Cytomorphometric Analysis of Buccal Mucosa of Tobacco Chewers

Dr. Amit Thahriani[1], Dr. Abhishek Khare[2], Dr. Anupama Mahendra[3], Dr. Amritaksha Bhattacharya[4], Mr. Anoop Kumar Bajpai[5]

Senior Lecturer[1], Reader[2], P.G Student[3], Research Scholar[5], Department of Oral Pathology[1,2,4], Department of Periodontology[3], Department of Anthropology[5], Awadh Dental College & Hospital, Jamshedpur[1], Career Dental College[2,3], Lucknow, Saraswati Dental College[4], Lucknow, Lucknow University[5]

Abstract :

Various environmental factors play a significant role in the pathogenesis of head and neck cancer. Different forms of tobacco have been considered as major risk factors for head & neck cancer. Exposure to various end products of tobacco causes alteration in various cytological parameters such as nuclear size, cell size, nuclear to cytoplasmic ratio, nuclear shape etc. Of these parameters, the nuclear size, cytoplasmic size and their ratio have been shown to be significant.

Aim: To assess the effect of tobacco on cytomorphology of exfoliative buccal mucosal cells.

Objective: To confirm the cause effect relationship between tobacoo usage and quantitative cellular and nuclear alterations.

Materials & Method: The study group consisted of 25 normal subjects (N), 25 subjects using some form of tobacco for more than 5 years but not having any oral lesion (H) and 25 patients having habit of using tobacco for more than 5 years and having a potentially malignant oral lesion (L). Since area is a better indicator of size as compared to diameter, comparison was made between cellular area, nuclear area and the ratio between nuclear and cytoplasmic area.

Results: Revealed significantly increased n/c ratio among tobacco users caused principally by reduction in cytoplasmic area rather than an increased nuclear area. Patients with lesions showed both a significant decrease in cytoplasmic area along with increased nuclear area.

Keywords: Oral Exfoliative Cytology, Cytomorphometry

Introduction

Exfoliative cytology, which is a simple, noninvasive diagnostic technique, could increase the chances of earlier detection of premalignant and malignant lesions[1]. In exfoliative cytology, the quantitative parameters are objective and reproducible; they may be important aids in the making of a cytopathologic diagnosis in such situations. One such quantitative parameter is morphometry. The smear obtained by exfoliative cytology can be analyzed quantitatively and qualitatively. With advancements in the field of quantitative oral exfoliative cytology, various parameters such as nuclear size, cell size, nuclear-to-cytoplasmic ratio, nuclear shape, texture can be evaluated collectively in order to confirm the diagnosis [2]. Of these parameters, the nuclear size, cytoplasmic size and their ratio have been shown to be significant in the evaluation of oral lesions [3,4]. The variations obtained in these parameters have been attributed to exposure to carcinogenic agents like tobacco. The concept of cellular or nuclear alteration on exposure to varying forms of tobacco can be best explained by reviewing the nature of cellular response to stimuli from the end products of different types of tobacco usage. Decrease in the cellular diameter and increase in the nuclear size are two significant morphologic changes that occur in actively proliferating cells[5].

Materials & Methods

Sample size consist of 75 patients with age group between 21 to 75 years of age. The patients were divided into 3 groups, Group A: 25 Normal healthy subjects (N) , Group B: 25 Patients with habit of consuming tobacco in any form for more than 5 years without any visible mucosal changes(H) , Group C: 25 Patients with habit of consuming tobacco in any form for more than 5 years with tobacco associated lesion on buccal mucosa.

The patients who presented with the history of systemic illness, tobacco use or alcoholic consumption were excluded from the study.

Scrapings were made from the buccal mucosa with plastic spatula or moistened wooden spatula, smeared on to a clear glass slide and immediately fixed with 95% ethanol for a minimum of 15 minutes. The smear was visualized in 200X objective and focused on the stage micrometer scale. In all the cases the Nuclear area and the Cellular area were measured in both the horizontal and vertical axes in micrometer. Only clearly defined cells were measured, excluding the clumped or folded cells. 50 cells were measured from each smear and the mean Nuclear area and the Cellular area were obtained for each case. The Nuclear Cytoplasmic (NC) Ratio was also calculated for each case using papanicolau stain(PAP).


Fig 2. Showing photograph of patients having habit of using tobacco for more than 5 years and having a potentially malignant oral lesion.

Fig 1. Showing photograph of Normal healthy subjects.

**Fig 3.photograph showing, Scrapings made from the buccal mucosa with plastic spatula**

Staining

**Fig 4.photograph showing. PAP Stain, Slides, cytology brush, 95% ethanol**

Morphometry
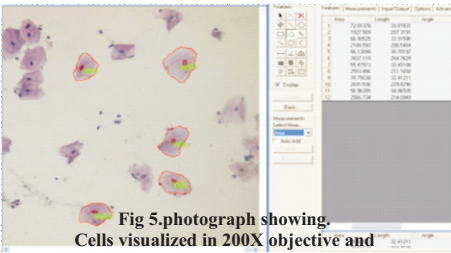
Fig 5.photograph showing. Cells visualized in 200X objective and focused on the stage micrometer scale. In all the cases the Nuclear area and the Cellular area were measured in both the horizontal and vertical axes in micrometer.
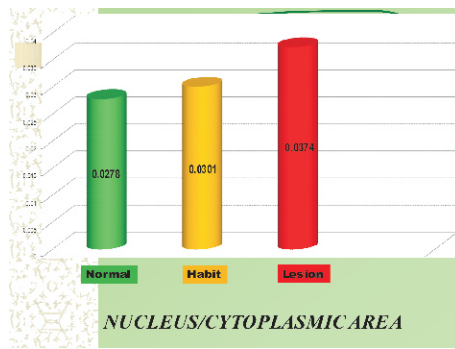
Result & Observation
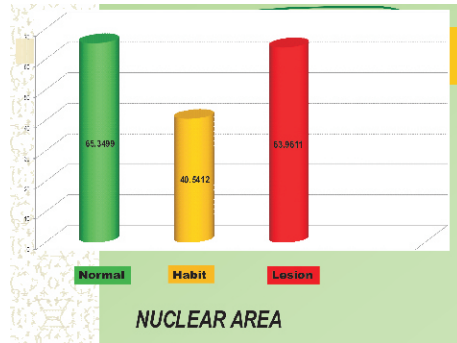
NUCLEAR AREA

Photograph showing Mean value of the nuclear area among the three groups

Lesion vs Normal : p=0.169

Lesion vs Habitual : p=0.989

Normal vs Habitual : p=0.217
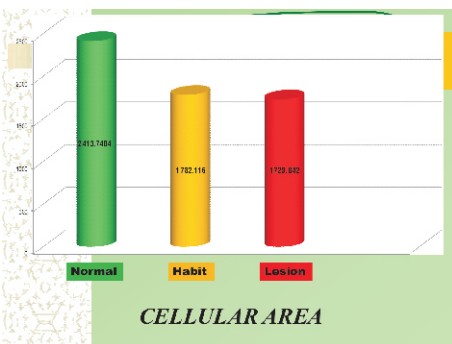
CELLULAR AREA

Photograph showing Mean value of the cellular area among the three groups

Lesion vs Normal : p=0.922

Lesion vs Habitual : p<0.001

Normal vs Habitual : p<0.001

NUCLEUS/CYTOPLASMIC AREA

Photograph showing Mean value of the nucleus/cytoplasmic area among the three groups

Lesion vs Normal : p<0.001

Lesion vs Habitual : p=0.003

Normal vs Habitual: p=0.540

Discussion

Users of smokeless tobacco exhibit oral cancer preferentially in areas where the quid is held, that is the Cytomorphometric analysis of buccal mucosa of tobacco chewers buccal or alveolar surfaces. The habit of placing tobacco mixed with lime (Khaini) in the mandibular sulcus,usually in the canine premolar region is widespread in the rural population of Central Maharashtra, India (Bhonsle RB et al., 1979)[6]. It is a thick, yellowish white intra oral lesion, occasionally with loose tags of tissue that occurs at the site where tobacco-lime is placed in combination as a quid and was seen more often among men especially in premolar region. Bhonsle RB et al. (1979) suggested that the tendency for the superficial layers of the lesion to be scraped off is probably due to the caustic action of the mixture (pH 8.3).

During transformation of normal tissue to premalignancy or malignancy, cellular changes occur at the molecular level before they are seen under the microscope and much before clinical changes become evident. Identification of high-risk oral premalignant lesions and intervention at premalignant stages could constitute one of the keys in reducing the mortality, morbidity and cost of treatment associated with oral squamous cell carcinoma. In addition, certain patients are known to be at high risk for head and neck cancer, specifically those who use tobacco or alcohol and those over 45-year-old.Such patients can be screened by clinical examin-ation,as early-stage disease, if diagnosed, is curable[7].

Tobacco induced mucosal changes have been identified in exfoliated cells[8].The morphology of the exfoliated cells depends on the nature of the changes taking place in the epithelial layer; conversely, alteration in cytological pattern may be attributed to the changes occurring in the epithelial layer[9]. Applying this possibility exfoliative cytological techniques have been applied to examine the effect of tobacco on the oral mucosa[10,11].

Comparision of cytological and nuclear area showed decrease in both the parameters in group B and decrease cytological area and increased nuclear area in group C relative to group A.

This decrease in cell size from group A to group C could be an early indication of malignant change as reported by cowpe et al in 1985 and Einstien et al in 2005[2,3].

The N/C ratio increased from group A to group C thereby suggesting changes in the nuclear content which is required for replication.

The decrease in cytoplasmic and nuclear area of group B could possibly reflect tobacco induced apoptosis and subclinical kearatinization leading to an increased number of cells exhibiting pyknosis with cell shrinkage and may be an early indication of genotoxic effect of tobacco.

References

1. Freitas Md, García–garcía A, Carneiro Jlm, Crespo–abeleira A, Gándara–rey Jm, Exfoliative Cytology Of The Oral Mucosa: A Cytomorphometric Comparison Of Healthy Oral Mucosa In Oral Cancer Patients And Healthy Subjects. Revista Brasileira De Patologia Oral 2003, 2(4):2–6.

2. Einstein Tb, Sivapathasundharam B, Cytomorphometric Analysis Of The Buccal Mucosa Of Tobacco Users. Indian J Dent Res 2005, 16(2):42–46.

3. Cowpe Jg, Longmore Rb, Green Mw, Quantitative Exfoliative Cytology Of Normal Oral Squames: An Age, Site And Sex-related Survey. J R Soc Med 1985, 78(12):995–1004.

4. Cowpe Jg, Longmore Rb, Green Mw, Quantitative Exfoliative Cytology Of Abnormal Oral Mucosal Smears. J R Soc Med, 1988, 81(9):509–513.

5. Frost Jk, Pathologic Processes Affecting Cells From Inflammation To Cancer. In: Bibbo M (ed), Comprehensive Cytopathology, 2nd Edition, W.b. Saunders Company, Philadelphia, 1997, 68–78.

6. Bhonsle Rb, Murti Pr, Daftary Dk, Mehta Fs, An Orallesion In Tobacco-lime Users In Maharashtra, India J Oral Pathol 1979, 8(1):47–52.

7. Mehrotra R, Gupta A, Singh M, Ibrahim R, Application Of Cytology And Molecular Biology In Diagnosing Premalignant Or Malignant Oral Lesions. Mol Cancer 2006, 5:11.

8. Ghose Ur, Parida Bb, Cytological Study Of Exfoliated Buccal Mucosa Cells Of Tribes In Orissa State (india) With High Risk For Oral Cancer. Indian J Cancer 1995,32(3):95–99.

9. Hillman Rw, Kissin B, Oral Cytologic Patterns In Relation To Smoking Habits. Some Epithelial, Microfloral, And Leukocytic Characteristics. Oral Surg Oral Med Oral Pathol 1976,42(3):366–374.

10. Ogden Gr, Cowpe Jg, Green Mw, Quantitative Exfoliative Cytology Of Normal Buccal Mucosa: Effect Of Smoking. J Oral Pathol Med 1990, 19(2):53–55.

11. Zimmermann Er, Zimmermann Al, Effect Of Race, Age,smoking Habits, Oral And Systemic Disease On Oral Exfoliative Cytology. J Dent Res 1965, 44:627–631.