of 2.8% [2]. Adolescents, particularly those in the 15-19 and 20-24 age groups, have shown a strong interest for vaping, with prevalence rates of 10.5% and 7%, respectively [2]. This popularity is attributed to several factors, including e-cigarette fashionable designs, diverse flavours, pleasant aromas, and the perception of being safer than conventional cigarettes [3].

However, the negative impacts of vaping on dental and oral health are significant [4, 5]. E-liquids, comprising propylene glycol, glycerine, and flavours, have been found to facilitate the attachment of *Streptococcus mutans*, a common etiological agent of caries, which is prevalent in Indonesia [2, 6]. Some e-liquid products also contain nicotine, which has been linked to various oral cavity changes, including reduced oral acidity, increased *Streptococcus mutans* density on tooth surfaces, enhanced colonization of *Porphyromonas gingivalis* in gingival epithelial cells, and advanced growth and virulence of *Candida albicans* [7–10]. These changes can lead to prevalent dental and oral health issues such as caries, periodontitis, and oral candidiasis [2, 11]. Furthermore, the aerosol produced during vaping can result in decreased saliva flow rate, potentially leading to xerostomia and various other health issues, as indicated by research conducted by Hasan et al. in 2021 [12]. Exposure to toxic substances such as heavy metals (nickel, cadmium, lead, etc.) and carcinogens (formaldehyde, acetaldehyde, nitrosamines, and acrolein) has also been reported due to the aerosol produced [13, 14]. Saliva, as a notable oral fluid, is believed to undergo various changes due to direct exposure to e-cigarette aerosol and exhibits diverse biomarkers [3, 15]. This study sought to determine the saliva profile (acidity level, flow rate, viscosity) and the

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quantity of salivary microbiota (*S. mutans*, *P. gingivalis, C. albicans*) between smokers and non-smokers. The Aim of this study was to compare the salivary profiles of adolescent smokers and non-smokers, concentrating on salivary pH, flow rates, viscosity, and the concentrations of *S. mutans, P. gingivalis*, and *C. albicans.*

Materials and Methods

This analytical observational study with a cross-sectional design was conducted at Universitas Trisakti, Jakarta, Indonesia recruited 390 subjects randomly aged 17-25 years, categorized into five groups : e-cigarette smokers, e-cigarette and former conventional cigarette smokers, dual users, conventional cigarette smokers, and non-smoker groups to address possibility impact of cigarette types in saliva profile. The sample size was determined using the G*Power application, employing the Chi-square test, based on prior research conducted by Rahayu et al., which reported a 28.9% prevalence of keratosis among smokers associated with vaping [16]. The confidence level (α value) was set at 5% (0.05), and the test power was established at 0.95 (95%). Consequently, the minimum required sample size was calculated to be 166 individuals, with an additional 10% accounted for potential outliers (failure rate). The smoker group comprised subjects who had used electronic cigarettes and/or conventional cigarettes for a minimum of one year, while the non-smoker group included subjects who had never smoked. Exclusion criteria encompassed subjects with systemic diseases, drug and alcohol consumption. The study received approval from the Research Ethics Commission of the Faculty of Dentistry, Universitas Trisakti under the reference number 688/S1/KEPK/FKG/7/2023.

Unstimulated Whole Saliva Collection

Saliva collection was preceded by instructing subjects to abstain from eating, drinking, or smoking for one hour and rinse their mouths with distilled water. Subsequently, the subjects were positioned in a relaxed, forward-bent posture, allowed saliva to accumulate in the mouth, and then requested to expel saliva into the provided 15mL falcon tube for ten minutes. During this process, subjects were prohibited from speaking or moving their tongues [17].

Saliva Profile Assessment

Initially, the salivary flow rate was quantified by calculating the volume of saliva collected over a duration of ten minutes [17]. Subsequently, the viscosity of the saliva was assessed visually by at least three calibrated examiners, who classified it into three categories: thin, normal, and thick. Following this assessment, the salivary pH was measured by combining 1 mL of saliva with 25 mL of double-distilled water, and this mixture was analyzed using a digital pH meter (Yieryi, Shenzhen, CN).

Oral Microbiota Quantity Assay

The examination of oral microbiota quantity involved DNA extraction from saliva samples using the Quick-DNA™ Miniprep Plus Kit (Zymo Research, Irvine, CA), followed by quantification of the sample DNA via qPCR using HOT FIREPol® EvaGreen® qPCR Mix (Solis BioDyne, Tartu, EST) and primers for each microbiota oral [18, 19] :

S. mutans (16s rRNA)

Forward : 5'-GCC TAC AGC TCA GAG ATG CTA TTC T-3'

Reverse : 5'-GCC ATA CAC CAC TCA TGA ATT GA-3'

P. gingivalis (16s rRNA)

Forward : 5'-TGC AAC TTG CCT TAC AGA GGG-3'

Reverse : 5'-ACT CGT ATC GCC CGT TAT TC-3'), as well as 18S rRNA primers for

C. albicans (18s rRNA)

Forward : 5'- CCC AGT CTT TCA CAA GCA GTA AAT-3'

Reverse : 5'- GTA AAT GAG TCA TCA ACA GAA GCC-3'

The qPCR procedure for DNA isolate examination comprised an initial denaturation for ten minutes at 95°C for one cycle, followed by 40 cycles consisting of denaturation at 94°C for 15 seconds, annealing at 60°C for one minute, and a final extension at 95°C for 15 seconds.

Data Analysis

Kappa test was conducted to evaluate inter-examiner agreement regarding saliva viscosity by three investigators. Categorical data was subjected to chi-square testing, while continuous data underwent independent t-tests and one-way ANOVA testing, with significance levels set at $p<0.05$.

Results

The study encompassed 390 subjects, comprising 195 smoking subjects and 195 non-smoking subjects. Table 1 illustrates that the highest proportion of smokers were male (77.95%) and within the 19-20 age group (49.23%). Regarding smoking habits, males predominantly engaged in dual usage of conventional and electronic cigarettes, while females primarily utilized e-cigarette (Figure 1).

Before conducting saliva profile analysis, a Kappa test was initially performed to evaluate the agreement between examiners regarding saliva viscosity. According to Altman (1991), the inter-examiner agreement was deemed to be good ($\kappa = 0.634$, 95% CI, 0.589 – 0.679, $p < 0.001$). Analysis of salivary physical characteristics across five groups – non-smokers, e-cigarette smokers, e-cigarette and former conventional cigarette smokers, dual users (e-cigarette and conventional cigarette smokers), and conventional cigarette smokers – revealed significant differences. The findings, presented in Table 2, indicated significant variations in saliva acidity ($p=0.039$) and flow rate ($p < 0.001$) but no substantial variance in saliva viscosity ($p = 0.070$). Particularly, marked differences in saliva acidity were evident between the

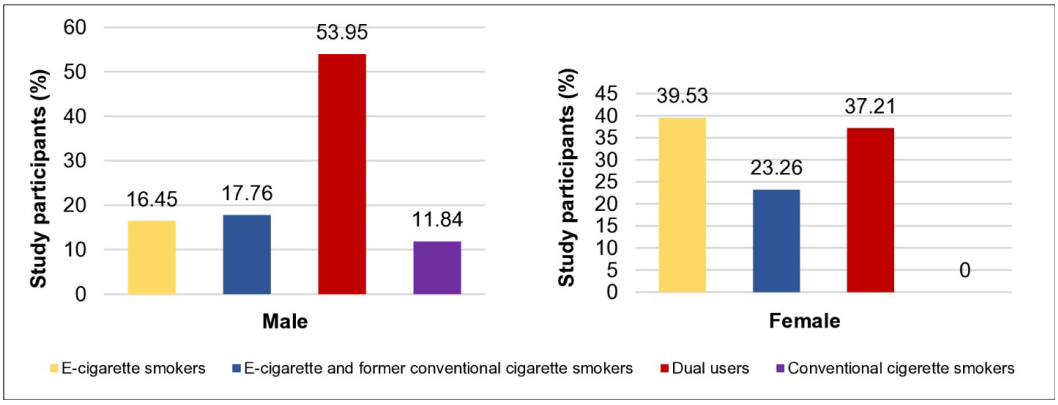

Figure 1. Percentage of Smoking Habits based on Gender

non-smoker group and dual users (p = 0.046) (Figure 2A). Conversely, significant variations in saliva flow rate were observed between the non-smokers group and all subgroups, including e-cigarette smokers (p < 0.001), e-cigarette smokers and former conventional cigarette users (p = 0.002), dual users (p < 0.001), and conventional cigarette users (p = 0.045) (Figure 2B).

In the assessment of oral microbiota quantity between groups (Figure 3), notable distinctions were identified in the quantity of *P. gingivalis* (p = 0.010) and *C. albicans* (p = 0.005) but no substantial difference in the quantity of *S. mutans* (p = 0.635) (Figure 3A). Notably, significant distinctions in the quantity of *P. gingivalis* were discerned between non-smokers and e-cigarette smokers (p = 0.011), as well as between the dual users (p = 0.006) (Figure 3B). Moreover, variations in the quantity of *C. albicans* were observed between non-smokers and the subgroup of e-cigarette smokers who had ceased conventional cigarette usage (p = 0.046), as well as with the dual users (p = 0.019) (Figure 3C).

Table 3 presents the correlation analysis between the variables under study. A noteworthy correlation was observed among the pH, flow rate, and viscosity of saliva. Furthermore, a significant correlation was identified between the quantity of *S. mutans* with *P. gingivalis* (Figure 4) and *C. albicans* (Figure 5).

Discussion

This investigation revealed a significant prevalence of

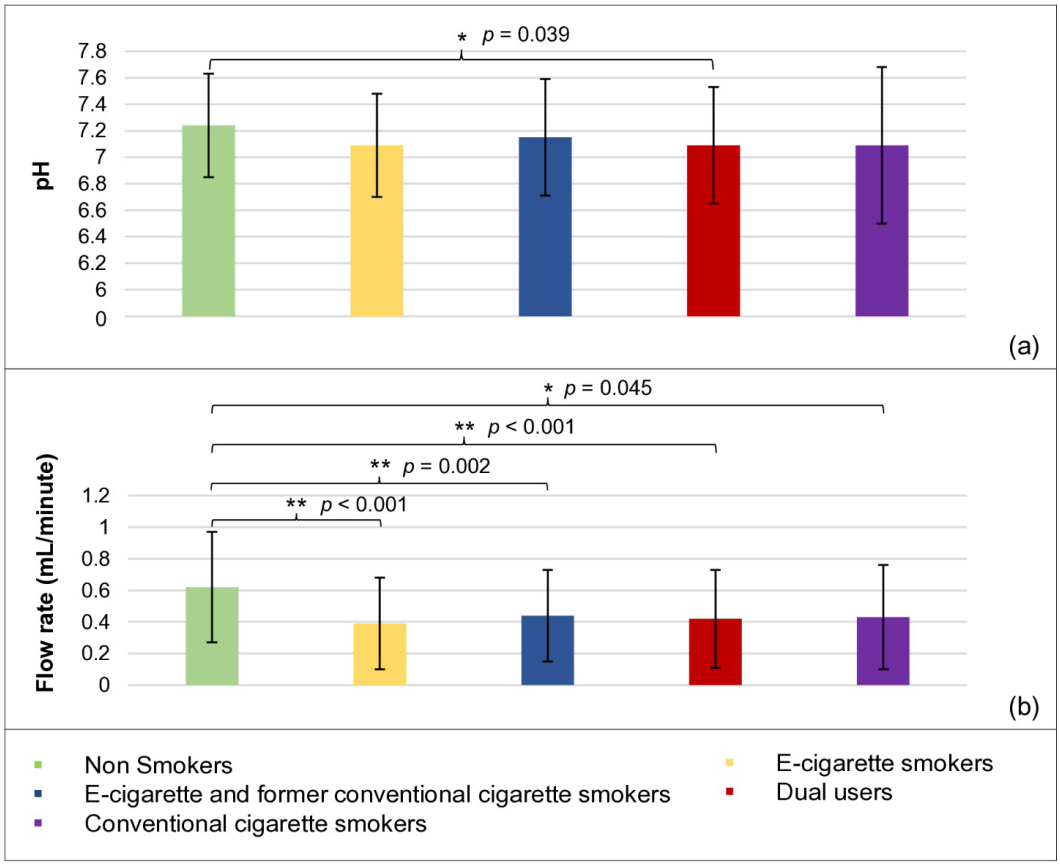

Figure 2. Saliva Profile between the Five Groups. The diagrams represent salivary pH (a) and salivary flow rate (b) between the five groups. The data represent the mean ± SD (*p<0.05, **p<0.01)

Table 1. Demographic Data of Study Participants

Variable	Non smokers	E-cigarette smokers	E-cigarette and former conventional cigarette smokers	Dual users	Conventional cigarette smokers
Gender [n (%)]					
Male	46 (23.23)	25 (12.62)	27 (13.64)	82 (41.41)	18 (9.09)
Female	149 (77.60)	17 (8.85)	10 (5.21)	16 (8.33)	0
Age (years) [n (%)]					
17-18	82 (73.87)	9 (8.11)	1 (0.90)	16 (14.41)	3 (2.70)
19-20	54 (36)	16 (10.67)	23 (15.33)	51 (34)	6 (4)
21-25	59 (45.74)	17 (13.18)	13 (10.08)	31 (24.03)	9 (6.98)

smoking among adolescents, particularly among males (76.77%) and in the 19-20 age group (64%) (Table 1). According to Amtha et al., the increase in cigarette use in Indonesia is connected to the societal approval of smoking [20]. This connection is especially evident among males, who view smoking as a sign of masculinity. The higher rate of smoking among teenagers may also be influenced by external factors, such as peer pressure [21].

In this study, electronic cigarettes were the most preferred smoking method among females (Table 1). This trend is consistent with previous National Health Surveys (Riskesdas) conducted in 2018 [2] and 2023 [22], which reported a doubling in the number of female smokers of both conventional cigarettes (37.2% to 50.5%) and electronic cigarettes (2.7% to 5.5%) [22]. The study also found that teenagers frequently use both vaping and traditional cigarettes, a trend that deserves attention because of the possible effects of vaping on saliva.

In this study, we hypothesized that cigarette (electronic and conventional cigarette) use could alter saliva profiles and the quantity of oral microbiota, potentially contributing to the onset of various diseases [23]. To

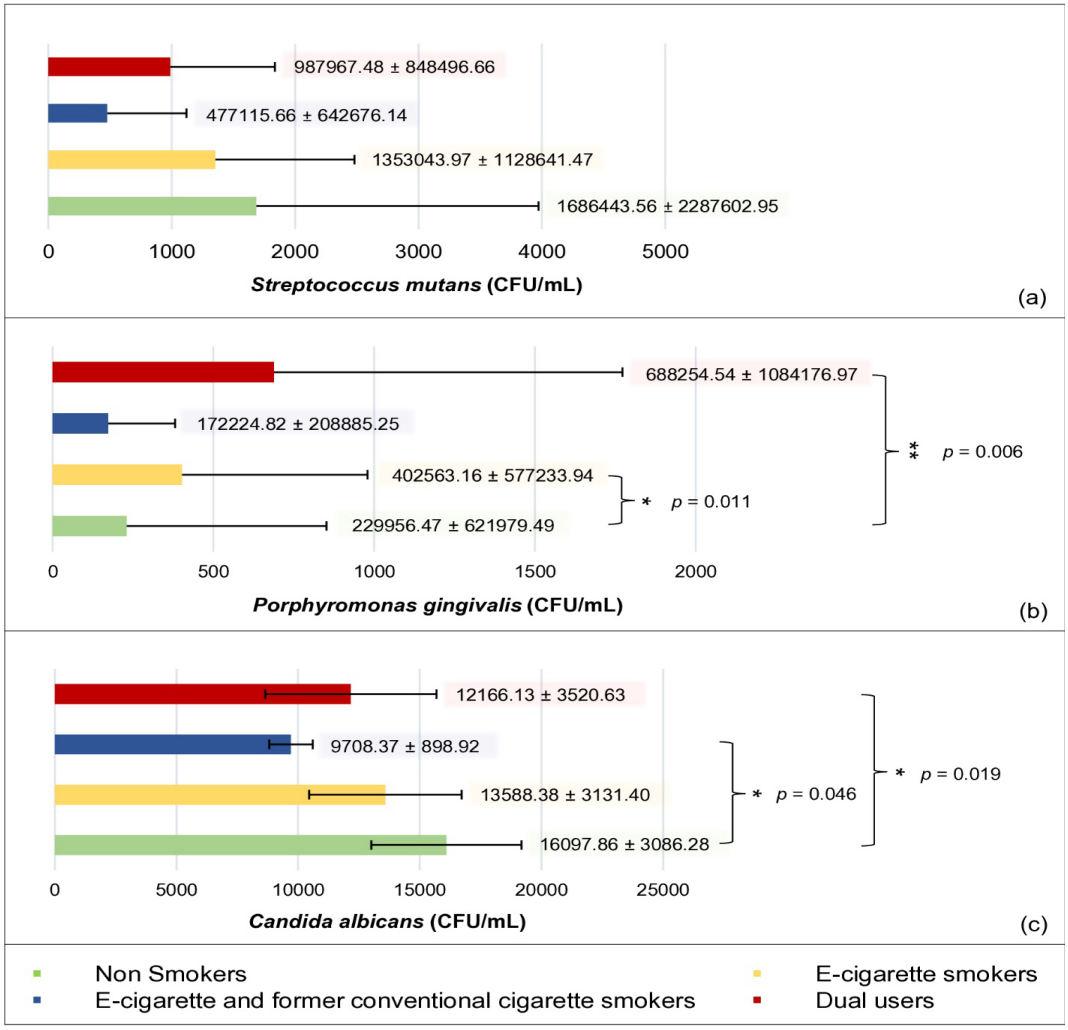

Figure 3. Microbiota Oral Quantity among the Four Groups. The diagrams represent the quantity of *Streptococcus mutans* (Kruskal-Wallis; p = 0.635) (a), *Porphyromonas gingivalis* (Kruskal-Wallis; p =0.010) (b), and *Candida albicans* (One-way ANOVA; p = 0.005) (c) among the four groups. The data represent the mean ± SD (*p<0.05, **p<0.01)

Table 2. Summary of Test Results, Significance of Acidity Degree, Flow Rate, and Viscosity of Saliva between the Five Groups

Variable	Non smokers (n = 195)	E-cigarette smokers (n = 42)	E-cigarette and former conventional cigarette smokers (n = 37)	Dual users (n = 98)	Conventional cigarette smokers (n = 18)	p-value	χ2
Salivary pH (x ± SD)	7.24 ± 0.39	7.09 ± 0.39	7.15 ± 0.44	7.09 ± 0.44	7.09 ± 0.59	0.039*, [a]	
Flow rate [mL/minute] (x ± SD)	0.62 ± 0.35	0.39 ± 0.29	0.44 ± 0.29	0.42 ± 0.31	0.43 ± 0.33	<0.001**, [b]	
Viscosity [n (%)]						0.070[c]	14,479
Thin	57 (29.23)	8 (19.05)	10 (27.03)	24 (17.91)	5 (27.78)		
Normal	126 (64.62)	26 (61.90)	23 (62.16)	12 (8.96)	8 (44.44)		
Thick	12 (6.15)	8 (19.05)	4 (10.81)	98 (73.13)	5 (27.78)		

[a], One-way ANOVA; [b], Kruskal-Wallis; [c], Chi square; *p<0.05; **p<0.01

further investigate this hypothesis, we divided the smoker group into five subgroups to examine the differences between users of conventional and electronic cigarettes. The findings revealed significant changes in saliva pH, a decrease in salivary flow rates, and an increase in *P. gingivalis,* indicating a reduction in saliva quality, particularly among dual users. Conversely, the quantities of the other two types of oral microbiota exhibited distinct results, with the non-smoker group displaying higher levels of *S. mutans* and *C. albicans.* These findings will be discussed in further detail in the subsequent paragraph.

In the analysis among the five groups also demonstrated

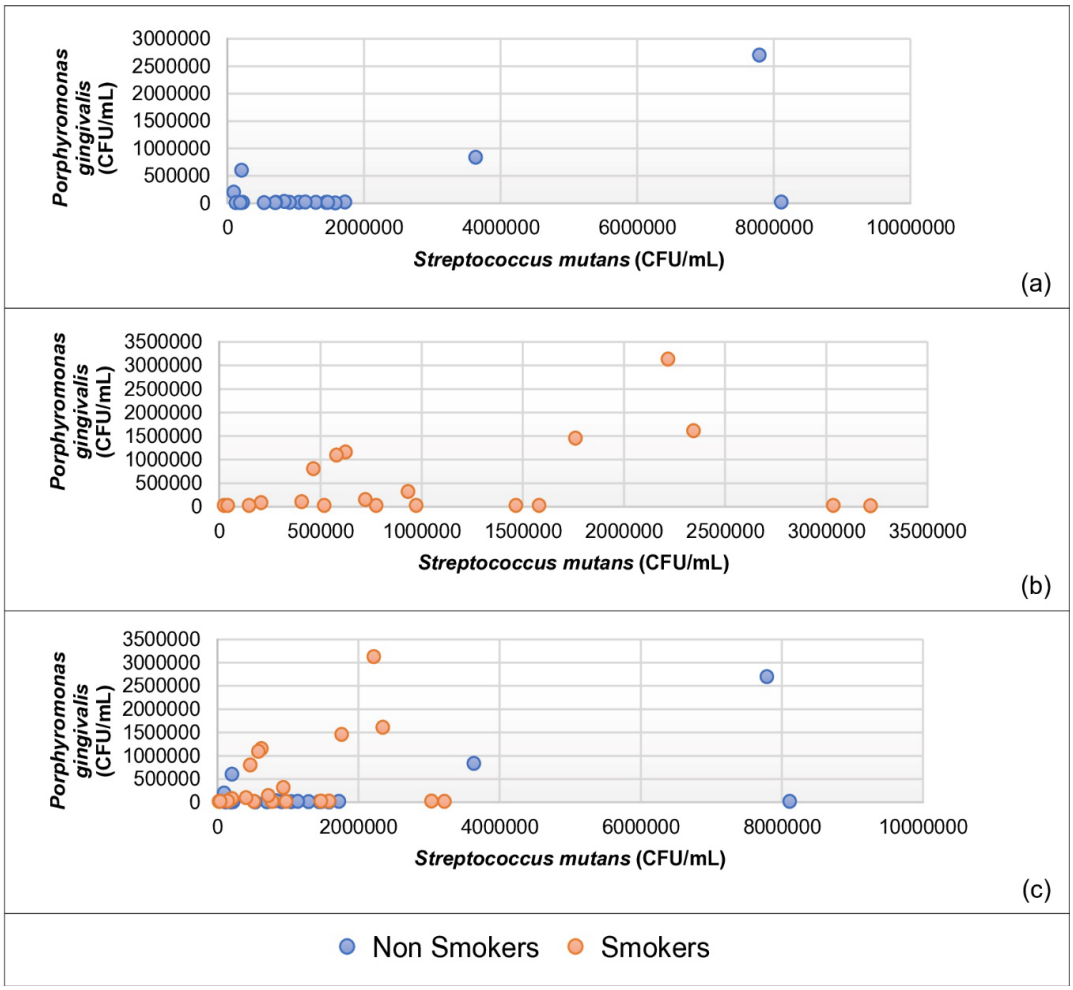

Figure 4. Distribution of *Porphyromonas gingivalis* and *Streptococcus mutans* in non smokers (a), smokers (b), non smokers and smokers (c)

Table 3. Correlation between Dependent Variables

		Salivary pH	Saliva viscosity	*S. mutans*	*P. gingivalis*	*C. albicans*
Salivary flow rate	r	0.335**	-0.223**	0.196	0.11	0.007
	p-value	<0.001	<0.001	0.225	0.944	0.965
Salivary pH	r		-0.266**	0.033	-0.009	0.239
	p-value		<0.001	0.841	0.955	0.137
Saliva viscosity	r			-0.72	-0.160	-0.101
	p-value			0.660	0.324	0.537
S. mutans	r				0.395*	0.405**
	p-value				0.012	0.010
P. gingivalis	r					-0.077
	p-value					0.636

r Pearson correlation; *p<0.05; **p<0.01

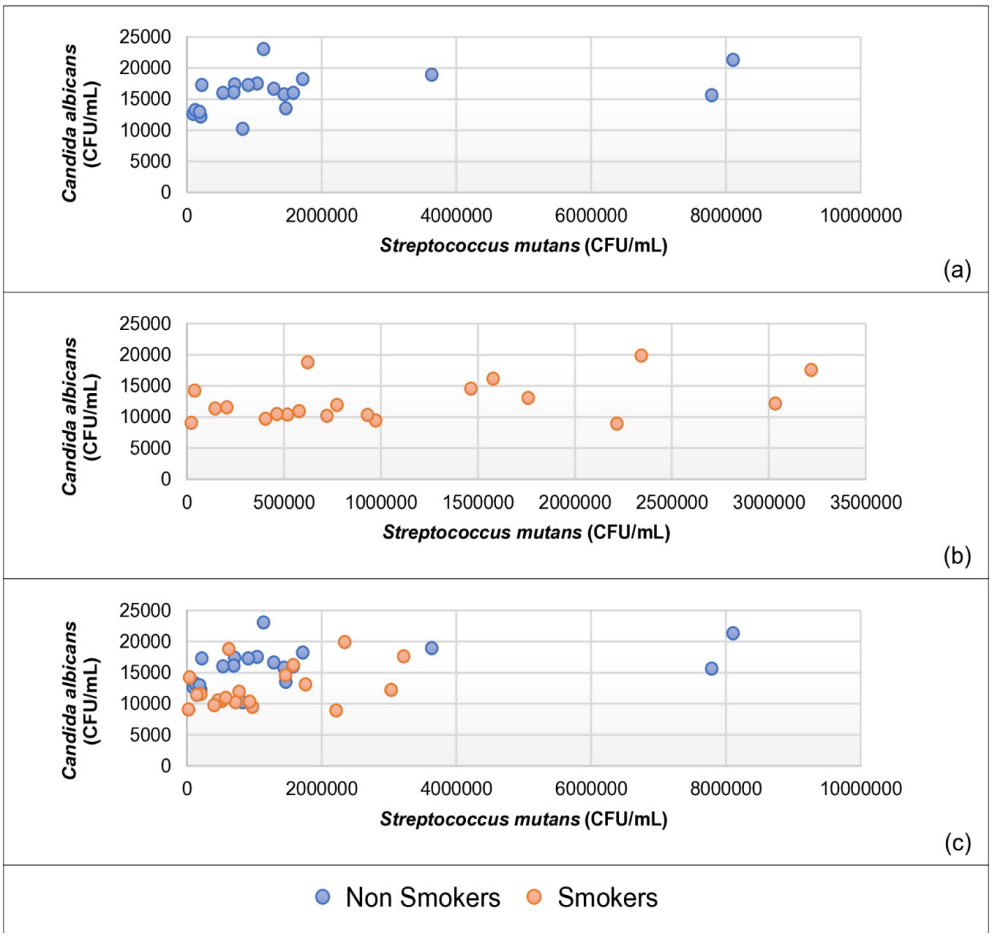

Figure 5. Distribution of *Candida albicans* and *Streptococcus mutans* in non smokers (a), smokers (b), non smokers and smokers (c)

a noteworthy disparity (p = 0.039), particularly observed within dual users (7.09 ± 0.44), exhibiting a significantly lower salivary pH (p=0.046) in comparison to non-smokers (7.24 ± 0.39). These findings are consistent with the outcomes reported by Kusumaningrum et al. and Zafar et al., indicating that the level of salivary acidity is lower in smokers compared to non-smokers [24, 25]. This phenomenon may be attributed to a reduction in bicarbonate ions in the saliva of smokers. E-cigarette users are consistently exposed to heated smoke and nicotine, while conventional cigarette users are exposed to heated smoke, nicotine, tar, and carbon monoxide [26]. Prolonged exposure to these elements can disrupt saliva secretion, resulting in decreased secretion of vital components in saliva, including bicarbonate ions [27]. In addition, both conventional and electronic cigarettes release aerosols that contain aldehydes. These aldehydes are believed to alter the physical and chemical properties of saliva, resulting in a decreased salivary pH [28, 29]. Nevertheless, it is essential to recognize that changes in saliva pH are influenced by various factors, such as the timing of saliva collection, saliva flow rate, dietary patterns, and individual

circadian rhythms. Notably, saliva collected at night or in the morning tends to exhibit higher acidity levels compared to daytime collection [30].

Moreover, the evaluation across the five groups demonstrated significant variances ($p < 0.001$) between the non-smoking group and all types of smoking groups, including the e-cigarette group (0.39 ± 0.29, $p < 0.001$), e-cigarette users who had ceased conventional cigarettes (0.44 ± 0.29, $p < 0.002$), the dual users (0.42 ± 0.31, $p < 0.001$), and conventional cigarette users (0.43 ± 0.33, $p = 0.045$). The findings of this study indicate that both e-cigarette and conventional cigarette usage can lead to a reduction in saliva flow rate. These results are consistent with the research by Lestari et al., which reported lower saliva flow rates in e-cigarette smokers compared to non-smokers, and are also corroborated by Nigar et al., which noted lower saliva flow rates in conventional smokers compared to non-smokers [8, 17]. Several studies have suggested that the heated smoke from e-cigarette and conventional cigarettes can disrupt blood flow in the oral mucosa, resulting in decreased receptor sensitivity and a subsequent decline in the salivation reflex [8, 17, 26, 27]. Additionally, the nicotine content in e-cigarette and conventional cigarettes has been linked to decreased saliva flow rate. Continual intake of nicotine can circulate in the blood, affecting cholinergic receptors, which play a crucial role in the salivation mechanism and can influence the vascularization of the salivary glands [8, 17, 27, 31]. Even electronic cigarette liquids without nicotine may not be free from the risk of negatively impacting saliva quality. This is because both conventional cigarette smoke and the aerosol produced by electronic cigarettes contain acrolein, an aldehyde derivative known to irritate the mucous membranes of the mouth, which are crucial for saliva secretion [32]. However, it is important to note that this study reported relatively normal saliva flow rates among smokers, ranging between 0.3-0.4 mL/minute [33]. Numerous factors can influence an individual's saliva flow rate, including the ambient air temperature during saliva collection, preceding physical activity, and the water intake of each subject [34–36]. Moreover, the saliva collection method can also impact the rate of saliva flow, as inadvertent mouth movements by the subjects may stimulate the salivary glands, leading to increased saliva production.

While previous studies have demonstrated differences in saliva viscosity, the present analysis of five groups revealed no statistically significant variations ($p = 0.070$) [24, 37]. However, an examination of Table 2 suggests a trend toward thicker saliva viscosity among individuals who use both electronic and conventional cigarettes (73.13%). Numerous studies explicate that the escalation in saliva viscosity among smokers is attributed to the heated smoke produced by e-cigarette or conventional cigarettes, along with the nicotine content, which disrupts the salivary glands, particularly the parotid glands. Consequently, there is a reduction in saliva secretion, particularly of a serous nature. The diminished saliva secretion by the parotid gland is compensated by the submandibular and sublingual glands, which predominantly secrete mucus or thick saliva [31, 37].

This study also examined the quantity of oral microbiota, and the analysis revealed that no significant differences were observed between the groups excluding the conventional cigarette smokers ($p = 0.635$). However, the analysis across the four groups (Figure 3A) indicates that the quantity of *S. mutans* in non-smokers is higher than in the three groups of smokers. These findings diverge from the findings of Rouabhia et al, which stated an increase in the quantity of *S. mutans* in the biofilm among e-cigarette smokers [7]. It is imperative to emphasize that this research focused on saliva, wherein the *S. mutans* present were in the form of planktonic cells. In this context, the quantity of *S. mutans* in the form of planktonic cells is lower in smokers, as reported by Huang et al, owing to the inhibitory effect of nicotine in concentrations of 2-4 mg/mL on the growth of *S. mutans* [38]. Moreover, El-Ezmerli et al. reported that nicotine could upregulate the expression of the glucan-binding protein (Gbps) and glucosyltransferase (Gtfs) genes in *S. mutans* planktonic cells, while downregulating Gbps and Gtfs in *S. mutans* biofilm cells. Consequently, this mechanism leads to an increased attachment of *S. mutans* in planktonic form to the biofilm, affecting the quantity of *S. mutans* in the form of planktonic cells [39].

In contrast to *S. mutans*, an analysis of across the four groups indicated a significant difference ($p = 0.010$), with both e-cigarette smokers ($p = 0.011$) and dual users ($p = 0.006$) demonstrating significantly higher levels of *P. gingivalis* compared to non-smokers (Figure 3B). These findings align with the findings of Jiang et al., which assert that the use of conventional cigarettes escalates the colonization of potentially harmful subgingival bacteria, such as *P. gingivalis* [40]. This escalation was elucidated by Shin and Lee et al. and Baek et al., who indicated that initial exposure to nicotine leads to a reduction in the growth of *P. gingivalis*, but subsequent exposure results in a twofold increase in its growth due to its ability to adapt to this substance [41, 42]. Another study by Guglielmetti et al. unveiled that the increase in *P. gingivalis* corresponds to the rise in carbon monoxide levels in the oral cavity resulting from the combustion of conventional cigarettes. It is well-established that *P. gingivalis* is an obligate anaerobic bacterium that thrives in low-oxygen conditions in the oral cavity; hence, increased carbon monoxide levels and reduced oxygen in the oral cavity stimulate the proliferation of *P. gingivalis* [43].

In the analysis of *C. albicans* quantity, a significant difference was observed among the four groups ($p = 0.005$), as shown in Figure 3C. Furthermore, Figure 3C illustrates that the non-smoker group exhibited a significantly higher quantity of *C. albicans* when compared to the group of e-cigarette users who had ceased using conventional cigarettes ($p = 0.046$) as well as the dual users ($p = 0.019$). These findings are consistent with the research conducted by Haghighi et al., which indicated that the nicotine content in e-cigarette and conventional cigarettes can impede the growth of *C. albicans* [44]. However, a distinct study by Mokeem et al. presented contrasting results to this study, demonstrating that the smoking group had a higher quantity of *C. albicans* than the non-smoking group [45]. It is crucial to note that the

outcomes of this study cannot be directly construed to imply that the smoking group faces a lower risk of oral candidiasis due to lower levels of *C. albicans* compared to non-smokers. This is because oral candidiasis can arise from various interconnected factors. Bardellini et al. reported that Candida's ability to invade the superficial layers of the epithelium is contingent upon the pH of the oral cavity, where a more acidic pH optimizes its ability [46]. Additionally, El-Sakhawy et al. [47] explicated that oxidative molecules present in cigarettes can activate nuclear factor erythrocyte 2-related factor 2 (NFERF2), which can regulate NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, potentially leading to adverse effects that may impair the oral mucosal defense response and increase susceptibility to *C. albicans* infection. Furthermore, this research affirms that nicotine can influence the characteristics of the pathogen *C. albicans*, including its impact on the expression of genes associated with virulence [47].

Besides examining the impact of electronic and traditional cigarettes on each variable, this study also explored the connections between the variables. Analysis of Table 3 yields a significant correlation among the pH, flow rate, and viscosity of saliva. This investigation reveals that a reduction in saliva flow rate leads to a decrease in saliva acidity and an increase in saliva viscosity. The interrelationship among these factors has been previously elucidated, indicating that the noxious substances present in e-cigarette and conventional cigarettes, along with their byproducts, can impact the organs involved in saliva secretion. This includes the cholinergic receptors that regulate the central nervous system's activity related to saliva secretion, blood flow in the oral cavity, and vascularization of the salivary glands. Consequently, disruption of all three aspects results in reduced saliva flow rate and thicker saliva viscosity [8, 17, 24, 26, 27, 31, 37]. Moreover, a decline in saliva flow rate ultimately diminishes the secretion of bicarbonate ions, thereby lowering the acidity of saliva [27].

A significant association was also identified between *S. mutans* and *P. gingivalis* ($p = 0.012$, $r = 0.395$), indicating that an increase in the number of *S. mutans* colonies corresponds to a decrease in the number of *P. gingivalis* colonies (Figure 4). This finding aligns with the research findings of Tu et al. [48], which assert an antagonistic relationship between *S. mutans* and *P. gingivalis*. The study reported a potential decrease in the detection rate of *P. gingivalis* in the presence of certain gram-positive cariogenic bacteria, such as *S. mutans* [48].

In addition to *P. gingivalis*, this study has revealed a significant correlation between *S. mutans* and *C. albicans* ($p = 0.010$, $r = 0.405$). Based on Figure 5, it is evident that *S. mutans* synergistically supports the growth of *C. albicans* in specific quantities. However, when the quantity of *S. mutans* exceeded 2 million CFU/mL, a decrease in the quantity of *C. albicans* was observed. This phenomenon has been elucidated by several studies, indicating that *S. mutans* can exhibit both synergistic and antagonistic correlations with *C. albicans*. According to Kim et al., *S. mutans* facilitates the growth of *C. albicans* by providing sucrose breakdown products, as *C. albicans* exhibits less efficient sucrose metabolism [49]. Conversely, *C. albicans* supports the growth of *S. mutans* by enhancing the production of exopolysaccharides (EPS) to create a conducive physical environment for the accumulation and formation of *S. mutans* microcolonies [50]. Additionally, *C. albicans* produces fungal factors that activate and increase the activity of the *S. mutans* glucosyltransferase B (GtfB) gene, which in turn augments the production of insoluble glucans for bacterial attachment. Furthermore, *S. mutans* and *C. albicans* are also found to have an antagonistic correlation. As reported by Huang et al., *S. mutans* can suppress the growth of *C. albicans* by producing competence-stimulating peptide (CSP), which inhibits the formation of *C. albicans* germ tubes [38].

Limitation of this study was during randomized sampling for examination the oral microbiota quantity, the subjects who smoked conventional cigarettes did not randomly fulfil the group, resulting in the division of the oral microbiota quantity among smokers and non-smokers into only four groups, which differs from the salivary profile report.

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. However, no significant differences in saliva viscosity were observed between the five groups. Additionally, the analysis of oral bacteria showed lower quantity of *Streptococcus mutans* and *Candida albicans*, and higher levels of *Porphyromonas gingivalis*, in the smoker group compared to the non-smoker group.

Author Contribution Statement

Angelita Victoria Kurniawan: conceptualization; data collection; drafting the article. Alyah Heriandi: data collection. Rahmi Amtha: conceptualization; methodology; supervision; drafting the article; reviewing the manuscript. Indrayadi Gunardi: methodology; data analysis and interpretation; drafting the article; supervision; reviewing the manuscript. Elizabeth Fitriana Sari: supervision; reviewing the manuscript.

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General

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Approval

This research is part of student's thesis which was approved on January 22, 2024.

Ethical Declaration

The study received approval from the Research Ethics Commission of the Faculty of Dentistry, Universitas

Trisakti under the reference number 688/S1/KEPK/FKG/7/2023 and was approved on July 24, 2023.

Data Availability

The author will share data for reasonable request

Conflict of Interest

None.

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