

ORIGINAL ARTICLE

CLINOPATHOLOGICAL AND CYTOLOGICAL CHANGES IN ORAL MUCOSA OF PATIENTS HAVING TOBACCO SMOKING HABBIT

Avanindra Kumar¹, Avanish Kumar², Ritu³, Sufia Parveen⁴, Md. Jawed Akhtar⁵

HOW TO CITE THIS ARTICLE:

Avanindra Kumar, Avanish Kumar, Ritu, Sufia Parveen, Md. Jawed Akhtar. "Clinopathological and Cytological Changes in Oral Mucosa of Patients having Tobacco Smoking Habbit". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 66, December 01; Page: 14250-14256, DOI: 10.14260/jemds/2014/3911

ABSTRACT: BACKGROUND: Tobacco was introduced into Europe in the late 15th century. Portuguese traders introduced it to India in late 16th or early 17th century. Since then, tobacco use has spread with remarkable rapidity, into all sections of people. Now tobacco is used in different forms out of which some are in form of smoking like cigarette, bidi whereas some are smokeless e.g., chewing, application over the teeth & the gingiva. Among tobacco habituated Indian population, about 70% are in the smoking form.¹ Passive smoking is also a significant health hazard. There is a vital role of dental practitioners in identifying individuals at risk of mucosal disease, the importance of public education about the risk factors, and the necessity for counseling patients with precancerous lesions on avoiding further risk.² **AIMS AND OBJECTIVE:** To study clinico-pathological & cytological changes in oral mucosal cells of people with the habit of smoking tobacco by using exfoliative cytology and PAP stain. **MATERIAL AND METHODS:** The oral exfoliative cytology smears are taken from 60 person (30 smoking habit & 30 control) from the oral pathology department of K M Shah Dental College & Hospital. The smears are spread on the glass slide and are fixed with 95% ethyl alcohol. The slides are stained with papanicolaou stain and observed under microscope. **RESULTS:** The result showed that the anucleated cells (Precancerous feature) are increased in patient with smoking habit as compared to control group. Anucleated cells are highest in oral sub mucous fibrosis group of patients.

KEYWORDS: exfoliative cytology, smoking tobacco habit, pre-cancerous lesion, papanicolaou stain.

INTRODUCTION: The word tobacco was originally used to denote a "Y" shaped piece of cone or pipe called tobaco or tobaca that was used by Mexicans Indians to inhale powdered leaves of a plant. Later the plant came to be known by the name of the device as "tobacco". The generic name of the tobacco plant, Nicotiana is derived from the name of the French Ambassador to Portugal, Jean Nicot who introduced tobacco to the French court in 1560.¹ Tobacco was introduced into Europe in the late 15th century. Portuguese traders introduced it to India in late 16th or early 17th century. Since then, tobacco use has spread with remarkable rapidity, seeping into all sections of the society. Initially tobacco was smoked in India, but later it was used for chewing & application over the teeth & the gingiva as well i.e. smokeless form. In the course of time, large spectrums of methods of use are developed. Cigarette smoking is more popular in urban population than rural population. Indian cigarettes have lesser filter in comparison to some cigarette manufactured in some developed countries. Different types of tobacco smoking are bidi (most popular in India), Cigarette, Cigar, Cheroots, Chuttas, Dhumati, Chillum, Hookah etc. Tobacco is well known addictive and is harmful to health in many ways.

Oral mucosa is an index to the general health of the body. Everything, which passes through the oral cavity in the form of food and drink, comes first in contact with it. The oral mucosa face

ORIGINAL ARTICLE

insults in the form of various habits like chewing pan, tobacco, betel nut, smoking, alcohol drinking, which with the passage of years leave their marks on the oral mucosa. The most deleterious and dangerous entities associated with these habits are the oral cancer. In tobacco smoke along with nicotine 'thousands of other chemical substances are found.' which are irritant, toxic and carcinogenic. The potent carcinogens present in it are the tobacco-specific Nitrosamines, polycyclic aromatic hydrocarbons, tar & many others. Individuals with precancer run a risk that is 69-times higher for them to develop oral cancer as compared to tobacco users who do not have precancer. Therefore recognition and management of precancerous conditions, constitute a vital oral cancer control measure.³

It is true that cytology is no match for biopsy in a final diagnosis but exfoliative cytology is a simple, quick, painless, bloodless and non-invasive technique that can be repeated frequently with minimal discomfort to the patient. It is also suitable in patients with systemic diseases who are contraindicated for biopsy. It guards against false negative biopsy and post biopsy complications can be eliminated.⁴ Cytology can play a Major role in long term monitoring of wide spread innocuous but suspicious lesion and as part of a follow up protocol after treatment of oral cancer.⁵

AIMS AND OBJECTIVES: To study clinico-pathological & cytological changes in oral mucosal cells of patients with the habit of smoking tobacco by using exfoliative cytology and PAP stain and comparing it with control non-smoker group.

REVIEW OF LITERATURE: In a study of 50 healthy men of 20 to 30 years age Miller et al. (1951)⁶ reported as: a) dorsal surface of tongue showed 86% red staining cells and 14% blue staining cells. b) ventral surface of tongue 21% red staining cells and 79% blue staining cells. c) cheek 73% red staining cells and 27% blue staining cells. d) gingiva 89.4% yellow staining cells and 10.3% red staining cells. The degree of cornification was highest at the gingiva followed by dorsum of the tongue, cheek & the ventral surface of the tongue. Based on these findings, they suggested that the apparent constancy of this cornification pattern can be used as a normal base line which may be utilized in studies of abnormal conditions.

Montgomery P. W. (1951)⁷: Made a study on 75 clinically normal individuals by exfoliative cytology of oral mucosa. In his study soft palate and cheek showed predominance of blue cells and smaller number of red cells and a very few yellow cells. Vestibular area showed similar pattern except for an increase in the number of yellow cells. Two tongue region showed predominance of red cells & 1/3 of the cells were yellow type, posterior tongue regions showed a greater proportion of blue cells than anterior tongue region. Gingiva showed more number of yellow cells, red cell next in prominence and blue cells less commonly encountered. In addition they also noted variation in size, shape & staining qualities of cells and their nucleus. In some cells cytoplasm was quite clear while that of other was granular or showed particulate matter in the cytoplasm.

Silverman sol et al. (1958)⁸: Based on their findings, suggested that exfoliative cytology offers an excellent adjunct to biopsy & can be used for a routine procedure for lesions of unknown etiology when biopsy is delayed & following treated oral malignancies to evaluate the effectiveness of therapy.

ORIGINAL ARTICLE

Zimmermann et al. (1965)⁹: Reported increased keratinized cells in smokers. Patient with systemic disease such as endocrine disorders (diabetes mellitus) and respiratory disease had a significant reduction in keratinized cell count of hard palate but an increased count in the buccal mucosa compared to control.

Shklar G. et al. (1968)¹⁰: Carried out a correlated study of oral cytology and histopathology in 2,052 patients to determine the reliability or accuracy of cytodiagnosis. Based on their findings, they suggested that the reliability of oral cytology for diagnostic purposes is somewhat lower than that of biopsy.

Hillman R.W. et al. (1976)¹¹: Carried out study of Pap smears from the cheek & the superior & inferior surfaces of the tongue of 790 alcoholic patients to evaluate possible association between cytologic features & cigarette consumption. Preparations from smokers showed greater nucleus and cell size & high cell/nucleus ratio, greater frequencies of both precornified & late cornified cells and smaller concentrations of bacteria, fungi, filamentous forms and leukocytes.

Cowpe J.G. et al. (1985)¹²: Studied exfoliative cytology of oral squames from five oral sites: buccal mucosa, floor of mouth, labial mucosa, junction of hard & soft palate and dorsum of tongue in 105 patients all ranging from 12 to 87 years. Author found nuclear area varied significantly with advancing age, but there was only slight variation among the younger age groups which increased as age progressed, however there was no significant variation in total cell area with advancing age. They also found nuclear and cell size varied significantly between the five sites.

Mollaoglu et al. (2001)¹³: Did a study on 33 patients with squamous cell carcinoma in the floor of the mouth. Based on their findings, they concluded that the cytomorphometric assessment of the Papanicolaous stained oral smears collected from malignant & premalignant lesions can provide an additional diagnostic test for monitoring such lesions & thus detecting oral malignancy at an early stage.

Sandra alberti et al. (2003)¹⁴: Studied microscopic and cytomorphometric analysis of oral exfoliated cells in 10 control individuals and 10 type II diabetic patients to develop a new method for diabetes diagnosis, each group comprised 6 women & 4 men, all ranging from 27 to 72 years & enjoying good oral hygiene. Cytomorphometric analysis showed that the cells of all the three oral sites did not present statistically significant differences in the cytoplasmic areas, while the nuclear areas of these cells larger for the type II diabetic group compared to the control group. On average the CA/NA ratio in the diabetic group was 37.4% smaller.

Pektas Z.O. et al. (2006)¹⁵: Carried out brush biopsy of 22 patients with suspicious oral lesions to obtain dissociated epithelial cells before incisional biopsy. Based on their findings, they suggested that cytomorphometric analysis via oral brush biopsy is a valuable adjunct to biopsy for identification of premalignant & early stage cancerous oral lesions as a rapid and minimally invasive procedure with high specificity & sensitivity rates, requiring no topical or local anesthesia.

ORIGINAL ARTICLE

MATERIAL AND METHODS:

- Mirror & probe, Cotton swab, Glass marking pencil, Normal saline, wooden tongue spatula.
- Fixative solutions (95% Ethyl Alcohol).
- Staining material (PAP stain), Glass slide and cover slip, Microscope.

SAMPLE SIZE: Randomly 60 adult male (30 Smokers & 30 Non-smoker of tobacco as Control Group) have been included in the study from the OPD of KM Shah Dental College and hospital. In the smoker group only those persons are included who have been smoking more than 5 cigarettes per day for at least 3 years. The persons included in control group are having no history of smoking.

STUDY METHOD: Scraping of the lesions of oral mucosa, smears are obtained by using a standard wooden tongue spatula moistened with normal saline. In cases where a heavy keratinized surface is present, fissured or reddish areas are scraped to obtain the sample. The scrapings are smeared on plain glass slides and fixed immediately in 95% ethyl alcohol and stained with the Papanicolaou stain. 100 cells in each slide are randomly observed for different kind of normal and abnormal exfoliated cells under microscope so a total of $30 \times 100 = 3000$ cells are observed in smoking group and same number of cells are observed in control group.

OBSERVATIONS: The smokers included in the study are having habit of smoking either bidi or cigarette. Among 30 smokers in the present study, 24 smoke bidi while 6 smoke cigarette. In 22 smokers some kind of lesions in oral mucosa are present (Table-1). Out of 10 patients of leukoplakia 7 are having habit of bidi smoking while 3 are having habit of cigarette smoking. All the 8 patients of oral submucous fibrosis (OSMF) and 4 patient of ulcerative lesion are having habit of bidi smoking. (Table-2).

Habit	Total no. of patients	Lesion in oral mucosa	No. of patients	Percentage
Smoking	30	Lesion present	22	73.3%
		Lesion absent	8	26.7%

Table 1: Distribution of lesion in smokers

Type of lesion	No. of patients	Bidi	Cigarette
Leukoplakia	10	7	3
OSMF	8	8	0
Ulcerative lesion	4	4	0
No lesion	8	5	3
Total	30	24	6

TABLE 2: Type of lesions and their frequency in the patient

For quantitative evaluation 100 cells in each slide (Total $30 \times 100 = 3000$ cells) are observed under microscope randomly in smoking group and also in control group as well. Among smoking group 2080 are found anucleated cells, 670 are pink (Fig-1, a) and 231 (Fig-1, b) are blue. There is

ORIGINAL ARTICLE

significant difference from counting in control group (table-3). For qualitative evaluation different parameters are taken into consideration (table 4).

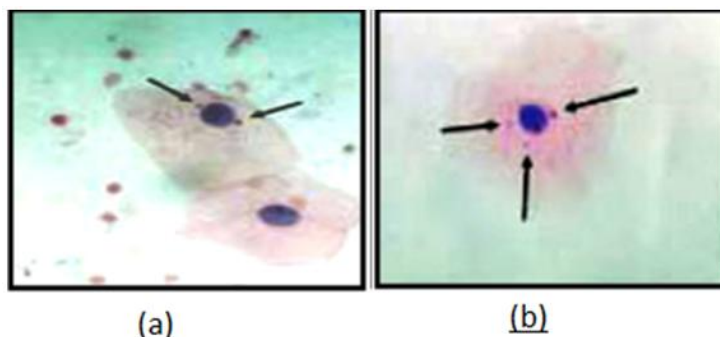


Fig. 1: Showing blue cell (a) and Pink cell (b)

Group	Total cells/ patient	Anucleated cells	Pink	Blue	Overlapped and improperly stained cells (Features not clear)
Smoking group (30)	3000	2080	670	231	19
Control (30)	3000	1117	1004	865	14

TABLE 3: Quantitative evaluation of different lesions in smoker group and control group

Group	Aniso Nucleosis	Altered N/C	Increased nuclear size	Hyper chromatasia	Anisocytosis	Karyo rexhesis	Karyo Lysis	Vacuo- lization	In- flamma- tory cells
Smoker Group (30)	0	0	0	4	0	6	0	12	8
Control (30)	0	0	0	0	0	0	0	3	0

TABLE 4: Qualitative evaluation of different lesions in smoker group and control group

DISCUSSION: The malignancies of the oral cavity arise from premalignant lesions, such as leukoplakia, erythroplakia and oral sub mucous fibrosis (Osmf). During transformation of normal tissue to premalignancy or malignancy, cellular changes occur at the molecular level before they are seen under the microscope & much before clinical changes become evident. Identification of high risk oral premalignant lesions & intervention at premalignant stages could constitute one of the keys in reducing the mortality & morbidity.

Cytology has been recommended for the early diagnosis of oral cancer and proved to be a reliable primary diagnostic test.¹⁰ But comprehensive literature reviewed on 'oral exfoliative cytology' by Tyler (1972)¹⁶ concluded that cytological examination of oral lesions does not constitute definitive diagnosis. The false negative rate for oral cytologic examination determined in this study was 31%. The reliability of the procedure varies from site to site, apparently depending to some extent on the degree of keratinization.

ORIGINAL ARTICLE

Anne J Krush,¹⁷ "Lebert's contribution to understanding of cancer and cancer genetics" emphasized the altered size of cells and nuclei as a basis of diagnosing cancer. Both nuclear & cytoplasmic areas are parameters known to be of significance in the diagnosis of malignancy. Hence Cowpe & Longmore¹⁸ suggested that quantitative techniques, based on the evaluation of parameters such as nuclear area (NA), cytoplasmic area (CA) and nuclear-to-cytoplasmic area ratio (NA/CA) may increase the sensitivity of exfoliative cytology for early diagnosis of oral cancer.

Findings of present study indicate the various cytological changes in oral mucosal cells which may progress towards a precancerous and cancerous condition.

SUMMARY & CONCLUSION: Present study of exfoliative cytology from oral cavity clearly supports the relation of tobacco smoking with abnormal cytological change in cells of buccal mucosa. These cytological changes may progress towards a premalignant and then in course of time become cancerous. As exfoliative cytology is easy and not inconvenient to the patient so it can be very helpful in diagnosing early precancerous condition.

REFERENCES:

1. Peter S, Sequeira PS. Epidemiology, Etiology and Prevention of Oral Cancer. In: Peter S, editor. Essentials of Preventive and Community Dentistry. New Delhi: Arya (Medi) Publishing House; 2003. pp. 468-505.
2. Kulasegaram R, Downer MC, Jullien JA, Zakrezewska JM, Speight PM. Case-control study of oral dysplasia and risk habits among patients of a dental hospital. *Oral Oncol, Eur J Cancer* 1995; 31: 227-31.
3. Mehta FS, Hammer JE. Tobacco Related Oral Mucosal Lesions and Conditions in India. Basic Dental Research Unit, Tata Institute of Fundamental Research Mumbai. New Delhi: Jaypee Brothers Medical Publishers Pvt. Ltd. 1993. p. 23-44.
4. Sivapathasundharam B, Kalasagar M. Yet another article on exfoliative cytology. *Journal of Oral & Maxillo Facial Pathology* 2004; 8 (2): 54-57.
5. Cowpe JG, Longmore V, Green M W. Quantitative exfoliative cytology of abnormal oral mucosal smears. *Journal of the Royal Society of Medicine* 1988; 81: 509-513.
6. Miller SC, Alexander S, Stahl S. A study of the cornification of the oral mucosa of young male adults. *Journal of Dental Research* 1951; 30 (1): 4-11.
7. Montgomery PW. A study of exfoliative cytology of normal human oral mucosa. *Journal of Dental Research* 1951; 30 (1): 12-18.
8. Silverman S, Becks H, Farber SM. The diagnostic value of intraoral cytology. *Journal of Dental Research*, April 1958; 37 (2): 195-205.
9. Zimmermann ER, Zimmermann AL. Effects of race, age, smoking habits, oral and systemic disease on oral exfoliative cytology. *Journal of Dental Research* 1965; 44: 627-31.
10. Shklar G, Meyer I, Cataldo E, Taylor R. Correlated study of oral cytology and histopathology. Report on 2,052 oral lesions. *Oral Surg Oral Med Oral Pathol.* 1968 Jan; 25 (1): 61-70.
11. Hillman RW, Kissin B. Oral cytologic patterns in relation to smoking habits: some epithelial, microfloral, and leukocytic characteristics. *Oral Surgery Oral Med Oral Pathology.* 1976; 42 (3): 366-74.
12. Cowpe JG, Longmore RB, Green MW. Quantitative exfoliative cytology of normal oral squames: an age, site and sex-related survey. *Journal of the Royal Society of Medicine* 1985; 78 (12): 995-1004.

ORIGINAL ARTICLE

13. Mollaoglu N, Cowpe JG, Walker R. Cytomorphologic analysis of papanicolaou stained smears collected from floor of the mouth mucosa in patients with or without oral malignancy. Turk J Med Sci 2001; 31: 225-228.
14. Alberti S, Spadella CT, Assis GF, Cestari TM. Exfoliative cytology of the oral mucosa in type II diabetic patients: morphology and cytomorphometry. J Oral Pathol Med 2003; 32: 538-43.
15. Pektas ZO, Keskin A, Günhan O, Karslioglu 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 Maxillofac Surg 2006; 64 (4): 628-35.
16. Tyler C Folsom, Charles P white, Louis Borner, Henery F. Canby, George E. Garrington Oral exfoliative study: Review of literature and report of three year study. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology. 1972; 33 (1): 61-74,
17. Anne J Krush: "Hermann Lebert's Contribution to the Understanding of cancer and cancer Genetics". Transaction of the Nebraska Academy of Sciences. 1977; 4: 436.
18. Cowpe JG, Longmore RB, Green MW, J R Quantitative exfoliative cytology of normal oral squames: an age, site and sex-related survey. Soc Med. 1985; 78 (12): 995-1004. PMID:; 4067983.

AUTHORS:

1. Avanindra Kumar
2. Avanish Kumar
3. Ritu
4. Sufia Parveen
5. Md. Jawed Akhtar

PARTICULARS OF CONTRIBUTORS:

1. Senior Lecturer, Department of Oral Pathology, Dr. B. R. Ambedkar Dental College, Patna, Bihar.
2. Associate Professor, Department of Anatomy, Indira Gandhi Institute of Medical Sciences, Sheikhpura, Patna, Bihar.
3. Senior Resident, Department of Forensic Medicine & Toxicology, Indira Gandhi Institute of Medical Sciences, Sheikhpura, Patna, Bihar.

4. Lecturer, Department of Prosthodontics, Maitri College Dentistry and Research Centre, Anjora, Durg, Chattishgarh.
5. Senior Resident, Department of Anatomy, Indira Gandhi Institute of Medical Sciences, Sheikhpura, Patna, Bihar.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Avanindra Kumar,
Senior Lecturer,
Department of Oral Pathology,
Dr. B. R. Ambedkar Dental College,
Patna-800014, Bihar, India.
Email: simsavanish.yt@gmail.com

Date of Submission: 08/11/2014.
Date of Peer Review: 10/11/2014.
Date of Acceptance: 26/11/2014.
Date of Publishing: 28/11/2014.