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Deciphering of the morphology of buccal epithelial cell from smoker and non-smoker group

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Abstract

The environmental and the occupational exposure can cause cytogenetic damage in exfoliated buccal epithelial cells, this damage is frequently tracked using liquid-based cytology (LBC) preparations in the micronucleus (MN) assay. It is possible to study aging markers (predictors) in the oral cavity. Research materials such as oral fluid, gingival fluid, saliva, buccal cells, dental plaque, etc. can be obtained noninvasively. Buccal epithelial cell is the term for the mucous membrane that borders the structures inside the mouth cavity. Oral exfoliative cytology is an easy, painless, and non-invasive technique that uses microscopic examination of cells taken from the surface of oral mucosa. Certain data suggest that biological age and lifestyle can be inferred indirectly from the morphological characteristics of the nuclei of buccal cells. This study aims to elaborate on the morphology of buccal epithelial cells in the smoker and non-smoker group. This prospective study was conducted from July to August 2023. Buccal swab samples were taken from each participant with informed consent. Clean cotton buds were used to create smears from the inner linings of cheek cells in 50 participants, consisting of 28 smokers and 22 non-smokers. Our results show that the apoptosis index in the smoker group is higher than in the non-smokers group. The cytogenetic index shows 7 and 1 between smokers and non-smokers, respectively. The proliferative index shows 57 and 39. The apoptosis index shows 86 and 49. The index of cytogenetic disorder accumulation shows 4.63 and 0.79. The repetitive index shows 26 and 19. This result shows that the abnormality of buccal epithelial cell/cytogenetic damage in the smoker's group was higher than in the non-smokers group.

Keywords: Buccal epithelial; Apoptosis; Cytogenetic index; Smokers

1. Introduction

Buccal epithelial cell is the term for the mucous membrane that borders the structures inside the mouth cavity. This is a moist soft tissue membrane that stretches from the palatopharyngeal folds in the back to the vermilion border of the lips and labial mucosa in the front (Brizuel, 2023). One source of material that can be obtained through noninvasive collection techniques is buccal cells (Feigelson, 2001). Oral exfoliative cytology has been used to identify changes in the oral cavity linked to cancer (Verma, 2015). PCR and other genotype tests can be used to analyze DNA from buccal cells (Aidar, 2007). Over several months, the DNA extracted from buccal cells remains stable at room temperature (Aidar, 2007). Numerous techniques have been employed to procure buccal cells for DNA analysis. These include the following: using cotton swabs, cytobrushes, a modified Guthrie card, and rubbing cheeks against teeth as a means of exfoliating cells (Mulot, 2005).

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Variations in the functional activity of buccal epithelial cells are a good indicator of the body's overall and localized homeostasis (Kiselev, 2013), as well as any deterioration brought on by aging. Because of this, scientists looked into buccal cells to use a variety of techniques to diagnose the body. In a different study, the telomere test was used to measure the rate of aging of an organism using buccal cells. Variations in the functional activity of buccal epithelial cells are a good indicator of the body's overall and localized homeostasis, as well as any deterioration brought on by aging. In both healthy individuals and Alzheimer's disease patients. Buccal cells have been shown to be a viable substitute material for telomere testing. Their non-invasive production process gives them an advantage over lymphocytes. (Zamora, 2015).

Certain data suggest that the biological age can be inferred indirectly from the morphological characteristics of the nuclei of buccal cells. An examination of the literature revealed that, when compared to similar indicators in young people, the number of micronuclei in buccal cytology can increase by 366% in elderly healthy donors, the number of heterochromatins can increase by 45.8%, the number of cells with karyorrhexis can increase by 439%, and the number of cells with a displaced nucleus can increase by 233%. The authors claim that the systemic processes of DNA damage, proliferation, and apoptosis of buccal epithelial cells during aging are reflected in the variations in morphological characteristics of buccal cell nuclei during normal and accelerated aging (Kiselev, 2013; Zamora, 2015).

In order to investigate the cellular and molecular causes of disease, human tissue is necessary. However, because of the possibility of patient trauma, the requirement that trained medical personnel participate in the sampling procedures, or the inaccessibility of tissue, access to patient cells may be restricted. In addition, getting enough tissue for analysis might not be achievable. The typical process for obtaining cells involves taking a tissue sample or venipuncture to draw blood. Due to the invasive nature of both procedures, patients may decline to take part in the research. When there are no medical personnel available, non-invasive methods of collecting material may be used for remote collection. They may also be used in screening programs as part of public health and preventative initiatives. This study aims to elaborate on the morphology of buccal epithelial cells in the smoking and non-smoking groups.

2. Materials and Methods

2.1. Study Design

This prospective study was conducted from July to August 2023, and ethical approval was obtained from the Ethical Committee of Ibnu Sina General Hospital with the registered number 071/070/437.76.46/2021.

2.2. Sample Collection

A total of 50 participants, consisting of 28 smokers and 22 non-smokers, were included in this study. All participants belonged to sewage workers in Ngipik, Gresik, East Java, Indonesia. Buccal swab samples were taken from each participant with informed consent. Clean cotton buds were used to create smears from the inner linings of cheek cells.

2.3. Staining and Microscopic Examination

Staining and microscopic observation of slides were carried out in the laboratory, and acid-fast bacilli smears were conducted based on the previous method (Schluger & Tschöp, 2019). The data were interpreted descriptively.

2.4. Data Analysis

The data were analyzed descriptively. Some indexes regarding the abnormality of epithelial cells were counted, consisting of the cytogenetic index (CI), proliferative index (PI), apoptosis index (AI), the index of cytogenetic disorder accumulation (ACI), and reparative index (RI). Below are the details of the formula for each index:

- **The cytogenetic index** was calculated as the sum of micronuclei.
- **The proliferative index (PI)** was calculated as the sum of binuclear cells.
- **The apoptosis index (AI)** was calculated as the sum of cells with condensed chromatin, karyorrhexis, karyolitic, and apoptosis bodies.
- **The index of cytogenetic disorder accumulation (ACI)** was calculated as CI x PI/AI.
- **The reparative index (RI)** was calculated as the sum of the cells with karyorrhexis.

3. Results

The mean ages of smokers and non-smokers were 41.15 and 47.88 years old, respectively. Among 50 participants, 3 participants (6%) had been diagnosed with T2DM and 17 (34%).

A total of 50 participants, including smokers and non-smokers, were examined for epithelial cells. A total of 3134 epithelial cells were observed, consisting of 2303 basal cells, 536 differentiated cells, 59 pyknotic cells, 44 karyorrhexis cells, 48 karyolitic cells, 148 binucleated cells, 2 cells with buds, and 37 trinucleated cells. The most frequently occurring epithelial type that was found is the basal cell. The morphological type of epithelial cell can be seen in Figure 1 below:

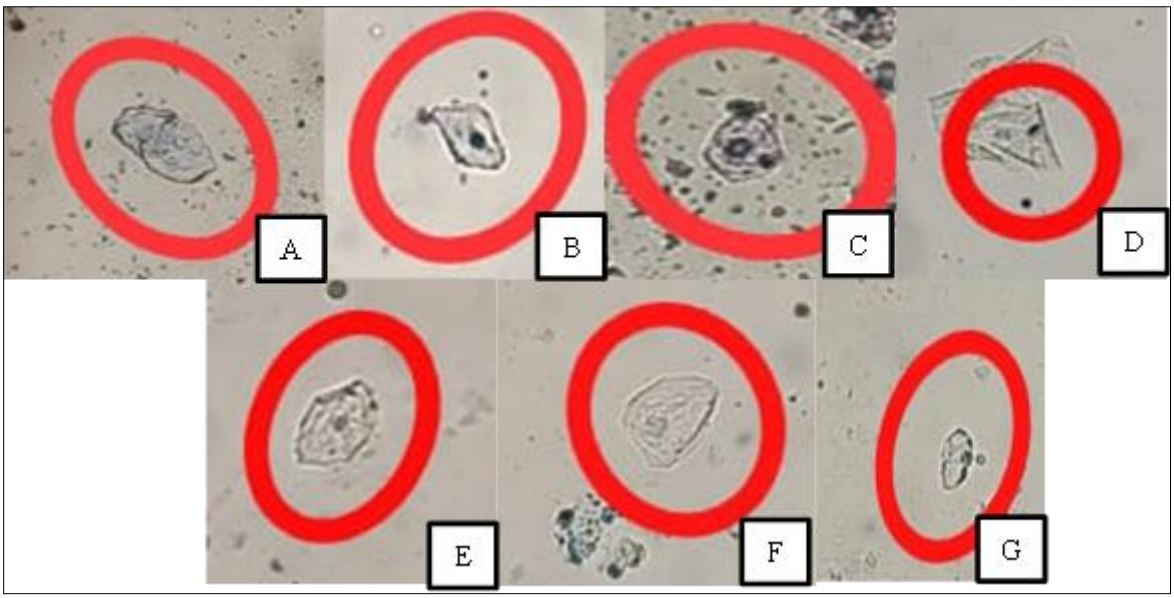

Figure 1 Morphological Type of Epithelial Cell: a.Kariolytic cell; b. Transitional cell; c. Basal cell; d. Micronuclei ; e. Differentiated cell; f. Karyorrhectic cell; g. Pyknotic cell

We examined 28 smoker participants with an average age of 41.15 years old. The morphological types of buccal epithelial cells that were examined consisted of basal cells, differentiated cells, pyknotic cells, karyorrhexis cells, karyolitic cells, binucleated cells, micronuclei, trinucleated cells, and condensed chromatin cells. The morphological type of epithelial cell is shown in Table 1.

Table 1 The morphological type of epithelial cell in smoker group

No.	Age	BC	DC	P	KR	KL	BCC	MNC	TNC	CC	Total
1.	58	6	13	0	0	0	0	0	0	2	21
2.	55	5	5	0	0	0	0	0	0	0	10
3.	61	23	3	2	3	15	0	3	0	0	49
4.	34	17	3	2	2	0	0	0	0	0	24
5.	38	11	10	2	1	0	2	0	2	1	29
6.	50	7	7	0	0	0	0	0	0	0	14
7.	24	80	6	3	1	2	9	0	11	0	112
8.	23	19	2	0	0	9	0	0	0	0	30
9.	26	130	17	2	6	3	25	1	6	2	192
10.	45	10	0	1	0	1	0	0	0	0	12
11.	39	15	17	1	1	0	0	0	0	0	34

12.	45	15	2	0	0	0	0	0	0	0	17
13.	50	30	6	2	3	3	0	1	0	0	45
14.	41	62	1	1	1	4	0	0	0	0	69
15.	26	35	5	2	0	0	2	0	0	0	44
16.	27	17	1	4	0	1	0	0	0	0	23
17.	28	24	2	0	0	0	0	0	0	0	26
18.	25	27	3	0	2	0	0	0	0	0	32
19.	36	85	10	1	0	0	10	0	2	0	108
20.	42	30	5	0	1	9	0	2	0	0	47
21.	50	35	10	2	2	1	0	0	0	0	50
22.	39	32	6	0	0	0	0	0	0	3	41
23.	48	15	3	2	2	0	3	0	0	1	26
24.	46	12	2	1	1	3	0	0	0	0	19
25.	62	80	8	0	0	0	5	0	0	0	93
26.	45	55	17	0	0	0	0	0	0	0	72
27.	58	15	8	0	0	0	0	0	0	0	23
28	56	15	0	0	0	0	1	0	0	0	
Total		907	172	28	26	51	57	7	21	9	
Average		32.39	6.61	1.86	2	4.63	7.12	1.75	5.25	1.8	
Percentage		70.97	13.45	2.19	2.03	0.19	4.46	0.54	0.08	0.25	

*Note: BC: Basal Cell; DC: Differentiated Cell; P: Pyknotic Cell; KR: Karyorrhexis; KL: Karyolitic; BCC: Binucleated Cell; MNC: Micronuclei cell; TNC: Trinucleated Cell; CC: Condensed Chromatin

Table 2 The morphological type of epithelial cell in non-smoker group

No.	**Age**	**BC**	**DC**	**P**	**KR**	**KL**	**BCC**	**MNC**	**TNC**	**CC**	**Total**
1.	37	40	4	2	1	3	0	0	0	0	50
2.	38	35	9	3	0	0	1	0	1	0	49
3.	58	50	11	1	0	0	3	0	0	0	65
4.	31	14	3	0	0	0	0	0	0	0	17
5.	50	33	11	2	0	2	1	0	0	0	49
6.	25	68	4	2	1	0	7	0	1	0	83
7.	69	10	3	1	0	1	0	0	0	0	15
8.	69	6	5	0	0	0	0	0	0	0	11
9.	28	36	5	0	3	3	0	1	0	0	48
10.	43	32	3	0	0	0	3	0	0	0	38
11.	21	23	8	1	0	0	7	0	0	1	43
12.	40	87	19	3	5	7	1	0	0	0	122
13.	40	7	0	1	2	2	0	0	0	0	12
14.	48	17	3	0	0	0	2	0	0	0	22

15.	50	23	3	0	0	1	1	0	0	0	28
16.	73	39	5	0	0	0	0	0	0	0	44
17.	68	70	26	0	0	0	2	0	0	0	99
18.	72	15	10	1	2	1	2	0	0	3	34
19.	60	24	15	0	0	0	2	0	0	0	41
20.	60	13	2	0	5	4	0	0	0	0	24
21.	52	89	33	2		2	7	0	0	0	133
22.	48	1	0	0	0	0	0	0	0	0	1
Total		732	182	19	19	26	39	1	6	4	1028
Percentage		71.2	17.7	1.84	1.84	2.52	3.79	0.09	0.58	0.38	

*Note: BC: Basal Cell; DC: Differentiated Cell; P: Pyknotic Cell; KR: Karyorrhexis; KL: Karyolitic; BCC: Binucleated Cell; MNC: Micronuclei cell; TNC: Trinucleated Cell; CC: Condensed Chromatin

3.1. Apoptosis Index

3.1.1. Cytogenetic index (CI)

In this study, the CI value of the smoker group was 7, while that of the non-smoker group was 1.

- **The proliferative index (PI)** was calculated as the sum of binuclear cells. In this study, the PI value of the smoker group was 57, and the PI value of the non-smoker group was 39.
- **The apoptosis index (AI)** was calculated as the sum of cells with condensed chromatin, karyorrhexis, karyolitic, and apoptosis bodies. In this study, the AI value of the smoker group was 86, and the AI value of the non-smoker group was 49.
- **The index of cytogenetic disorder accumulation (ACI)** was calculated as CI x PI/AI. The ACI value in the smoker group was 4.63, while in the non-smoker group it was 0.79.
- **The reparative index (RI)** was calculated as the sum of the cells with karyorrhexis. The RI value in the smoker group was 26, while in the non-smoker group it was 19.

4. Discussion

We performed this study to explore the various morphological types of epithelial cells in the smoker and non-smoker groups. Our results show that basal cells are the most frequent morphological type of epithelial cells, which were found in both smokers and non-smokers. In the smoker group, basal cells were found in an amount of 70.97%, while in the non-smoker group, they were found in an amount of 71.2%. Basal cells place the highest percentage of epithelial cells in this study, followed by differentiated cells, and the lowest in the smoker group was trinucleated cells (0.08%), while in the non-smoker group it was cells with buds (0.09%). Micronuclei cells in the smokers group (0.54%) had a higher percentage than in the non-smokers group (0.09%). As many as seven micronuclei were identified in four smokers. While in the non-smokers group, there was 1 participant with micronuclei epithelial cells.

The buccal cell was the first line of defense during ingestion or inhalation. Buccal cells have the ability to break down carcinogens (Holland, 2008). The carcinogens found in tobacco mixtures and smoke can either cause genetic damage and mutation of germ cells, which can lead to the accumulation of abnormal genes, or they can cause mutation of somatic cells, which can result in the appearance of micronuclei (MN) (Bansal, 2012). Extranuclear cytoplasmic bodies linked to chromosomal abnormalities are called micronuclei (Palve, 2008; Ramesh, 1998). The buccal mucosa is an available tissue that can be sampled noninvasively without causing stress to subjects. Smoking and chewing tobacco mixtures are risk factors for chromosome damage, which can lead to the appearance of MN-like bodies in exfoliated cells, which are significantly smaller than the main nucleus (Thomas, 2009). With a diameter less than one-third that of the main nucleus, micronuclei are located within the inner half of the cytoplasm, encircling the nucleus (Samatha, 2010). Furthermore, it can be easily extracted from buccal mucosal cells, distinguished from binucleated cells, and recognized by microscopic examination.

Our results show that all indexes, including the cytogenetic index, proliferative index, apoptosis index, the index of cytogenetic disorder accumulation, and the reparative index, are higher in the smoker group than in the non-smoker

group. This study was in line with other reports and showed that heavy smoking had a positive correlation with the micronuclei frequency. The association between the frequency of micronuclei and either daily cigarette consumption or cumulative pack-years of smoking demonstrated a significant positive trend. Every investigated confounder was negative. This is also supported by other studies, which show that smokers had a significantly higher average frequency of buccal cell micronuclei (1.50 ± 0.47%) than nonsmokers (0.55 ± 0.32%). Cells that were collected from female smokers had a higher frequency of micronuclei (1.54 ± 0.42%) than those from male smokers (1.31 ± 0.56%). Gender and age had no effect on the frequency of micronuclei in nonsmokers.

The little chromatin bodies known as micronuclei are formed when acrocentric chromosomal fragments or entire chromosomes condense in the cytoplasm and lag behind during cell division (Znaor, 2003). As a result, it is the only biomarker that makes it possible to assess the clastogenic and aneugenic effects simultaneously in a variety of cell types, all of which are readily identifiable in interphase cells (Speit, 2006). The MN assay has been applied to buccal mucosa cells as a biomarker of genetic damage. Certain lifestyle factors, including alcohol consumption, smoking, vitamin deficiencies, and supplementation, have been linked to cytogenetic damage (Jyoti, 2012). Additionally, smoking may raise the frequency of MN in the buccal epithelial cells that have been exfoliated (de Geus, 2008). It is difficult to distinguish the effects of alcohol from smoking on exfoliated buccal epithelial cells because both substances can raise MN frequencies. Nonetheless, compared to nonsmokers and nondrinkers, the synergistic effects of alcohol and smoking are more obvious. According to our research, smoking cigarettes significantly increased the risk of MN in both the case and control groups. However, there are no statistically significant differences in alcohol consumption between the controls and the cases. Stich, 1983).

The number of cigarettes smoked daily and the length of time spent smoking determine the impact of smoking as a risk factor for oral cancer. Heavy smokers are defined as those who have smoked for ten years or longer and/or more than two packs per day (Ayanian, 1999; Sayette, 2001). According to Shiffman et al. (2002) anyone who smoked more than a pack a day was classified as a heavy smoker. The participants in this study were adults who had smoked for a minimum of ten years and at least one pack per day. Many people have experienced various changes in their oral mucosa as a result of smoking. Numerous pathologies, from benign and curable lesions to oral cancer in the mucous membranes of the mouth, have been linked to smoking (Vellapaly, 2007; Bergstorm, 2003). Patients with lesions in the buccal mucosa, such as epithelial dysplasia, leukoplakia, erythroplakia, and squamous cell carcinoma, were excluded from the study because it focuses on the effects of smoking on normal buccal mucosa (Sham, 2003; Almeida, 2002). In conclusion, buccal swab could be a standard technique to collect a sample for analyzing morphological changes in cells to detect an early stage of oral abnormality development.

5. Conclusion

Our study concluded that the abnormality, or cytogenetic, of epithelial cells in smokers was higher than in non-smokers. The cytogenetic index shows 7 and 1 between smokers and non-smokers, respectively. The proliferative index shows 57 and 39. The apoptosis index shows 86 and 49. The index of cytogenetic disorder accumulation shows 4.63 and 0.79. The repetitive index shows 26 and 19.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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