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Research Article

The Influence of the Electronic Cigarette on the Buccal Mucosa

Taisa Maria Rodrigues Vilardi[1], Bruna Maria Rodrigues Vilardi[1], Gabriela Lopes dos Santos[3], Ana Paula Fernandes Ribeiro[3], Lucas Farineli[2] and Nancy Alfieri Nunes[1]*

[1]PhD in Oral Diagnosis, Lins School of Dentistry, Methodist University of Piracicaba, Brazil

[2]Dental Surgeon, Lins School of Dentistry, Methodist University of Piracicaba, Brazil

[3]Dentistry Undergraduates, Lins School of Dentistry, Methodist University of Piracicaba, Brazil

***Corresponding Author:** Nancy Alfieri Nunes, PhD in Oral Diagnosis, Lins School of Dentistry, Methodist University of Piracicaba, Brazil.

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Abstract

Currently, there has been an increase in the marketing of alternative methods to quit smoking. Electronic cigarettes are part of these methods. This study evaluated demographic data, systemic conditions, types of smoking and associated risk factors in volunteer patients, in the period of August 2015, through the analysis of a questionnaire. The 18 individuals were divided into 3 groups: control group, traditional cigarette smokers and electronic cigarette smokers. In addition, the research analyzed the cells collected from the buccal mucosa and the lateral border of the tongue by means of the Micronucleus Technique (MN). Statistical tests of Kolmogorov-Smirnov, Student's t- test and ANOVA (5%) were applied to the results. The male gender comprised 66.6% of the sample, white adults 77.7%, non-alcoholics 88.8%, median age was 34 years. The control group, electronic cigarette smokers and traditional cigarette smokers with regard to MN count showed a statistical difference between the control and traditional cigarette groups with the electronic cigarette group ($p = 0.039$) for MN found in the buccal mucosa. For the tongue, there was difference between the control group and the other groups ($p = 0.000$). It was concluded that electronic cigarettes are devices similar to traditional cigarettes, mainly in their use. The individuals who make use of these devices are predominantly young male adults. It was observed that the cells collected from the buccal mucosa and lateral tongue border of these patients had a higher incidence of micronucleus in electronic cigarette smokers. It is suggested that the available nicotine is absorbed, also, in the oral mucosa, attacking more strongly the tongue mucosa. It is concluded that there is a need for future research that evaluates the effect of electronic cigarette use in the long term both in the physical, chemical and psychological spheres.

Keywords: *Electronic Cigarettes; Oral Health; Smoking Habit*

Introduction

The use of tobacco causes a high mortality rate in the world. It is estimated that during the 20th century, one hundred million people died due to its consumption, with smoking accounting for 12% of adult mortality worldwide. Millions of people die each year from tobacco-related illnesses, and the estimate is that, given current consumption, that number is increasing, with 70% of such losses occurring in developing countries [1,2].

The action of cigarette components, hydrocarbons, nitrosamines and heat combined, favor deleterious action on the buccal mucosa [3-5]. This factor can be potentiated when associated with alcoholism [6-10].

Additionally, it was observed that in smokers there are significant cardiovascular alterations, besides other alterations in the respiratory, hematological and nervous systems. It was also noted that in the pediatric sector, passive smoking has induced complications in children's health. The authors suggest that in the literature there is a wide range of drugs that interact with the substances present in the cigarette and undergo biochemical changes that induce aggression [12,13].

Currently, there has been an increase in the marketing of alternative methods to quit smoking. Electronic cigarettes are part of these methods, and there is a significant increase in the use of these devices [12-16]. It has been observed that most users are young adults and of male gender [17,18]. Researchers believe that electronic cigarettes are well accepted for mimicking traditional cigarettes [14,15,19-21], providing during their use movements and patterns of user behavior similar to those of smoking [14,20,22].

Electronic cigarettes, also known as e-cigarettes, can generate lung, throat and mouth irritation. The substances used in these devices, as well as nicotine, vary widely in composition even though they belong to a single manufacturer [23].

Another aspect that should be addressed is that even though indirectly, electronic cigarettes are harmful to health. According with CAHN, SIEGEL [14] research on electronic cigarettes is scarce, including on their impact on the environment, such as the effects on air quality, disposal of the device in the environment and so on. This bias was observed through a literature review, with no other mention of this damaging effect on the environment. The manufacturing method produces pollutant substances for the environment are larger than the manufacture of traditional cigarette. Evaluation of planting, exposure of farmers, soil change should be more carefully researched. Furthermore, the aerosols produced by the evaporation of the liquids that supply these devices are harmful, as well as the chemical decomposition products of the discarded cigarettes that cause damage to the environment.

Worldwide there are difficulties for governmental bodies in classifying electronic cigarettes as tobacco or ancillary medicine in the use of nicotine [24,25]. The lack of supervision and norms on e-cigarettes has favored an increase in the number of users and types of products marketed [26]. In addition, its components, such as refills or cartridges, also have easy access and indiscriminate purchase [18,23,27,28], which has further favored its use [29].

The need for further studies to establish regulations to establish standards for the manufacture and marketing of e-cigarettes and their constituents [5,30-32], including the evaluation of their harmful potential to the oral mucosa, is well-known. Considering the data collected in the literature, it is observed the need for information on the influence of electronic cigarette on health, including in the oral mucosa. Therefore, this research proposes to evaluate exfoliated cells of the oral mucosa of users of electronic cigarettes, through the micronucleus technique, contributing to the knowledge of this product and possible risks that it offers.

Material and Methods

The research was carried out in the premises of the pathology laboratory of Lins School of Dentistry- Methodist University of Piracicaba, which obeyed the ethical and legal norms specified by the Research Ethics Committee of the Methodist University of Piracicaba under number 42.

Thirty individuals were recruited, who were invited to attend the Clinic of Stomatology of Lins School of Dentistry - UNIMEP, for clinical examination and identification of the profile. They were invited to participate in the research, obeying the ethical standards of Resolution 196 of the Ministry of Health and Ordinance 466 of 2012. However, only 18 individuals applied to participate in the study. The individuals were divided into three groups: Group I (control) - 6 individuals: non-smokers/non-alcoholics, group II - 6 electronic cigarette smokers; group III - 6 traditional cigarette smokers.

As inclusion criteria, the individuals were: age 18 and older, both genders, smokers of traditional cigarettes and/or electronic cigarettes and non-smokers and non-alcoholic. Among the exclusion criteria were people: younger than 18 years old, users of other drugs concomitant with cigarette smoking, lesions on the buccal mucosa and lateral border of the right-side tongue, who do not complete the research or give up. The Terms of Free and Informed Consent were also submitted and completed by the individuals themselves.

A form was elaborated with the purpose of obtaining personal data and health of each patient, as well as notes on health conditions, tobacco consumption (frequency, intensity and type) and general habits.

The results were submitted to the statistical tests of Kolmogorov-Smirnov and Student's t-test and ANOVA with significance at 5%.

Results

The initial sample consisted of 30 individuals; however, the final sample contained 18 people according to the inclusion criteria, 6 individuals divided into each of the 3 groups: control, smoker and vapers (users of electronic cigarettes). Among the 18 individuals, 12 were males (66.6%), 14 were white (77.7%), 16 were non-alcoholic (88.8%) and the median age was 34 years. The characterization of the sample can be verified in detail in table 1.

Gender	Female	6	33,3
	Male	12	66,6
Ethnic-racial group	White	14	77,7
	Black	1	5,5
	Asian	3	16,6
Age group	< 35 years	12	50
	> 35 years	12	50
Smokers	No	6	33,3
	Electronic cigarette	6	33,3
	Traditional Cigarette	6	33,3
Alcohol	Yes	2	11,1
	No	16	88,8
Changes in the oral cavity	Bruxism	3	16,6
	Leukoplakia	1	5,5
	Malocclusion	8	44,4

Table 1: *Characterization of the sample: demographic data of patients from the Stomatology Clinic of the Faculty of Dentistry of Lins-UNIMEP.*

In the group of smokers, 4 patients reported dyspnea with constancy and 1 patient reported feeling fatigue daily. These patients associated the symptoms with smoking addiction.

As for the associated habits, 8 patients (6 smokers and 1 vaper) frequently used chewing gum and candy, 1 smoker used toothpicks after meals. In addition, 4 smokers presented onychophagia.

The smokers reported dysgeusia and altered smell. The onset of addiction occurred in 83.3% between 17 and 20 years. All smokers claimed to have lived with smoking since childhood; the most common kinship was with siblings (66, 6%).

It was noted that in vapers, 66.6% due to the use of e-cigarettes reported changes in taste and 50% changes in smell.

Considering the total sample (18 patients), 55.5% patients who knew about the electronic cigarette, and the median time they knew the device was 5 years. As for e-cigarette users, the group consisted of patients who used the devices for 1 to 7 years. The median time was 3 years. All vapers claimed to know the health risks associated with e-cigarettes.

The Kolmogorov-Smirnov statistical test was applied, which demonstrated normality of the sample for all variables studied ($p > 0.20$).

When analyzing the exfoliated cells of the buccal mucosa, in both buccal and tongue mucosa, the micronucleus count (MN) did not present statistical differences ($p \geq 0, 355$) (Table 2).

MN	BM	T	p
Mean (SD)	2,8 (2,74)	4,5 (3,99)	0, 355

Table 2: *Mean and standard deviation of MN in buccal mucosa (BM) and tongue (T).*

Table 3 shows the comparison between control experimental groups (nonsmokers), smokers of electronic cigarettes (vapers) and smokers of traditional cigarettes, as regards the micronucleus count in keratinocytes (Figure 1A and 1B). In the data presented, there was a statistical difference between the control and traditional cigarette groups when compared to the electronic cigarette group ($p = 0.039$) for MN found in the buccal mucosa. For the tongue, there was difference between the control group and the other groups ($p = 0.000$).

Location	Control	Traditional Cigarette	Eletronic Cigarette	P
	Mean (SD)	Mean (SD)	Mean (SD)	
Buccal Mucosa	0,5 (0,5)[a]	3,7 (2,21)[a]	4,2 (3,08)[b]	0,039
Tongue	0,7 (0,5) [a]	6,3 (2,92)[b]	6,5 (4,07)[b]	0,000

Table 3: *Comparison of micronuclei between groups.*
SD: Standard Deviation. ANOVA test. Different horizontal letters represent statistical differences (Tukey test).

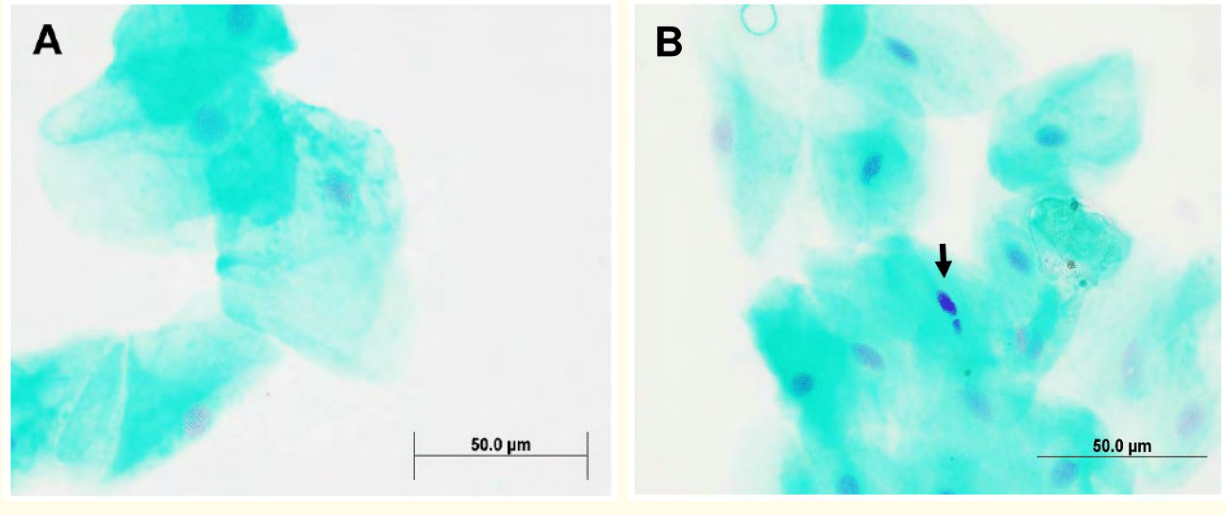

Figure 1: *Photomicrography A - Right-side buccal keratinocytes of the control patient in Fast-Green staining (100x). Photomicrograph B - Right side lateral tongue keratinocytes of the patient group of the electronic cigarette smokers in Fast-Green coloration (100x). Micronucleus presence (black arrow).*

Smoking did not correlate with the amount of MN. There was no difference between traditional cigarette consumption time and present amount of MN ($p \geq 0.334$), regardless of the location of the collection. Also, no statistical difference was observed between the daily quantity of traditional cigarettes and the amount of MN ($p \geq 0.237$), regardless of the location of the collection.

When we observed the electronic cigarette consumption, no statistically significant difference was found when considering MN numbers ($p \geq 0.370$), regardless of the location of the collection.

There was no correlation or difference between MN age ($p \geq 0.246$) nor between sex ($p \geq 0.276$), regardless of the location of the collection.

Discussion

Users of traditional cigarettes are exposed to changes in health [34]. These changes were classified by the World Health Organization [1,2] as changes as epidemic alterations of non-communicable diseases: cardiovascular changes, respiratory system changes, hematological changes [11,35] and oral cancer [36,28].

Margham., *et al*. (2016) [17] found that cells exposed to e-cigarettes significantly reduced cell survival, with increased rates of apoptosis and necrosis, regardless of the nicotine content of the vapor released. The cells also showed significant increase in tail length and accumulation of γ-H2AX foci, indicating larger ruptures of the DNA strand. However, studies that reproduce in vivo and the use of carcinogenic cells in the oral cavity, which are genotypically damaged, are still lacking.

Among the substances present in cigarettes stands out the nicotine that is toxic to cells [37]. This substance, during the act of smoking, produces alteration in the central nervous system by possessing properties considered psychoactive that lead the individual to the addiction. Nicotine may provide withdrawal episodes if the drug is not used [9,38].

Electronic cigarettes are also known as e-cigarettes/e-cigarettes [39] or Electronic Nicotine Delivery Systems (ENDS) [40-42]. These devices have been marketed as a supportive product to quit smoking, and are believed to aid in relieving withdrawal symptoms, and there are no restrictions on use in public places [22,43-47].

The literature review confirmed the fact that electronic cigarettes are devices that mimic the physical appearance of traditional cigarettes [48], with an e-cigarette model that looks different from a traditional cigarette [10,49,50] that was used by 100% of the participating vapers. The research already carried out corroborates to elucidate that electronic cigarettes do not produce high temperatures and smoke, since there is no combustion, and vaporization of liquids occurs through the action of lithium batteries [13,15,16,20,27,49,51-53].

The final sample of the research contained 18 individuals: 6 non-smokers, 6 vapers and 6 smokers. In 2014, D'Ruiz, Graff and Yan [45] evaluated a slightly larger sample of 23 e-cigarette smokers and traditional cigarette smokers. This study similar to ours had predominance of white individuals, but did not evaluate the predominance of sex, nor the presence of addictions like alcoholism. The mean age of the individuals (38.7 years) was close to the results of the current study (34 years).

According to Wang., *et al*. (2015) [18], the use of electronic cigarettes prevails more in male gender as seen in this research (66.6%). Another data with literary support was a greater incidence of the use of e-cigarettes in young people [11,16,38,43,44,48,54].

To date, no studies have been found to evaluate the use of e-cigarettes and their correlation with alcoholism or oral alterations, however, this research did not identify correlations.

Part of the sample, 55.5% of the individuals reported knowing what the electronic cigarette is [18]. The median activity time of the evaluated vapers was 3 years, with no other reports in the literature, but it is known that the knowledge about e-cigarettes is related to their use in the short term [49,25].

Although the micronucleus (MN) technique was considered efficient in evaluating oral mucosal lesion [55-60] no previous work was performed evaluating mouth mucosa cells with vapers, as developed in this study.

The results of this research showed that MN counts in exfoliated cells of the buccal mucosa and lateral border of tongue were not statistically significant ($p \geq 0, 355$). Studies in smokers did not compare the question of collection sites [5,55,58,59,61,62].

In this situation, the micronucleus count, there was a statistical difference between the control and traditional cigarette groups with the electronic cigarette group ($p = 0.039$) for MN found in the buccal mucosa. For the tongue, there was difference between the control group and the other groups ($p = 0.000$) [63,64].

There was no difference between traditional cigarette consumption time ($p \geq 0.334$) or quantity of traditional cigarettes ($p \geq 0.237$) and amount of MN ($p \geq 0.334$), regardless of the location of the collection. Unlike our results, Naderi., *et al*. (2012) [65], observed a higher number of micronuclei in keratinocytes of the oral cavity of smokers when compared to the control group (non-smokers) and others also did not detect difference between smokers with a history of smoking in a short or long period.

The cells collected from the oral mucosa of vapers, regardless of the location of the collection, did not present MN in number that made a statistical difference ($p \geq 0.370$). There was no correlation between the difference between age and MN ($p \geq 0.246$), nor between sex ($p \geq 0.276$), regardless of the location of the collection. In another type of smokeless tobacco, chewing tobacco, Bansal., *et al*. (2012) [55], in a sample of 25 individuals who chewed tobacco, 25 smokers and 25 non-smokers, found a higher number of MN in users of smokeless tobacco compared to traditional smokers and control group. There was a positive correlation between increased frequency of micronuclei and habits of chewing tobacco use. No articles were found investigating the influence of electronic cigarettes on oral mucosa cells.

Conclusion

Faced with the micronucleus count, a higher incidence was observed in e-cigarette smokers than in traditional or non-smokers, young and male individuals. It is suggested that the nicotine available in these devices is absorbed, also, in the oral mucosa, aggressively attacking the mucosa of the tongue.

It is concluded that there is a need for future research that evaluates the effect of electronic cigarette use in the long term both in the physical, chemical and psychological spheres.

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