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Cytomorphometric Changes In The Buccal Mucosal Cell In Smokers In Central Indian Populations.

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Abstract

Background: Smoking is currently the most preventable cause of diseases and death worldwide and is one of the causative risk factors for developing cancer in different organs. Hence, it is necessary to detect potentially malignant lesions at their incipient stage. Oral cancers commonly affects floor of the mouth, soft palate, lateral border of the tongue and other areas of the mouth. **Aim and Objective: T**he present study was the Cytomorphometric Changes In The Buccal Mucosal Cell In Smokers In Central Indian Populations. Material and Methods:A comparative study conducted among smokers of Central India population, was performed on 200 males under the age group of 20-60 years.The subjects were chosen randomly from IPD and OPD patients of Medicine Department of Index Medical College and Hospital, Indore. **Results:** There was significant difference observed between non smoking and smoking for cells with binucleation pyknosis, perinuclear halo, cytoplasmic granules, karyolsis, karyorrhexis, and micronuclei, in buccal mucosal cells but non significance difference was found for cytoplasmic vacuoles between nonsmoking and smokers. similar study of non-smoking populations.**Conclusion:** The present study indicates that almost all cytomorphological findings were high in smokers than non-smokers. Early detection of oral cancers becomes complex as they are mostly innocuous and asymptomatic during their initial stages, Cytomorphometric analysis can be used regularly to detect these cell alterations. Currently, use of exfoliative cytology has increased as an adjunct to screening of precancerous lesions and malignancies of the oral cavity.

Key words: Cytomorphometric, smoker, Buccal Mucosal, Central India

Introduction :

Smoking is currently the most preventable cause of diseases and death worldwide and is one of the causative risk factors for developing cancer in different organs.(1) Hence, it is necessary to detect potentially malignant lesions at their incipient stage. Oral cancers commonly affects floor of the mouth, soft palate, lateral border of the tongue and other areas of the mouth. Malignant changes in the epithelium are early indicators, unlike visible symptoms that can be seen in the mouth at later stages. Therefore, analysis and observation of cytological changes in the epithelium will help with early detection. Buccal mucosa of habitual smokers shows various cytomorphometric changes which can be studied using exfoliate cytology. Cytology screening is considered as fast, safe, non – invasive , and inexpensive method. Oral exfoliative cytology is particularly valuable for mass screening purposes; with a sensitivity of 94%, and specificity of 100%. (2) Cytometry is a technique for characterization and measurement of cells and cellular specifications like: nucleus size, cytoplasm size, nuclear-cytoplasmic ratio, aneuploidy and diploidy analysis of nucleus.(3) These cells can be collected from the epithelial surfaces by lightly scraping the surface, by swabbing, aspirating or washing the surfaces. During malignant conditions or during infection, the exfoliation becomes exaggerated and the epithelial cells show variation in morphology. Such exfoliated cells, when collected and appropriately stained, give information on the living epithelium from which they are derived. These characteristic cellular and nuclear appearances in cells thrown off from healthy epithelium, differ distinctly from those, derived from inflamed or malignant lesions. Thus by studying the alterations in morphology of the exfoliated cells and their pattern, the diagnosis of various pathologic conditions can be made.(4)

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It was believed that chronic exposure to smoking causes alteration in mucosa especially buccal, labial and tongue mucosa, as the fact established that tobacco smoke contains more than 40 known carcinogens such as tar, nitrosamines etc. as well as heat generated by smoking plays important role in mucosal changes. The most common type of oral cancer is squamous cell carcinoma, develops from the stratified squamous epithelium that lines the oral cavity and pharynx. Tobacco use affects mainly the surface epithelium, resulting in changes in the appearance of tissues. Behavioral intervention to quit smoking may be efficient if smokers are assigned a perceptible and visual individual risk of dysplastic changes. Buccal cells are shed spontaneously (e.g. exfoliative cell) and daily from healthy buccal mucosa. The exfoliative buccal cells are end-stage cells of differentiation and seldom display mitotic figures. Tobacco-associated buccal cell changes have been reported to be the biomarkers of disease progression. Presence of two or more of the following features were consistent with atypia: nuclear enlargement, associated with the increased nuclear/cytoplasmic ratio, nuclear hyperchromatism, chromatin clumping with prominent nucleation, irregularity of nuclear membranes, bi or multi-nucleation, increased keratinization.(5)

Materials and Methods:

A comparative study conducted among smokers of Central India population, was performed on 200 males under the age group of 20-60 years.The subjects were chosen randomly from IPD and OPD patients of Medicine Department of Index Medical College and Hospital, Indore. Buccal smears of these patients were processed in the Department of Anatomy. Before starting the study, informed consent of the patients were taken.

Patients were grouped on the basis of different pack year. Three groups were formed, as shown below.
Group A: Pack Year <5
Group B: Pack Year 5-10
Group C: Pack Year >10
Group D will be the control group of 50 samples.

Sample Collection:

The area of buccal mucosa from where the sample has to be collected was dried using a piece of sterile gauze. The exfoliated buccal cells was obtained by scraping the sides of the cheek 3 to 4 times using a wooden spatula. Samples were spread on a clean glass slide and then immediately fixed with fixation spray to avoid exposure to dry air. The slides were then stained with Rapid Papnicoalaou (PAP) staining technique and examined under the light microscope. Various cytomorphological changes like Binucleated Cells, Pyknosis, Perinuclear Halo, Cytoplasmic granulation, Karyolysis, Karyorrhexis, Cytoplasmic vacuoles, and Cells with Micronuclei were observed.

EXCLUSION CRITERIA

Patients wearing denture, underwent or followed radiation or chemotherapy, alcoholic, anaemic, diabetic, those with malignant, premalignant lesion of oral cavity, addicted to other form of tobacco or alcohol or having painful oral lesions were excluded from study. All subjects with history of maxillofacial trauma, oral malignancies, TM joint diseases, was excluded from the study.

INCLUSION CRITERIA

Case group (smokers) included non anaemic and non diabetic male patients with clinically healthy mucosa and having only diabetic male patients with clinically healthy mucosa and having only history of smoking and not received radiotherapy or chemotherapy in last 1 month.
Control group (Non-smoker) consisted of 50 subjects with no history of smoking and without any systemic illness /anaemia and Diabetes. Person with submucosal fibrosis persons in mouth were included in the study.

Results

Table 1. Comparison of binucleation, pyknosis, perinuclear halo, cytoplasmic granules, karyolysis, karyorrhexis, cytoplasmic granules and micronuclei amongst smoker **(cases)** and non-smoker **(control)**

	Non smokers		**Smokers**		**P-Value**
	Mean	SD	Mean	SD	
Binucleations	0.87	0.83	1.64	1.06	0.001 (Significant)
Pyknosis	0.93	0.65	2.76	1.26	0.001 (Significant)
Perinuclear Halo	0.51	0.58	1.47	0.86	0.001 (Significant)

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Cytoplasmic Granules	0.61	0.49	1.67	0.67	0.001 (Significant)
Karyolysiss	0.08	0.34	1.22	0.82	0.021 (Significant)
Karyorrexix	0.06	0.24	0.46	0.57	0.035 (Significant)
Cytoplamic vacuolus	0.081	0.34	0.29	0.43	0.65**(Nonsignificant)**
Micronuclei	0.67	0.77	3.01	0.94	0.034 (Significant)

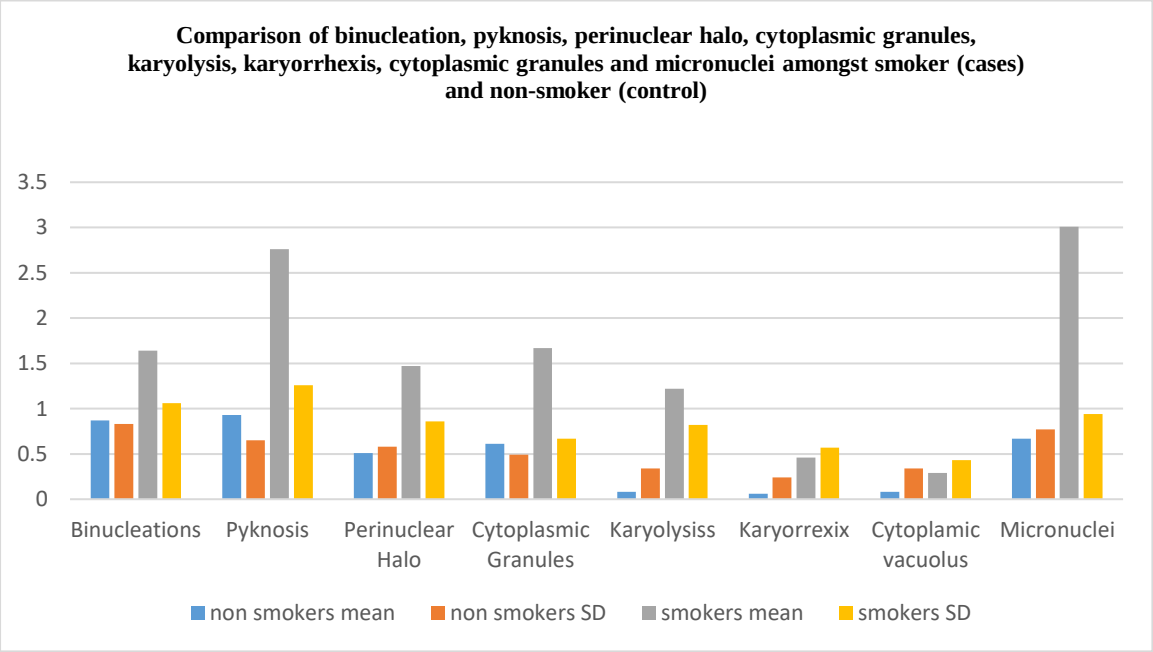

TABLE 2. Comparison of binucleation, pyknosis, perinuclear halo, cytoplasmic granules, karyolysis, karyorrhexis, cytoplasmic vacuoles and micronuclei amongst smoker groups based on pack year

	PACK <5		pack year <5-10		pack year>10	
	MEAN	**SD**	**Mean**	**SD**	**Mean**	**SD**
Binucleations	1.52	0.84	1.64	1.22	1.87	0.94
Pyknosis	2.26	1.09	2.38	1.18	4.04	0.85
Perinuclear Halo	1.36	0.89	1.67	0.84	1.25	0.73
Cytoplasmic Granule	0.73	0.65	1.58	1.04	2.83	1.27
Karyolysiss	0.42	0.6	0.67	0.63	2.45	0.97
Karyorrexix	0.52	0.69	0.44	0.5	0.54	0.58
Cytoplasmic Vacuolus	0.66	0.9	0.58	0.49	0.62	0.57
Micronuclei	1.21	0.63	3.11	0.94	4.5	1.1

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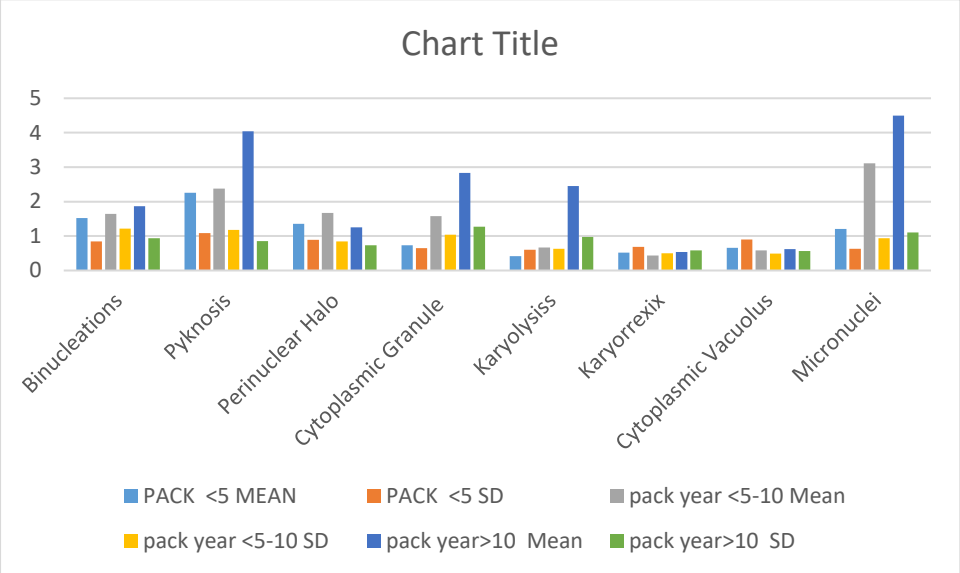

The binucleated cells in non-smokers was 0.87 ± 0.83 while in smoker mean of binucleated cells was 1.64 ± 1.06. mean of pyknosis cells in non-smoking and smoking was 0.93 ± 0.65 and 2.76 ± 1.26. mean value of perinuclear halo in non-smokers and 0.51 ± 0.58 and smokers 1.47 ± 0.86. cytoplasmic granules is mean value of non-smokers 0.61 ± 0.49 smokers 1.67 ± 0.67. mean cells karyolitic cells of non-smokers 0.008 ± 0.34 and smokers 1.22 ± 0.82. mean cells of karyorrhexis in non – smoking and smoking 0.06 ± 0.24 and 0.46 ± 0.57 respectively. mean cells of cytoplasmic vacuoles non-smoking and smoking was 0.081 ± 0.34 and 0.29 ± 0.43 respectively. Mean cells of with micronuclei in non-smoking and smoking was 0.67 ± 0.77 and 3.01 ± 0.94 respectively.

There was significant difference observed between non smoking and smoking for cells with binucleation pyknosis, perinuclear halo, cytoplasmic granules, karyolsis, karyorrhexis, and micronuclei, in buccal mucosal cells but non significance difference was found for cytoplasmic vacuoles between nonsmoking and smokers. similar study of non-smoking populations.
The prameters discussed under table 1 were analysed across different pack years, as shown in table 2. There was significant difference between all pack year groups for pyknosis, cytoplasmic granules, perinuclar halo, karyolysis, and micronuclei but no significant difference was observed for binucleation, karyorrhexis, cytoplasmic vacuoles.

Discussion:

The various cancers prevalent across the world, oral cancer ranks 6th globally. India being second to china in tobacco consumption has the highest rates of oral cancer. Researchers have shown that people tend to neglect their oral hygiene status, as they are not aware about the relationship between systemic disorders and oral hygiene. Due to innocuous and asymptomatic nature of this disease, early detection of oral cancer becomes difficult. Detecting potentially malignant lesions at its incipient has become the need of the hour. Despite the easy accessibility of oral cancer to self-examination, it is usually diagnosed at advanced stages, resulting in poor prognosis, and survival rate among patients.(6) Advanced therapeutic techniques, expensive treatment costs, and complications failed to decrease the morbidity and mortality rates in oral cancer.

Exfoliative cytology is a noninvasive technique that is well accepted by patients and is a good diagnostic modality for the early diagnosis of oral mucosal lesions.(7) Recent advances in technology facilitates the use of reliable quantitative techniques such as cytomorphometry, histometry, and computer-assisted image analyzer.(2)
The only way to cure problem of rising trends of oral cancer is by early detection, histopathological investigation, creating awareness for tobacco cessation and treating tobacco related oral cancer patients especially in their premalignant state, which may be the only hope in reducing burden of it. In our study, cytomorphological changes were studied in buccal mucosal cells. The study was performed on 150 male smokers of central India population. Pyknosis, binucleation, karyolysis, karyorrhexis, and micronuclei were the nuclear changes observed in the buccal mucosal cells.
The presence of two nuclei within a cell is called binucleation. A nuclear abnormality often seen in dysplastic cells. Studies suggest that the frequency of binucleation to be increased in smokers. Therefore, binucleation is

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considered as indicator of cytotoxicity. In the present study a significant increase in the binucleation was seen as compared to the control group. Binucleation was statistically significant between smokers and nonsmokers. The mean value for binucleated cells were found to be 1.64 ± 1.06 in smokers and 0.87 ± 0.83 in nonsmokers. Twinky M Thomas in 2017, Parmar et al in 2019, Sharma, et al 2021 were few of the similar studies on binucleation of buccal mucosal cells of smokers.(8–10). Pyknosis is defined as cells with small shrunken nucleus having high density of nuclear material, which is intense, stained all over. They may represent an alternative mechanism of nuclear disintegration different than process of karyorrhectic cell death stages. Our study found that the pyknosis value to be more in smokers to that of nonsmokers. The occurrence of pyknosis found to be statistically significant and positive correlation obtained with different pack years. In our study, the pyknosis value found to be 1.64±1.06, which was similar to Sharma, et al 2021 and Parmar et al 2019. In their study, the pyknosis value found to be 3.041 ± 0.916 and 2.71 ± 1.74 respectively(9,10). Hugo V et al in their study in 2015, found no statistical significance for pyknosis among smokers and non-smokers.(11)

Perinuclear Halo is a morphologic finding referring to the presence of a vacuolated area that surrounds the nucleus. It results from nuclear shrinking. Our study of perinuclear halo 1.47± 0.86 and Parmar et al 2019 1.45 ± 1.63 similar study was found. Ogenyi et al. 2019 also concluded that there was a gradual increase of perinuclear halo 42.731.(9,12)

Cytoplasmic granules

Disintegration and dissolution of the nucleus of a necrotic cell is karyolysis. These cells give a ghost like appearance on staining. In the current study, the mean karyolytic value in smokers was 1.22 ± 0.82 which was significantly lower in nonsmokers i.e. 0.08 ± 0.34. Navya BN et al. (2017), Parmar et al (2019) and Prihastuti, Et Al (2022) also observed karyolytic cells in buccal mucosal cells of smokers.(9,13,14) Karyorrhexis, is a characterized by the presence of nuclear fragmentation. Nuclear disintegration is a step in apoptosis of cells, resulting in loss of integrity of the nucleus.in our study, Karyorrhexis in smokers was more than nonsmokers, i.e 0.46 ± 0.57 and 0.06 ± 0.24, respectively. Parmar et al 2019 and Yarmohammadi and Jalayer Naderi 2023 are a few of the studies with similar findings on karyorrhexis.(9,15)

Cells with cytoplasmic vacuoles show multiple clear spherical vacuolization of variable size. They are due to partial or temporary disturbances in the cell membrane permeability.(15) Parmar et al 2019 also noted of cytoplasmic vacuoles mean value 0.18 ± 0.55 and the mean value of our study found to be similar i.e. 0.29±0.43.(9) Sharma VL. 2013 et al. in their study showed cytoplasmic vacuoles to be 27 %, and Seifi S et. Al in their study in 2014 found the occurrence of cytoplasmic vacuoles to be 30.8 %.(3,16)

Micronucleus assay is a potential biomarker for malignancy. A micronucleus (MN) is a small extra nucleus separated from the main one, generated during cellular division by late chromosomes or by chromosome fragments. It is a microscopically visible round to oval cytoplasmic chromatin mass in the extra nuclear vicinity. (17) The mean value of micronuclei for smoker were significantly higher than nonsmoker, i.e 3.01 ± 0.94 and 0.67 ± 0.77, respectively. Twinkle S Patel et al in their study on micronuclei in tobacco and related habits observed a stepwise increase in the micronuclei counts normal to potentially malignant to carcinoma suggested a link of this biomarker with neoplastic progression.(17) Across all the studies conducted on histopathological findings of malignant cells, micronuclei was found to be more in damaged cells than normal cells.

On comparison of the 8 parameters across different pack years, Binucleation, Karyorrhexis and Cytoplasmic Vacoules were not statistically significant. This shows that these parameters are independent of the duration of exposure to smoking. According to Noushin Jalayer Naderi et al the cytotoxicity effect of cigarette smoking was not significantly correlated to time exposure. Parmar et al also found no significant difference in karyorrhexis, binucleation and cytoplasmic vacuoles were not statistically significant across different pack years. (9,18) Pyknosis, Perinuclear halo, Cytoplasmic granule, Karyolysis and Micronuclei shows significant correlation across different pack years.

Conclusion

The present study indicates that almost all cytomorphological findings were high in smokers than non-smokers. Early detection of oral cancers becomes complex as they are mostly innocuous and asymptomatic during their initial stages, Cytomorphometric analysis can be used regularly to detect these cell alterations. Currently, use of exfoliative cytology has increased as an adjunct to screening of precancerous lesions and malignancies of the oral cavity.

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