International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 6; Issue 8; August 2017; Page No. 5010-5014
DOI: http://dx.doi.org/10.24327/ijcar.2017.5014.0633

Research Article

STUDY OF EXFOLIATED CELLS IN THE BUCCAL MUCOSA OF SMOKERS AND NON-SMOKERS – A CYTOLOGICAL COMPARATIVE STUDY

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ARTICLE INFO

Article History:

Received 18th May, 2017
Received in revised form 10th June, 2017 Accepted 5th July, 2017
Published online 28th August, 2017

Key words:

Buccal mucosa, Exfoliated cells, Haematoxylin &Eosin stain, Smokers, Tobacco

ABSTRACT

Oral exfoliative cytology is popular as an oral cancer screening tool, as it is painless, non-invasive procedure and is well accepted by patients as it cause less discomfort. Oral habits like smoking, chewing tobacco are documented as initiators of dysplastic changes in the oral mucosa. Tobacco smoking has been attributed as a major risk factor. **Aim:** To compare the cytology of apparently normal mucosa in tobacco smokers with that of non-smokers. **Materials and Methods:** Study population comprised of tobacco smokers who constituted the study group and non-smokers who constituted the control group. Smears of buccal mucosa were collected from 10 smokers and 10 non-smokers and stained by Haematoxylin and Eosin stain. **Results:** We found that cytology from smokers showed more clumping of cells, pleomorphism, binucleation and micronuclei compared to non-smokers. Non-smokers showed more of normal cells. **Conclusion:** The results of this study indicate that mild to moderate pleomorphism, clumps of cells, binucleation, micronuclei were seen in the oral cavity of smokers compared to non-smokers. This was a short study conducted in our department to motivate smokers to quit smoking by interpretation of the results.

INTRODUCTION

In many developing countries oral cancer is the fourth most common cancer among males and the sixth most common cancer in females.[1] Of all carcinomas diagnosed in India oral cancer is a major health problem, which accounts for 50-70% of all carcinomas diagnosed. Tobacco smoking have been attributed to as major risk factor for carcinogenesis.[2] Smoking is a common etiologic factor for oral cancers.[3] Despite a decrease in the incidence of head and neck cancers in some communities, the incidence of oral cancers has not fallen in recent years, one reason of which is the increased use of cigarettes and tobacco in those communities.[4] There is a geographic variation in the incidence of oral cancer among different countries of the world and among different regions within a country. This indicates that environmental factors may play an important role in the pathogenesis of cancer of the head and neck.

Despite advances in surgery, radiotherapy and chemotherapy, the 5-year survival rate of OSCC patients has remained approximately 50%. Lack of early detection and treatment has led to poor survival rate among oral cancer patients. Hence, it is necessary to detect potentially malignant lesions at their incipient stage. Early diagnosis and initiation of appropriate treatment of early malignant lesions offer the best hope of improving the prognosis.[5]

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As per the normal physiology, the oral epithelium renews itself rapidly (probably every 2 weeks). The rationale of oral exfoliative cytology is based on this physiological process, examining cells that are desquamated from the surface of the oral mucosa.[6]

Different in vivo and ex vivo strategies are being pursued for improving oral cancer mortality. In ex vivo strategies, biopsies of normal and malignant tissues, or scrapings containing exfoliated buccal cells, have been explored for many years. In in vivo strategies, dyes like toluidine blue has been used as a mouth stain for identifying high-risk primary oral premalignant lesions.[7]

Exfoliative cytology technique is a non-invasive method for initial and early diagnosis of cancers as an adjunct to clinical examination. This technique has been in use since the 1960s and 70s as a cancer evaluation and diagnostic technique with acceptable sensitivity and specificity.[8] Cytology can be used as a diagnostic technique in detecting early changes of diseases even in the absence of clinical manifestations. This technique has some advantages, including easy and fast implementation, adequate diagnostic value, non-invasiveness, low cost and reproducibility.[9]

Oral exfoliative cytology is a simple, non-invasive, and painless method that involves microscopic analysis of cells collected from the surface of the oral mucosa.[10] However; this method had been abandoned because of problems such as

inadequate tissue samples, technical errors, and the incorrect interpretation of findings.[11]

The oral mucosa of smokers exhibits many changes. Exfoliative cytological methods have been employed to examine these changes, especially in cells collected from the buccal mucosa.[12] In healthy individuals; epithelial cells of the buccal mucosa in the oral cavity are naturally exfoliated every day. Thus, buccal mucosa cells are similar to vaginal epithelial cells, and can be collected through excavation. Exfoliated buccal cells are at the final stage of cell differentiation, and rarely display mitotic features.[13]

In smokers with clinically normal oral mucosa it has been determined there exist some changes such as: a higher rate of proliferation of epithelial cells, nuclear and cytoplasmic alterations, as well as an increase in the number of keratinized cells.[14]

Characteristic squamous cells are shed from the mucosal surfaces in the incipient stages of tobacco induced changes prior to the conventionally accepted clinical signs. This allows for smears which are obtained by a simple, quick and non-invasive method for tissue analysis. Oral exfoliative cytology was employed in the present study to observe cellular morphological changes if any in smears from buccal mucosa of smokers.[15] Haematoxylin and eosin stain was used as a standard stain to delinate clinical changes in the buccal mucosa of both smokers and non-smokers group. The Hematoxylin and Eosin stain (H&E) is the most widely used histological stain. Its popularity is based on its comparative simplicity and ability to demonstrate clearly an enormous number of different tissue structures.[16]

It is envisioned that cell changes from smears will be used as an adjunct to clinical examination of smokers, which identifies them as being of high risk for oral cancer. Suspect individuals could then be monitored clinically.[1]

MATERIALS AND METHODS

The study was conducted in the Department of Oral Pathology, Vinayaka Missions Sankarachariyar Dental College Salem. The aim of the study was to compare the cytology of apparently normal mucosa in tobacco smokers with that of non-smokers

The present study was carried out in 2 groups with 10 persons in each group who reported as smokers and non-smokers. Ethical clearance was obtained from the ethical committee of the institution. Consent form was signed from all of the patients.

Inclusion Criteria

1. All the patients who had habit of smoking more than 1 year were included in study.
2. Only male patients aged 18-25 years

Exclusion Criteria

1. People who presented any mucosal lesion were excluded from study.
2. Any tobacco related habits other than smoking were excluded from the study.
3. Participant with any known systemic illness such as anemia, diabetes etc. were excluded as these factors may alter nuclear cytoplasmic ratio.
4. Female participants were excluded as hormonal changes may result in alteration in exfoliating mucosal cells.
5. Patients who have exposed to ionizing radiation within past 6 months were excluded from present study.

General mucosal screening was done. Questionnaire/ interview were used to record the subject data and habits. In the presence of habit, detailed history was recorded as to the type, period and frequency of the habit and a written consent was signed by the patients. All the individuals with smoking habit were considered as the study group population. Individuals without any habit of tobacco served as the control group population of the study.

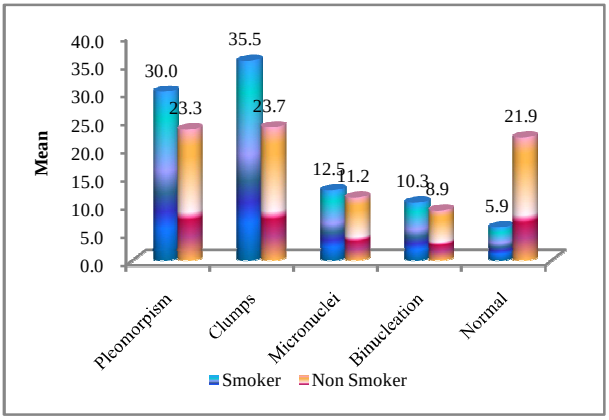
Two groups, smokers (n =10) and nonsmokers (n = 10) without clinically apparent lesions were randomly selected. Both, the study and the control group included healthy volunteers; all of them males aged 18 years to 25years. The examiner collected smears from the buccal mucosa. Smear was taken using a cytobrush. Cells were sampled using a gentle scraping motion. The smears were spread on to a clean microscopic glass slide, which was then fixed and stained using H&E staining protocol.

The smears were then observed under 40X & 100X magnifications. An eyepiece grid was used and 100 cells per slide were counted to note the changes. The cytological features in the smears were grouped as follows: a) Architectural changes; agglomerations or clumping of squamous cells and altered nuclear cytoplasmic ratio, b) Cellular changes; cellular pleomorphism and presence of micronuclei, c) Nuclear changes; binucleation

	Group	N	Mean	SD	Median	Mean Difference	t	p
Pleomorphism	Smoker	10	30.00	3.46	30.00	6.70	2.45	0.025*
	Non Smoker	10	23.3	7.93	22.50			
Clumps	Smoker	10	35.5	7.21	35.50	11.80	4.04	0.001**
	Non Smoker	10	23.7	5.77	25.50			
Micronuclei	Smoker	10	12.5	4.09	12.50	1.30	0.82	0.425
	Non Smoker	10	11.2	2.94	11.50			
Binucleation	Smoker	10	10.3	2.71	11.00	1.40	0.91	0.373
	Non Smoker	10	8.9	4.01	7.50			
Normal	Smoker	10	5.9	5.97	3.50	-16.00	-5.36 < 0.001**	
	Non Smoker	10	21.9	7.31	21.50			

* Significant at 5 %; ** Highly significant (Significant at 1 %)

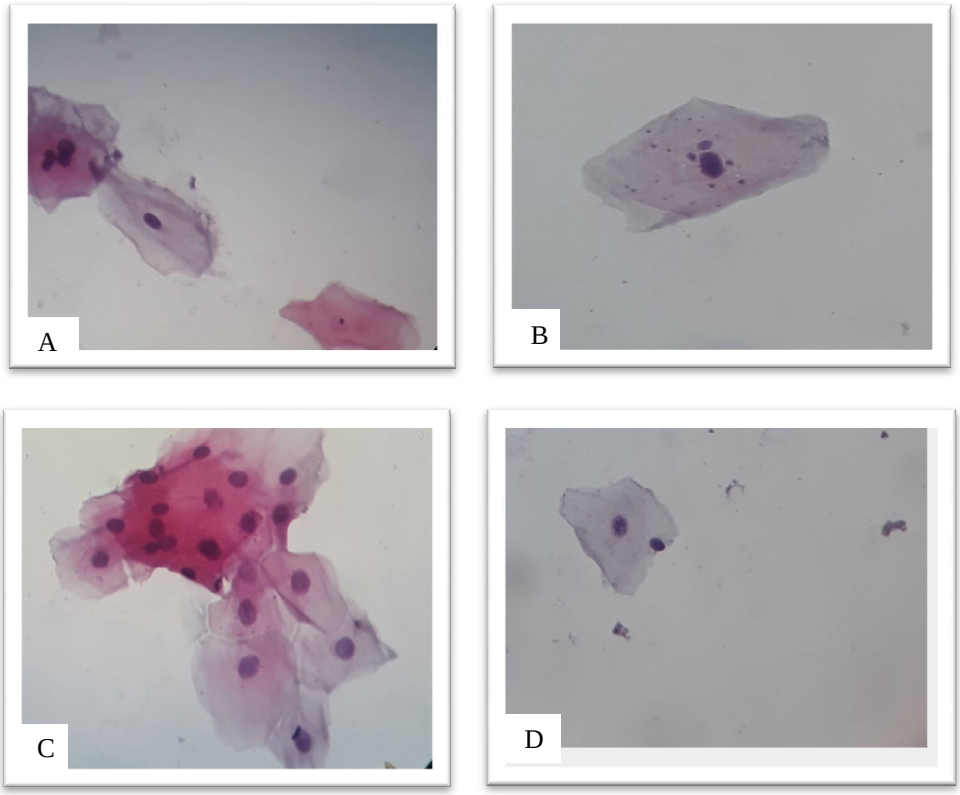
In the present study of exfoliated cells in the buccal mucosa of smokers and non-smokers we observed using "t test" that higher mean numbers of clumps are present in smokers compared to non-smokers and the difference between them is found to be statistically significant ($P < 0.05$). Highly statistically significant difference was noticed between smokers and non-smokers with respect to cellular pleomorphism ($P < 0.001$). Higher mean cellular pleomorphism was found in smokers compared to non-smokers. Mean micronuclei were found to be higher in smokers compared to non-smokers and the difference between them was found to be statistically significant ($P < 0.05$). Smokers recorded a higher mean binucleation compared to non-smokers and the difference between them is found to be statistically significant ($P < 0.05$).



more than 40 known carcinogens such as tar, nitrosamines etc. as well as heat generated by smoking plays important role in mucosal changes.[5] The most common type of oral cancer is squamous cell carcinoma, which 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.[18]

Agglomerations or clumping of cells without layering appears to occur more in inflammations and malignancy. Agglomerations indicate high turnover rate. This is very significant in view of the fact that clumping of cells is an accepted sign of dysplasia.[19]

The size and shape of cells and nuclei in dysplasia usually differs (pleomorphism) from normal cells of the same origin.[20] In our study, cellular pleomorphism was seen more in smokers.



Photomicrographs: Pleomorphism, Micronuclei, Clumps, Binucleation

DISCUSSION

It was believed that chronic exposure to smoking causes alteration in mucosa specially buccal, labial and tongue mucosa, as the fact established that tobacco smoke contains

The frequency of occurrence of micronuclei is a measure of chromosome breakage in early cell divisions. The number of micronuclei is known to increase with carcinogenic stimuli.[21]

Higher frequency of micronuclei in cells collected from smokers was also observed in our study.

Binucleation is the presence of two nuclei within a cell. It is a nuclear abnormality seen in dysplastic cells and is found to be increased in smokers.[22] Binucleus formation is considered as indicator of cytotoxicity.[23] In the present study significant increase in binucleus frequency was observed in smokers group.

Normal cells were collected from both smokers and non-smokers. The presence of normal cells was found to be higher in non-smoker as compared to smoker because the former showed not much features of dysplasia due to absence of habits.

Recent advances in the clinical visualization and detection of the oral mucosa have made the viability of cytological procedures more specific and sensitive. Contact endoscopy and use of autofluorescence devices are the forerunners in this group.

A screening test must have the five characteristics of being:[24]

1. Simple, safe and accepted by the population
2. Able to detect the disease early
3. Able to detect those lesions that are prone to progress;
4. Able to detect those lesions that are treatable
5. Have high sensitivity (few false negatives) and high positive predictive value.

The procedure of contact endoscopy satisfies almost all the requirements of good screening procedure.

Significant differences in the fluorescent spectra of normal buccal mucosa and dysplastic oral tissues in the excitation bandwidth of 410nm form the basis of modern day autofluorescent devices. Commercial devices approved by the FDA (VizLite, VelScopeetc) are available for the clinician to peruse in diagnosis of potential malignant lesions.[25]

Due to low feasibilities of such devices the benchmark of diagnosis will be microscopic tissue examination. Hence cytological smears will always be highly specific, sensitive, easy to use and reproducible procedures in routine screening of population for potentially and malignant conditions of the oral cavity.

CONCLUSION

This study convince evidence that tobacco have definitive deleterious effect on oral mucous membrane in terms of nuclear cytoplasmic changes, which are the key features of malignancy. Such evidence could be used for early diagnosis of mucosal changes. Thus, dentists have the responsibility to educate the population regarding the adverse effect of tobacco as well as to encourage quitting such habits.[5]

Acknowledgment

On the recollection of so many and great favours and blessings, I now with a high sense of gratitude, presume to offer up my sincere thanks to the almighty, the creator and preserver. I also take this opportunity to thank STAT. Nandakumar SIR for helping me with the results in this study.

References

1. M. A Hashemipour, M.Aghababaie, T. R Mirshekari,et.al Exfoliative Cytology of Oral Mucosa among Smokers, Opium Addicts and Non-smokers: A Cytomorphometric Study
2. Sharma VL, Chowdhary DS, Agarwal SK, Jain Aarushi, Sharma Vijeta, RawatShivani, A Comparative Study Of Oral Epithelium In Tobacco And Alcohol Consumers In Central Rajasthan Population, *Int J Biol Med Res.* 2013; 4(3):3355- 9.
3. Orellana-Bustos AI, Espinoza-Santander IL, Franco-MartínezME, Lobos-James-Freyre N, Ortega-Pinto AV. Evaluation of keratinization and AgNORs count in exfoliative cytology of normal oral mucosa from smokers and non-smokers. *Med Oral.* 2004; 9: 197 - 203.
4. Saedi B, Razmpa E, Sadeghi M, Mojtahed M, Mojtahed A. The epidemiology of laryngeal cancer in a country on the esophageal cancer belt. *Indian J Otolaryngol Head Neck Surg.* 2009; 61: 1 - 5.
5. NeeldipsinhJadeja, Mona Shah, GaurangThakker et.al Exfoliative Cytology of Clinically Normal Buccal Mucosa in Smokers and Non Smokers: A Cytomorphometric Study. *J Res Adv Dent* 2015; 4:1:186-191.
6. Khandelwal S, Solomon MC. Cytomorphological Analysis of Keratinocytes in Oral Smears from Tobacco Users and Oral Squamous Cell Carcinoma Lesions -A Histochemical Approach, *Int J Oral Sci,* 2010, 2(1): 45-52.
7. Ahmed HG, Babiker AA. Assessment of cytological atypia, AgNOR and nuclear area in epithelial cells of normal oral mucosa exposed to toombak and smoking. *Rare tumors* 2009; 1:234-6.
8. Sampaio HC, Loyola AM, Gomez RS, Mesquita RA. AgNOR count in exfoliative cytology of normal buccal mucosa effect of smoking. *ActaCytol*. 1999; 43: 117 - 120.
9. Orellana-Bustos AI, Espinoza-Santander IL, Franco-MartínezME, Lobos-James-Freyre N, Ortega-Pinto AV. Evaluation of keratinization and AgNORs count in exfoliative cytology of normal oral mucosa from smokers and non-smokers. *Med Oral.* 2004; 9: 197 - 203.
10. Diniz FM, Garcia GA, Crespo AA, Martins CJL, Gandara RJM. Applications of exfoliative cytology in the diagnosis of oral cancer. *Med Oral* 2004; 9: 355-61.
11. Pektaş ZÖ, Keskin GÖ, Karslioğlu Y. Evaluation of nuclear morphometry and DNA ploidy status for detection of malignant and premalignant oral lesions: quantitative cytologic assessment and review of methods for cytomorphometric measurements. *J Oral MaxillofacSurg* 2006; 64: 628-35.
12. Einstein TBA, Sivapathasundharam B. Cytomorphometric analysis of the buccal mucosa of tobacco users. *Ind J Dent Res* 2005; 16: 42-6.
13. Proia NK, Paszkiewicz GM, Nasca MA, Franke GE, PaulyJL.Smoking and smokeless tobacco-associated human buccal cell mutations and their association with oral cancer-a review. *Cancer Epidemiol Biomarkers Prev*. 2006; 15: 1061-71.

14. Bustoc OAI, Santander EIL, Martiniez FE, Freyre LJN, Pinto OAV. Evaluation of keratinization and AgNORs count in exfoliative cytology of normal oral mucosa from smokers and non-smokers. *Med Oral* 2004; 9(3):197-203.
15. Silverman S, Jr. Intraoral Exfoliative Cytology. *CA Cancer J Clin* 1959; 9; 125-8.
16. C. F. A. CULLING THIRD EDITION
17. KomaliYerlagudda, VenkateshVishwanath Kamath, KrishnanandSatelur Morphological Assessment of Oral Cytological Smears Before and After Application of Toluidine Blue in Smokers and Nonsmokers *International Journal of Oral & Maxillofacial Pathology*. 2012; 3(1):08-14
18. Ahmed HG. Assessment of oral cytological changes associated with exposure to chemotherapy and/or radiotherapy. *Cyto J* 2009; 6:8-10.
19. Silverman S, Jr. Intraoral Exfoliative Cytology. *CA Cancer J Clin* 1959; 9; 125-8.
20. Koss L. ed. Diagnostic Cytology and its Histopathological Bases. Vol II, 4th edn. Philadelphia: JB Lippincott Co., 1992. 425-26.
21. Proial K, Paszkiewicz MG, Nasca MAS, Franke GE, Pauly JL. Smoking and Smokeless Tobacco-Associated Human Buccal Cell Mutations and Their Association with Oral Cancer - A Review. *Cancer Epidemiology, Biomarkers & Prevention* 2006:15:1061.
22. Celik A, Cavas T, Gozukara SE. Cytogenetic biomonitoring in petrol station attendants: micronucleus test in exfoliated buccal cells. *Mutagenesis* 2003; 18:417-21.
23. Ahmed HG, Babiker AA. Assessment of cytological atypia, AgNOR and nuclear area in epithelial cells of normal oral mucosa exposed to toombak and smoking. *Rare tumors* 2009; 1:234-6.
24. Lingen MW, Kalmar JR, Karrison T, Speight PM. Critical evaluation of diagnostic aids for the detection of oral cancer. *Oral Oncol* 2008; 44:10-22.
25. Ingrams DR, Dhingra JK, Roy K, Perrault Jr DF, Bottrill ID, Kabani S, *et al.* Auto fluorescence characteristics of the oral mucosa. *Head Neck* 1997; 19(1):27-32.

How to cite this article:

Twinky Merlin Thomas *et al* (2017) 'Study of Exfoliated Cells in the Buccal Mucosa of Smokers and Non-Smokers - A Cytological Comparative Study ', *International Journal of Current Advanced Research*, 06(08), pp. 5010-5014.
DOI: http://dx.doi.org/10.24327/ijcar.2017.5014.0633

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