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Salivary Thiocyanate Levels and Buccal Mucosal Cells Changes in E-cigarette Users and Traditional Smokers

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Abstract:

Background: The rising use of electronic cigarettes (e-cigarettes) has become a significant public health issue. While e-cigarettes are seen as a safer alternative to traditional smoking, they may still expose users to toxic chemicals that could adversely affect oral health. This study aims to investigate the cytomorphometric changes in buccal mucosal cells among different smoking groups, including e-cigarette vapers, traditional smokers of varying intensities, and non-smokers, and to correlate these findings with salivary thiocyanate levels, a biomarker for cyanide exposure.

Methods: A cross-sectional study included 73 male participants (aged 18-50 years) conducted in Al Muqdadiyah City, Diyala Governorate, Iraq. The participants were classified into five groups: electronic cigarette vapers (ECV, n=25), heavy regular cigarette smokers (Heavy RCS, n=12), moderate regular cigarette smokers (Moderate RCS, n=12), mild regular cigarette smokers (Mild RCS, n=12), and non-smoking controls (NSC, n=12). Salivary thiocyanate levels were assessed using atomic absorption spectrophotometry. Oral exfoliative cytology was utilized, with the cytological evaluation of the buccal mucosal smears focusing on nuclear abnormalities, including total micronuclei (MN), micronucleated cells (MN cells), binucleated (BN) cells, nuclear buds (NB), karyorrhectic (KR) cells, karyolytic (KL) cells, and pyknotic (PK) cells, which were stained using Papanicolaou (PAP) staining. Statistical analyses were performed for group comparisons and correlation tests.

Results: Mean salivary thiocyanate levels in heavy RCS and e-cigarette vapers (5.51 ± 0.153 mM/L and 3.674 ± 0.422 mM/L) were higher than those in non-smokers (1.145 ± 0.15 mM/L), showing a highly significant difference ($p=0.011$) in thiocyanate levels. Additionally, the results indicated a significant increase in all nuclear abnormalities among smokers and e-cigarette users compared to non-smokers ($P < 0.05$). Heavy smokers displayed the most pronounced cytological changes, followed by e-cigarette users, moderate smokers, and mild smokers. However, the

correlation between salivary thiocyanate and cytomorphometric changes was weak and inconsistent across all study groups.

Conclusion:

This study shows that e-cigarette use causes significant oral cellular damage comparable to moderate cigarette smoking, challenging the perception of e-cigarettes as safe alternatives. Both smoking types increased salivary thiocyanate levels and nuclear abnormalities in buccal cells. The weak correlation between biomarkers and cellular damage suggests multiple assessment tools are needed. These findings demonstrate that e-cigarettes pose substantial oral health risks and are not harmless.

Keywords: E-cigarettes, oral buccal cytological changes, salivary thiocyanate, vaping.

Introduction: The landscape of tobacco and nicotine consumption has undergone a significant transformation with the introduction and widespread adoption of electronic cigarettes (e-cigarettes) over the past decade. Initially marketed as safer alternatives to traditional cigarettes and potential smoking cessation aids, e-cigarettes have gained substantial popularity, particularly among young adults and former smokers (Callahan-Lyon, 2014).

This popularity is often fueled by perceptions of reduced harm compared to traditional smoking. However, a growing body of scientific evidence challenges these perceptions, highlighting potential adverse health effects, with the oral cavity being a primary site of concern due to direct exposure to the inhaled aerosol (Haneen, 2024; Ralho et al., 2019). Conventional cigarette smoking is a well-established major risk factor for numerous diseases, including oral cancers and periodontal disease, driven by nicotine addiction and exposure to a myriad of combustion-derived toxicants (Warnakulasuriya et al., 2010). While e-cigarette aerosol lacks many products of tobacco combustion, it is not merely water vapor. It typically contains nicotine, propylene glycol, vegetable glycerin, flavoring agents, and varying levels of potentially harmful chemicals, including carcinogens (e.g., formaldehyde, acetaldehyde), heavy metals, and fine particulate matter (Etter, 2014) The diverse range of devices, e-liquid compositions (including high-concentration nicotine salts), and user behaviours (vaping topography) adds layers of complexity to assessing their health impact (Filippidis et al., 2017) Emerging research increasingly links e-cigarette use to detrimental oral health outcomes. Documented effects include potential periodontal tissue damage, xerostomia (dry mouth), increased risk factors for dental caries, and alterations to the oral microbiome (Irusa et al., 2022; Javed et al., 2017; Ralho et al., 2019). Beyond these clinical observations, understanding the impact at the cellular level within the oral mucosa is crucial for evaluating potential long-term risks, including carcinogenesis.

Salivary thiocyanate has emerged as a valuable biomarker for assessing exposure to tobacco smoke and related toxicants. Thiocyanate (SCN^-) is formed through the detoxification of hydrogen cyanide, a toxic compound present in tobacco smoke, by the enzyme rhodanese in the liver (Schulz, 1984). The measurement of thiocyanate concentrations in saliva provides a non-invasive, reliable method for quantifying tobacco smoke exposure and has been extensively

validated in epidemiological studies(San Gabriel et al., 2020). Unlike cotinine, which primarily reflects nicotine exposure, thiocyanate levels indicate exposure to combustion products and can therefore provide insights into the different exposure profiles associated with traditional smoking versus e-cigarette use(Hovinen et al., 1999). The utility of salivary thiocyanate as a biomarker extends beyond simple exposure assessment. Studies have demonstrated that thiocyanate levels correlate with the intensity and duration of smoking habits, making it particularly valuable for distinguishing between different levels of tobacco use(Kalburgi et al., 2014). Furthermore, thiocyanate measurements can detect exposure to environmental tobacco smoke and have been used to validate self-reported smoking status in clinical and research settings(Sulistyarti et al., 2020).

Cytomorphometric analysis of exfoliated buccal mucosal cells represents another powerful tool for assessing the biological effects of tobacco and nicotine exposure on oral tissues. This non-invasive technique involves microscopic examination of cells naturally shed from the oral mucosa (Noor Saeed Aneed / Ali Khalaf Ali / Maitham Abdel Kazem, 2024), allowing for the detection of cellular and nuclear abnormalities that may indicate genotoxic or cytotoxic damage (Kokila et al., 2021). The buccal mucosa, being directly exposed to inhaled substances, serves as an ideal site for monitoring the early effects of tobacco and e-cigarette use on oral tissues. The cytomorphometric parameters commonly assessed in tobacco-related studies include micronuclei formation, nuclear budding, binucleation, and various forms of cell death, including karyorrhexis, karyolysis, and pyknosis(Babuta et al., 2014a). Micronuclei, in particular, have gained significant attention as biomarkers of chromosomal damage and genomic instability. These small, extranuclear bodies form during cell division when chromosome fragments or whole chromosomes fail to be incorporated into daughter nuclei(Saeed & Younis, 2012). The frequency of micronucleated cells in buccal mucosa has been shown to increase with tobacco use and is considered a reliable indicator of genotoxic exposure (Al-Rawi et al., 2014). Nuclear budding represents another important cytomorphometric parameter, characterized by the formation of small nuclear protrusions that may eventually separate from the main nucleus. This phenomenon is thought to reflect cellular attempts to eliminate damaged genetic material and has been associated with various forms of environmental and occupational exposures(N. Holland et al., 2008). Binucleated cells, characterized by the presence of two nuclei within a single cell, may indicate disrupted cell division processes and have been observed with increased frequency in tobacco users (N. Holland et al., 2008). The various forms of cell death detectable through cytomorphometric analysis provide additional insights into the cytotoxic effects of tobacco and e-cigarette exposure. Karyorrhexis, characterized by nuclear fragmentation, karyolysis, involving the dissolution of nuclear material, and pyknosis, marked by nuclear condensation, all represent different pathways of cell death that may be triggered by exposure to toxic substances(Bolognesi et al., 2013). Previous studies investigating the cytomorphometric effects of traditional cigarette smoking have consistently demonstrated increased frequencies of nuclear abnormalities in buccal mucosal cells of smokers compared to non-smokers (Prakruthi et al., 2018). These changes have

been shown to correlate with smoking intensity, duration, and cumulative exposure, suggesting a dose-response relationship between tobacco use and cellular damage (Krupina et al., 2021). However, the comparative effects of e-cigarette use on buccal cytomorphometry remain less well characterized, with limited studies providing conflicting results regarding the extent and nature of cellular changes associated with vaping (Hasan et al., 2024). The relationship between biochemical exposure markers such as thiocyanate and cellular damage indicators represents an important area of investigation that can provide insights into the mechanisms underlying tobacco-related oral health effects, some studies have reported correlations between salivary biomarkers and cytomorphometric parameters, the strength and consistency of these relationships vary considerably across different populations and exposure scenarios(Prakruthi et al., 2018). The present study was designed to investigate salivary thiocyanate levels and buccal cytomorphometric alterations among e-cigarette users, traditional cigarette smokers of varying intensities, and non-smoking controls. By examining these parameters in well-defined exposure groups, this research aims to provide new insights into the comparative oral health effects of different forms of tobacco and nicotine use and to explore the relationships between biochemical exposure markers and cellular damage indicators.

Material and Methods: This cross-sectional comparative study was conducted in Al Muqdadiyah City, Diyala Governorate, Iraq, from March to June 2024, and it was approved as research involving 73 residents aged 18 to 50 years. Participants included 12 mild regular cigarette smokers (Mild RCS; subjects who had smoked traditional cigarettes for at least six months and smoked fewer than 10 cigarettes daily), 12 moderate regular cigarette smokers (Moderate RCS; subjects who had smoked traditional cigarettes for at least six months and smoked between 10 and 20 cigarettes daily), and 12 heavy regular smokers (Heavy RCS; subjects who had smoked traditional cigarettes for at least six months and smoked more than 20 cigarettes daily). Additionally, 25 subjects were classified as electronic cigarette vapers (ECV), who had used electronic cigarettes for at least six months and vaped at least 125 puffs per day without cigarette use in the previous three months. Lastly, 12 Non-Smoking Controls (NSC) were included, who did not smoke traditional cigarettes or e-cigarettes. Those with other habits (e.g., alcohol, chewing tobacco, etc.), systemic diseases (e.g., diabetes, hypertension, etc.), and other chronic health conditions were excluded from the study. The study included only male participants. Ethical approval was obtained from the University of Baghdad, College of Dentistry (Approval No: 873), and written consent was provided by each participant in the study. Following this process, comprehensive oral examinations will be conducted for all participants.

Buccal mucosal cells were collected using a standardized exfoliative cytology technique. Participants were instructed to rinse their mouths thoroughly with distilled water and to refrain from eating, drinking, or smoking for at least two hours before sample collection. Using sterile disposable cytological brushes(Hadi & Yas, 2020), cells were scraped gently from the buccal mucosa in a consistent manner, applying approximately 10-15 gentle strokes to the middle third of the buccal mucosa on both sides of the mouth. The collected cells were immediately

transferred to pre-labeled, clean glass slides and fixed with 95% ethanol for subsequent cytomorphometric analysis(Ogden et al., 1994). Unstimulated whole saliva samples were collected using the spitting method under standardized conditions(Mohammed et al., 2024). Participants were seated comfortably and instructed to allow saliva to accumulate in their mouths for one minute before expectorating into sterile collection tubes, repeating this until approximately 5 ml of saliva was collected. This process occurred before mucosal smearing. All saliva samples were collected between 9:00 AM and 12:00 PM to minimize circadian variations in salivary composition(Mareim Radhi Abd Al Nabby / Abbas, 2024). Samples were immediately centrifuged and stored at -20°C until analysis(Abdul-Jabbar & Hoobi, 2025; misanjas et al., 2024). Fixed buccal cells were stained using the Papanicolaou staining technique, which provides excellent nuclear and cytoplasmic detail necessary for accurate cytomorphometric evaluation. The staining procedure involved sequential treatment with hematoxylin for nuclear staining, followed by counterstaining with eosin and other chromatic agents to enhance cellular contrast and morphological detail. The slides were carefully examined under a light microscope using a zigzag technique, counting 1,000 cells per slide (Alahmed et al., 2016). The cytomorphometric analysis included the evaluation of multiple nuclear abnormalities, each representing different aspects of cellular damage and dysfunction. Micronuclei (MN) were identified as small, round, extranuclear bodies with the same staining characteristics as the main nucleus, typically measuring 1/16 to 1/3 the diameter of the main nucleus. Micronucleated cells were counted separately to provide information about the frequency of cells containing one or more micronuclei. Binucleated (BN) cells were identified as cells containing two distinct nuclei of approximately equal size within a single cytoplasm. Nuclear buds (NB) were characterized as small nuclear protrusions connected to the main nucleus by a narrow chromatin bridge. Karyorrhectic (KR) cells showed evidence of nuclear fragmentation with multiple nuclear fragments of varying sizes. Karyolytic (KL) cells exhibited nuclear dissolution with faint or absent nuclear staining. Pyknotic (PK) cells were characterized by nuclear condensation with intensely stained, shrunken nuclei. The frequencies of cytological biomarkers were scored based on the criteria from Tolbert et al. (1992)(Tolbert et al., 1992). Salivary thiocyanate concentrations were determined using atomic absorption spectrophotometry, a well-established method for thiocyanate quantification in biological samples(Degiampietro et al., 1987). The analytical procedure involved several critical steps to ensure accuracy and reproducibility. Saliva samples were first thawed at room temperature and centrifuged at 3000 rpm for 10 minutes to remove cellular debris and particulate matter. The supernatant was then diluted appropriately to bring thiocyanate concentrations within the linear range of the analytical method. Standard solutions were prepared using potassium thiocyanate (KSCN) in distilled water, with concentrations ranging from 0.1 to 10.0 mg/L, to establish a calibration curve (Hovinen et al., 1999). The atomic absorption spectrophotometer was operated under optimized conditions, with measurements performed at the characteristic wavelength for thiocyanate detection(Chebotarev et al., 2019). Quality control measures included the analysis of blank samples, duplicate

measurements, and the use of certified reference materials to ensure analytical accuracy. The spectrophotometric method utilized the formation of iron(III)-thiocyanate complex, which exhibits characteristic absorption properties that allow for the quantitative determination of thiocyanate concentrations (Sulistyarti et al., 2020). All data were converted into a computerized file format without noise by checking for errors using mean and rational data sampling through cleaning methods.

Sample size computation: The sample size computation was conducted using statistical analyses performed by utilizing a statistical package for social sciences (SPSS) version 28 with Microsoft Excel 2019. The findings of the whole number of participants during the period from March 2024 to June 2024 were 73. The α error was 0.05, and the power was 0.90 for sample size computation. Frequency distribution for selected variables describing the detailed participants with samples was applied. The whole test was distributed as variables introduced by means, standard deviation (SD), and standard error mean. A one-way ANOVA test was used to compare the groups, and post-hoc analyses, including Duncan's test, were conducted at a significant level of 0.05.

Results: Out of the 73 participants, the age factor can be divided into three main groups: (18-29), (30-39), and (40-50) years, which can be efficiently dealt with. The most affected age group was (18-29 yrs) (21) participants of ECV, followed by (7) participants for the age group (18-29 yrs) of Non-smoker control. It can be observed that there is a diverse age distribution, with a distinguished concentration of younger individuals (18-29 yrs) in the ECV group, while the heavy RCS group has a higher frequency (7) of individuals in the age group (30-39 yrs). As shown in Table 1.

Table 1: Frequency distribution of age in the study groups

Groups	Age groups			
	18-29 years	30-39 years	40-50 years	Total
ECV	21	2	2	25
Heavy RCS	2	7	3	12
Moderate RCS	5	4	3	12
Mild RCS	5	5	2	12
Non-Smoker Control	7	3	2	12
Total	40	21	12	73

Salivary Thiocyanate Levels. The comparison of thiocyanate levels across five groups indicated varying levels of exposure to tobacco-related substances. Group 2 (Heavy RCS) has the highest mean thiocyanate level at 5.51 ± 0.153 (a), significantly higher than the other groups. Group 1 (ECV) follows with a mean of 3.674 ± 0.422 (b), indicating a notable drop in exposure. Both Groups 3 (Moderate RCS) and 4 (Mild RCS) show similar and lower means of 2.881 ±

0.208 (c) and 2.785 ± 0.02 (c), respectively. The Control group (non-smokers) has the lowest mean level at 1.145 ± 0.15 (d). The ANOVA test results in a p-value of 0.011, confirming a highly significant difference among the groups. The letter designations clearly show that Group 2 is significantly different from all others, while Groups 1, 3, and 4 are comparable but lower than Group 2. See Table 2 and Figure 1.

Table 2: Comparison across groups according to the thiocyanate levels, mM/L

Groups / Thiocyanate	Group1 ECV	Group2 Heavy RCS	Group 3 Moderate RCS	Group 4 Mild RCS	Control Non-Smoker
Mean ± S.D.	3.674 ± 0.422 b	5.51 ± 0.153 a	2.881 ± 0.208 c	2.785 ± 0.02 c	1.145 ± 0.15 d
ANOVA test P-value	3.446 0.011 S				

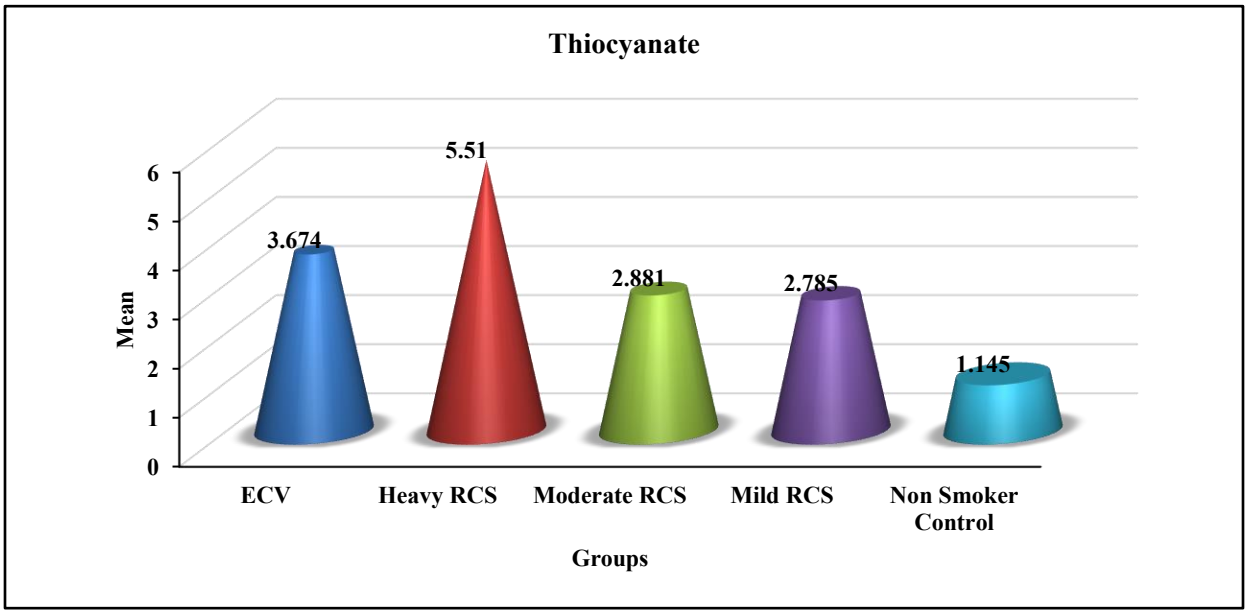

Figure 1. Comparison among groups according to the thiocyanate(mM/L) level.

Buccal Cytomorphometric Findings. In this study, the slides were scaled under 40x magnification using a light microscope; complete slides were observed in a zigzag design for the presence of MN and other nuclear anomalies, BN, N-Buds, KL, KR, and PK as displayed in Figures 2 to 8.

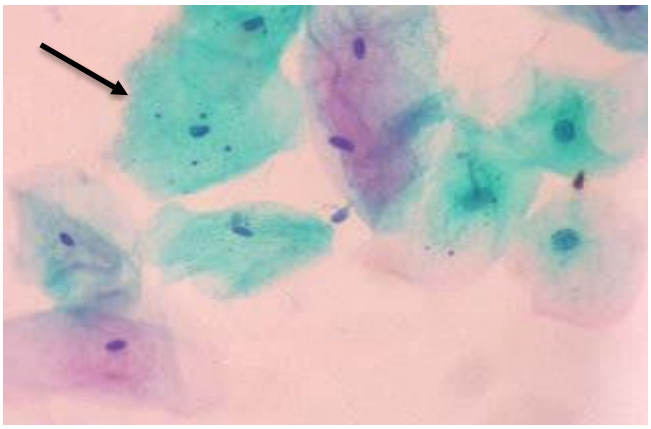

Figure 2. A microscopic view of buccal mucosal cells of E. cigarette users in a smear stained by the PAP method reveals the presence of four MNs in the cell under 40x.

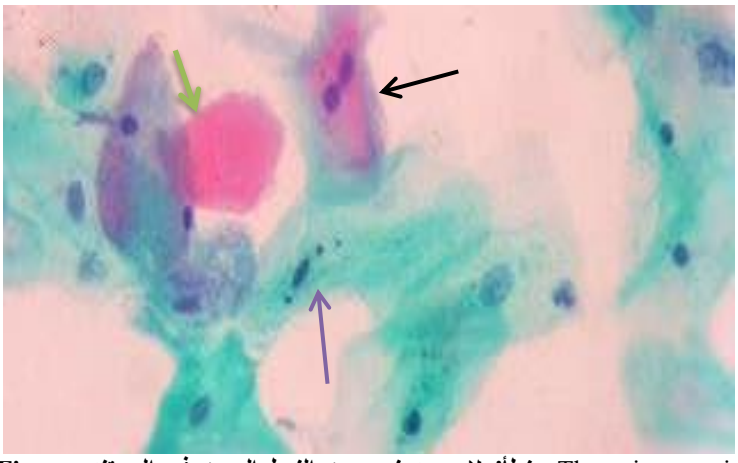

Figure .خطأ! لا يوجد نص من النمط المعين في المستند. The microscopic picture revealed
MN, BN, and KL cells in heavy RCS (X40)

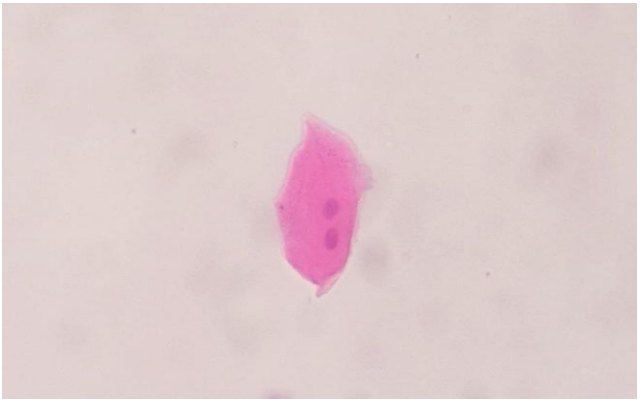

Figure 4. Binucleated buccal cell in (ECV) (X40)

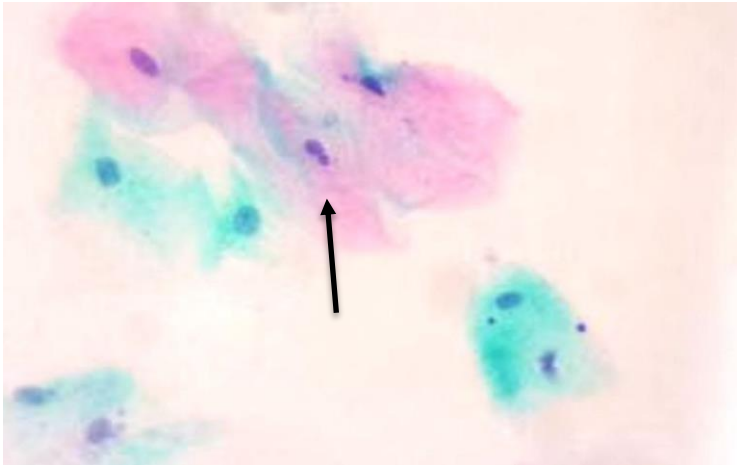

Figure 5. Nuclear Buds (N-buds) in heavy(RCS) (X40).

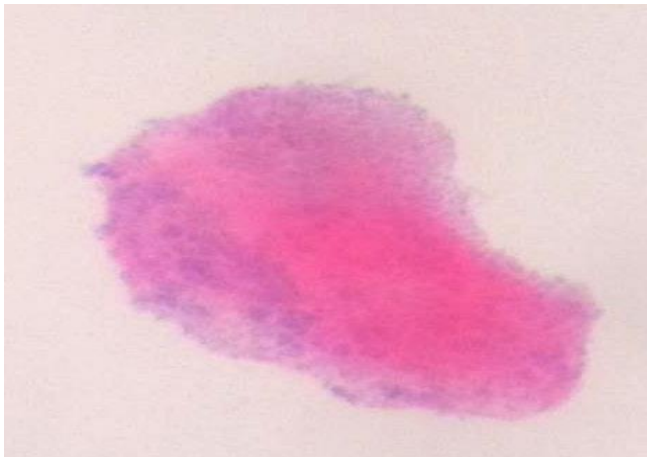

Figure 6. Karyolytic buccal cell (X40)(zoom in)

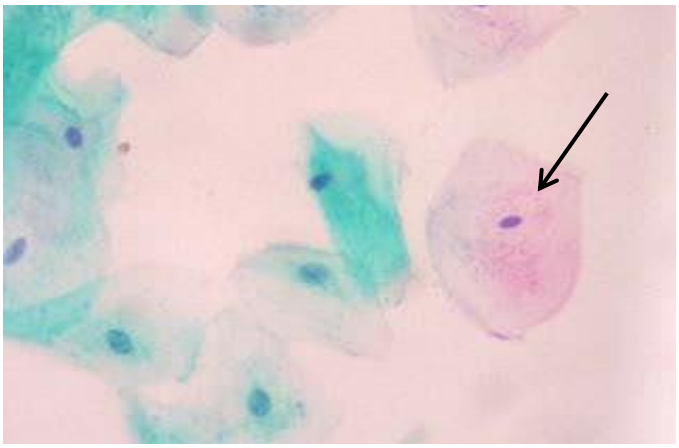

Figure 7. Karyorrhectic buccal cell in (ECV) (X40)

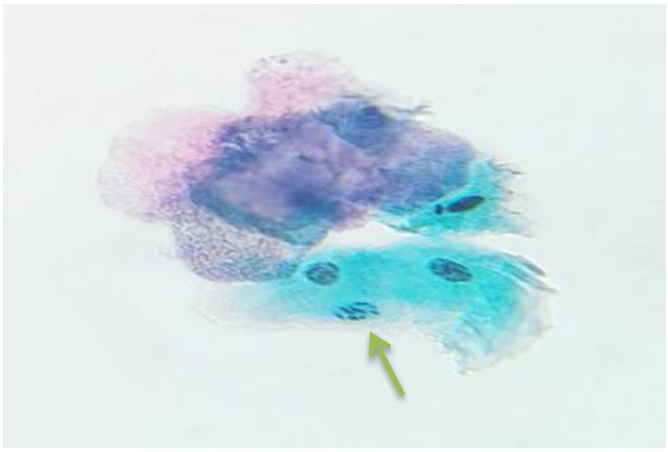

Figure 8. Pyknotic buccal cell in (ECV) (X40)

The mean frequencies (± Standard Deviation, S.D.) of nuclear anomalies per 1000 cells observed in the oral buccal mucosa for each study group are presented below. Statistical comparisons were performed using ANOVA, followed by Duncan's post-hoc test where appropriate.

Micronucleus (MN) levels and Micronucleated (MN) Cells

MN Levels: The comparison of MN levels among the five groups in the oral buccal mucosa site showed significant differences. The ANOVA test indicates a highly significant difference among at least one pair of groups with a p-value of 0.002. The Duncan post-hoc test confirms that groups with different letter designations are statistically distinct. Group 1 (ECV) shows a mean micronucleus level of 8.92 ± 0.988 (a), while Group 2 (Heavy RCS) has a mean of 9.333 ± 1.303 (a). Both groups are statistically similar, as indicated by the same letter designation. Groups 3 (Moderate RCS) and 4 (Mild RCS) display lower means of 6.08 ± 1.145 (b) and 6.33 ± 1.075 (b), respectively, demonstrating significant differences from Groups 1 and 2. The Control group (non-smoker) exhibits the lowest mean of 2.58 ± 0.313 (c), highlighting minimal exposure effects: Table 2 and Figure 9. **MN Cells**: The mean frequency of micronucleated cells also varied significantly across groups (ANOVA, p = 0.007). The Heavy RCS group had the highest mean (6.33 ± 0.881), followed by the ECV group (5.56 ± 0.596). The Moderate RCS (5.17 ± 0.991) and Mild RCS (4.42 ± 0.83) groups had lower means, and the NSC group had the lowest mean (1.92 ± 0.229). The Duncan Post-hoc tests revealed specific inter-group differences (Table 2, Figure 9).

Binucleated (BN) Cell:

The comparison of BN cells among the five groups in the oral buccal mucosa revealed significant cellular response differences. Group 2 (Heavy RCS) has the highest mean count of BN cells at 3.083 ± 0.587 (a), indicating a marked increase compared to the other groups. Group 1 (ECV) shows a mean of 2.16 ± 0.505 (b), while Groups 3 (Moderate RCS) and 4 (Mild RCS) both present mean counts of 2.08 ± 0.434

(b) and 2.08 ± 0.452 (b), respectively, suggesting that they are not significantly different from each other or Group 1. The Control group (non-smokers) exhibits the lowest mean of 0.666 ± 0.322 (c), reflecting minimal exposure effects. The ANOVA test yields a p-value of 0.025, confirming a highly significant difference among the groups. See Table 2 and Figure 9.

Nuclear Buds (N-Buds):

The comparison of N-Buds cells among five groups in the oral buccal mucosa indicates significant differences in cellular responses to various exposures. Group 2 (Heavy RCS) has the highest mean count of N-Buds at 1.083 ± 0.531 (a). Group 1 (ECV) follows with a mean of 0.48 ± 0.261 (b), while Group 3 (Moderate RCS) has a mean of 0.58 ± 0.193 (b), and Group 4 (Mild RCS) shows a mean of 0.42 ± 0.193 (b), all indicating no significant difference among these groups. The Control group (non-smokers) has the lowest mean of 0.08 ± 0.083 (c). The ANOVA test yields a p-value of 0.013, confirming a highly significant difference among the groups. The letter designations indicate that Group 2 is significantly different from the other groups, while Groups 1, 3, and 4 show similar responses. See Table 2 and Figure 9.

Karyorrhectic (KR) Cells:

The comparison of KR cells among the five groups in the oral buccal mucosa highlights significant differences. Group 2 (Heavy RCS) shows the highest mean KR levels at 5.25 ± 1.142 (a), followed by Group 1 (ECV) with a mean of 4.6 ± 0.926 (ab), while Group 3 (Moderate RCS) has a mean of 4.33 ± 0.398 (b), and Group 4 (Mild RCS) shows a mean of 3.66 ± 0.405 (c), all reflecting a gradual decrease in KR cell levels. The Control group (non-smokers) has the lowest means at 1.16 ± 0.355 (d). The ANOVA test results in a p-value of 0.019, confirming a highly significant difference among the groups. See Table 2 and Figure 9.

Karyolytic (KL) Cells:

The comparison of KL levels among the five groups in the oral buccal mucosa indicates significant variations. Group 2 (Heavy RCS) has the highest meaning of KL frequencies at 7.833 ± 1.254 (a), Group 1 (ECV) follows with a mean of 6.80 ± 1.325 (b), while Group 3 (Moderate RCS) shows a mean of 6.92 ± 1.151 (b), indicating no significant difference between these groups. Group 4 (Mild RCS) has a lower mean of 4.58 ± 0.434 (c), and the Control group (non-smoker) has the lowest mean of 1.33 ± 0.313 (d). The ANOVA test yields a p-value of 0.009, confirming a highly significant difference among the groups. The letter designations indicate that Group 2 is significantly different from all other groups, while Groups 1 and 3 show similar KL levels. See Table 2 and Figure 9.

Pyknotic (PK) Cell levels:

The comparison of PK cell levels across five groups in the oral buccal mucosa highlights significant differences. Groups 1 (ECV) and 2 (Heavy RCS) exhibit similar

high mean PK levels at 3.32 ± 0.336 (a) and 3.416 ± 0.515 (a), respectively. In contrast, Groups 3 (Moderate RCS) and 4 (Mild RCS) show lower mean levels of 2.916 ± 0.279 (b) and 2.833 ± 0.271 (b), respectively. The Control group (NSC) has the lowest means of 1.166 ± 0.297 (c). The ANOVA test results in a p-value of 0.016, confirming a highly significant difference among the groups. The letter designations indicate that Groups 1 and 2 are not significantly different from each other, while they are significantly higher than Groups 3, 4, and the Control group. See Table 2 and Figure 9.

Table 2: Comparison of Cytological Parameters for Buccal Mucosa Among Study Groups.

Cytological Parameter	ECV	Heavy RCS	Moderate RCS	Mild RCS	Non-Smoker Control	P-value
MN	8.92 ± 0.988 a	9.333 ± 1.303 a	6.08 ± 1.145 b	6.33 ± 1.075 b	2.58 ± 0.313 c	0.002 S
MN Cells	5.56 ± 0.596 ab	6.33 ± 0.881 a	5.17 ± 0.991 b	4.42 ± 0.83 c	1.92 ± 0.229 d	0.007 S
BN	2.16 ± 0.505 b	3.083 ± 0.587 a	2.08 ± 0.434 b	2.08 ± 0.452 b	0.666 ± 0.322 d	0.025 S
N-Buds	0.48 ± 0.261 b	1.083 ± 0.531 a	0.58 ± 0.193 b	0.42 ± 0.193 b	0.08 ± 0.083 c	0.013 S
KR	4.6 ± 0.926 ab	5.25 ± 1.142 a	4.33 ± 0.398 b	3.66 ± 0.405 c	1.16 ± 0.355 d	0.019 S
KL	6.80 ± 1.325 b	7.833 ± 1.254 a	6.92 ± 1.151 b	4.58 ± 0.434 c	1.33 ± 0.313 d	0.009 S
PK	3.32 ± 0.336 a	3.416 ± 0.515 a	2.916 ± 0.279 b	2.833 ± 0.271 b	1.166 ± 0.297 c	0.016 S

Note: Different letters indicate statistically significant differences between groups according to Duncan's multiple range test (post hoc test). S: Significant.

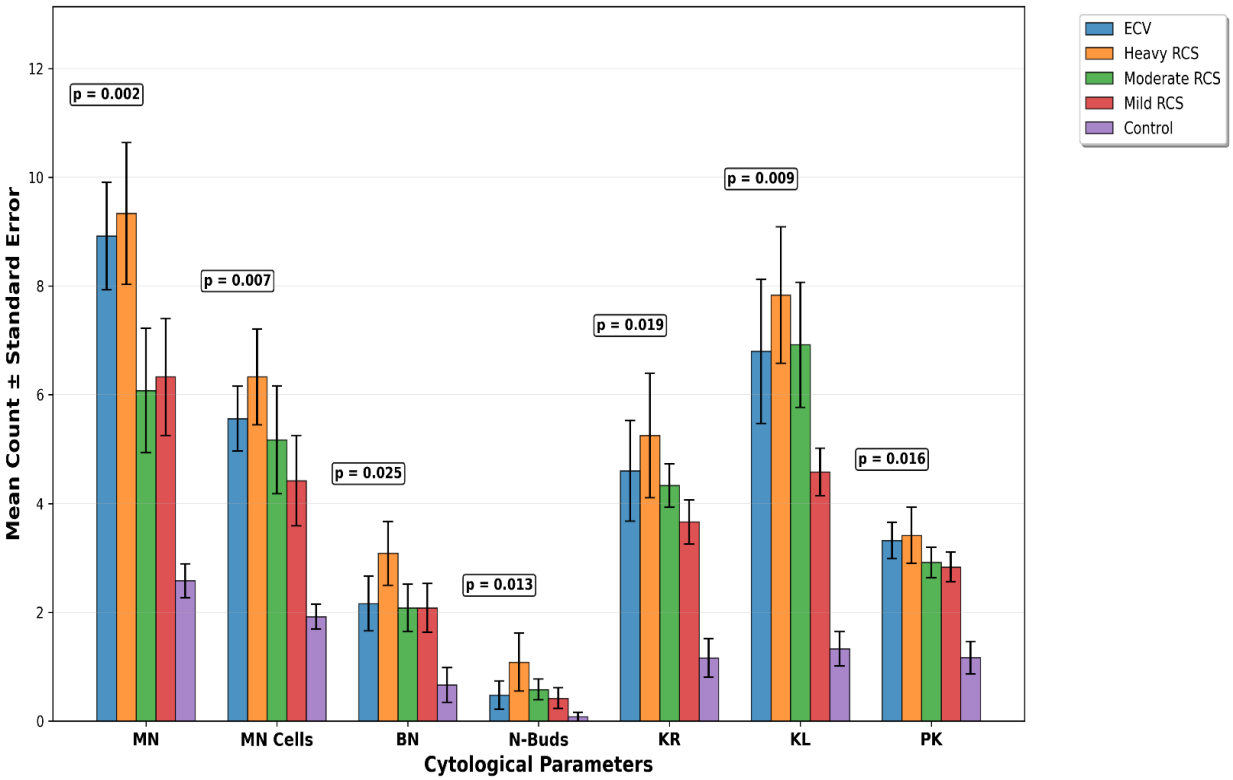

Figure 9: Cytological Parameters in Buccal Mucosal Cells Across Study Groups

Correlation Analysis Between Thiocyanate Levels and Cytomorphometric Parameters

The whole calculations of the mean, SD, and computing the P-value of cytological findings of oral buccal mucosa and salivary thiocyanate level findings for each group are done. Correlations of cytological results and salivary thiocyanate level findings were achieved with the help of Pearson correlation analysis, which exhibited the correlation coefficient of cytological findings and salivary thiocyanate level findings among groups as weak (negative or positive) and did not show a linear relationship. A significant difference and a weak (negative or positive) correlation were obtained between the cytological findings of oral buccal mucosa and salivary thiocyanate level findings like BN in G1 group, which was a weak negative correlation and a significant difference, (r=-.341) and (P-value=0.046), NB in G2 group that was a strong positive correlation however a highly significant difference (r=.661) and (P-value=0.019) was found, KR in G3 group that was a weak negative correlation and a significant difference, (r=-.564) and (P-value=0.046) and PK in G4 group that was a strong positive correlation and a significant difference, (r=.586) and (P-value=0.045). Furthermore, it has a non-existent relationship between MN and the G2 group (r=-.006). No significant difference among all groups; however, it can be clearly shown a different correlation (negative or positive) between cytological findings of oral buccal mucosa and salivary thiocyanate level findings of the whole results, as shown in Table 3.

Table .خطأ! لا يوجد نص من النمط المعين في المستند. **The correlation coefficient of cytological results of oral buccal mucosa and salivary thiocyanate level.**

Cytological kind		Salivary thiocyanate level				
		Groups				
		G1	G2	G3	G4	G5
MN	r	-.087	-.006	-.092	.372	.158
	P-value	.679	.985	.777	.234	.623
MN cell	r	-.122	.233	-.038	.326	.280
	P-value	.561	.467	.905	.301	.378
BN	r	-.341	-.393	.050	.117	-.287
	P-value	.046	.206	.877	.718	.366
N-Buds	r	-.172	.662	-.233	.557	-.218
	P-value	.412	.019	.467	.048	.496
KR	r	-.283	-.226	-.564	.120	.485
	P-value	.171	.480	.046	.710	.110
KL	r	.163	.107	.144	.261	-.085
	P-value	.435	.741	.656	.412	.792
PK	r	-.316	-.166	.370	.586	-.171
	P-value	.123	.606	.236	.045	.595

Discussion: This study investigated the incidence of cytological changes in buccal mucosal cells among e-cigarette users compared to traditional cigarette smokers of varying intensity and non-smokers and correlated these findings to the salivary thiocyanate levels. The results reveal valuable insights into the potential genotoxic and cytotoxic effects of e-cigarette use on oral mucosal cells. The findings show a significant elevation in salivary thiocyanate levels in both e-cigarette vapers and traditional cigarette smokers compared to non-smokers, confirming substantial exposure in these groups. The markedly higher thiocyanate levels in heavy RCS compared to all other groups, including ECV, represent a key finding of this study. This difference likely reflects the higher exposure to hydrogen cyanide in conventional cigarette smoke compared to e-cigarette aerosol. Hydrogen cyanide is primarily produced during the combustion of tobacco and is largely absent or present in much lower concentrations in e-

cigarette aerosol, which is generated through heating rather than combustion (Farsalinos et al., 2015). These findings are aligned with those reported by Flieger et al. (Flieger et al., 2019), who found significantly elevated salivary thiocyanate levels in tobacco smokers compared to non-smokers, with e-cigarette users showing intermediate levels. Their study reported thiocyanate concentrations in the range of 121.25–187.54 mg/L for tobacco smokers, (121.24–244.11 mg/L) for e-cigarette smokers, and (33.03–79.49 mg/L) for non-smokers. While the absolute values differ from this study due to methodological differences, the relative pattern is consistent. The elevated thiocyanate levels in ECV compared to non-smoking controls, despite the absence of combustion in e-cigarettes, are an intriguing finding. This may be attributed to several factors, including:

(1) Previous smoking history among e-cigarette users, as thiocyanate has a relatively long half-life of 10-14 days (Scherer, 2006).
(2) Potential exposure to cyanide compounds from certain e-liquid components or their thermal degradation products (Sleiman et al., 2016).
(3) Dual use or occasional conventional cigarette smoking among self-reported exclusive e-cigarette users.

The gradient of thiocyanate levels across the smoking intensity groups (heavy > moderate ≈, mild) further supports the dose-dependent relationship between cigarette consumption and exposure to combustion-derived toxicants. The similar levels between moderate and mild RCS groups suggest a potential threshold effect or limitations in the sensitivity of thiocyanate as a biomarker at lower smoking intensities. The presence of micronuclei in oral epithelial cells is considered a reliable biomarker of genotoxic damage and chromosomal instability (Bolognesi et al., 2015). Micronuclei formation occurs when chromosome fragments or whole chromosomes fail to incorporate into daughter nuclei during cell division, resulting in small, extranuclear chromatin bodies (Fenech et al., 2011). The comparable MN levels between ECV and heavy RCS groups are particularly noteworthy and suggest that e-cigarette aerosols may induce genotoxic damage like that caused by heavy traditional smoking. This challenges the perception that e-cigarettes represent a substantially safer alternative to conventional cigarettes. Bustamante et al. (Bustamante et al., 2018) similarly reported significant DNA damage in oral cells of e-cigarette users, though their study found slightly lower levels compared to conventional smokers. The increase in the number of MN levels supplies evidence that tobacco users of any form can be at substantial risk for emerging oral cancer compared to the non-smoking group (Szukalska et al., 2020). It can be observed that the percentage decrease of MN cells in the buccal mucosa of the nonsmokers group; however, the highest mean percentage was found in Heavy RCS (Group 2). This pattern is consistent with the findings of a previous study by Upadhyay et al. (Upadhyay et al., 2019). MN cells indicate how many cells are affected (genotoxic exposure), while MN count suggests the severity of damage (cells with multiple micronuclei indicate more severe damage). In all groups, MN counts were higher than MN cells, indicating that some cells contained multiple micronuclei. This suggests more intense genotoxic damage. Sarkar et al. (2021)

(Krupina et al., 2021) reported that cells with multiple MN show higher chromosomal instability. Christine J. Ye et al. (Ye et al., 2019) emphasized that high MN/MN cell ratios may be predictive of carcinogenic transformation.

Beyond micronuclei, this study evaluated several other nuclear anomalies, including binucleated (BN) cells, nuclear buds (N-Buds), karyorrhexis (KR), karyolysis (KL), and pyknosis (PK). In most parameters, heavy RCS exhibited the highest frequencies of these anomalies, followed closely by ECV, with moderate and mild RCS showing intermediate levels, and non-smoking controls consistently displaying the lowest frequencies. Binucleated cells, which result from cytokinesis failure following nuclear division, were significantly more prevalent in heavy RCS (3.083 ± 0.587 in buccal mucosa) compared to other groups. The findings of BN in the lateral surface of the tongue across groups are similar to findings in buccal mucosa, which are significantly higher in the ECV (2.72 ± 0.897) and Heavy RCS groups (2.833 ± 0.549). This was in line with the study by Biswas et al. (Biswas et al., 2014), which revealed the statistical significance of BN for smokers and nonsmokers.

Nuclear buds, representing gene amplification or elimination of nuclear material, also showed a similar pattern, with heavy RCS exhibiting the highest levels (1.083 ± 0.531 in buccal mucosa). These findings align with those of Holland N, et al. (Nina Holland et al., 2008), who reported increased frequencies of nuclear anomalies in smokers compared to non-smokers, with a dose-dependent relationship. These findings suggest that higher exposure to tobacco-related substances induces DNA damage and repair attempts, as reflected by the increased formation of N-Buds, a highly significant difference in N-Buds among the five groups that was in line with the study by Babuta et al. (Babuta et al., 2014b). Karyorrhexis, karyolysis, and pyknosis, which represent different stages of cell death or nuclear degeneration, were also significantly elevated in tobacco users compared to controls. The high prevalence of these anomalies in the ECV group, often comparable to heavy RCS, suggests that e-cigarette aerosol may induce cellular damage and death mechanisms similar to those triggered by conventional cigarette smoke. Antonija Tadin et al. (Tadin et al., 2024) similarly reported increased markers of cellular damage in e-cigarette users, though they found these to be generally lower than in conventional smokers. The overall pattern of nuclear anomalies observed in this study indicates that both e-cigarette vaping and traditional smoking induce significant cytotoxic and genotoxic effects on oral mucosal cells, with the severity generally correlating with exposure intensity. The absence of strong correlations between thiocyanate levels and cytological parameters across most groups suggests that hydrogen cyanide exposure may not be directly linked to the specific cellular changes measured in this study. In the oral, buccal mucosa, significant correlations were observed between thiocyanate and BN ($r = -0.341$, $p = 0.046$) in the ECV group, N-Buds ($r = 0.661$, $p = 0.019$) in the heavy RCS group, and PK ($r = 0.586$, $p = 0.045$) in the Mild RCS group. This is consistent with research by Khlystov and Samburova (Khlystov & Samburova, 2016), who suggested that other components of tobacco smoke and e-cigarette aerosol, such as aldehydes and reactive oxygen species, may play more significant roles in inducing cellular

damage, and another study by Silwal et al (Silwal et al., 2020) that evaluated salivary thiocyanate levels in tobacco smokers and nonsmokers and discovered a fairly weak relationship between thiocyanate levels and cytological change in the oral mucosa.

Conclusions: This study demonstrates that both traditional cigarette smoking and e-cigarette use cause significant alterations in salivary thiocyanate levels and buccal cell morphology. E-cigarette users showed cellular damage comparable to moderate cigarette smokers, challenging the perception of e-cigarettes as harmless alternatives. The findings reveal substantial genotoxic and cytotoxic effects in e-cigarette users, including increased micronuclei, nuclear buds, binucleated cells, and cell death markers. While salivary thiocyanate serves as a reliable exposure biomarker, its correlation with cellular damage was weak, suggesting that comprehensive risk assessment requires multiple biomarkers. These results indicate that e-cigarettes pose significant oral health risks and should inform clinical practice and public health policy.

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Conflicts of Interest Statement.....

Manuscript title: ... Salivary Thiocyanate Levels and Buccal Mucosal Cells Changes in E-cigarette Users and Traditional Smokers

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