CYTOMORPHOLOGICAL CHANGES IN BUCCAL EPITHELIAL CELLS OF ERYTHROPLAKIA, LEUKOPLAKIA, LICHEN PLANUS AND ORAL SQUAMOUS CELL CARCINOMA PATIENTS

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Abstract

Chewing of tobacco as a part of custom in rural India is followed for many centuries. Superficial cells of the oral mucosa are constantly being shed, as a result of constant abrasion of the surface tissue. Any defects in this procedure can lead to developmental anomalies as well as cancerous growth. Cytomorphometric examination of these cells is one such priceless quantitative method to assess the influence of tobacco chewing on buccal mucosa. Micronuclei are extra nuclear cytoplasmic bodies, induced in cells by numerous genotoxic agents that damage the chromosomes. The data of the different nuclear anomalies in buccal mucosal cells was analyzed on the basis of precancerous and cancerous oral lesions. The data of the present study clearly revealed that cytomorphological changes were much more evident in patients with oral cancerous lesions in comparison to patients with precancerous oral lesions.

Key words: Chewing tobacco, Oral lesions, Micronuclei assay.

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Introduction

Buccal epithelial cells form the first barrier in the oral mucosa exposed to chewing tobacco. Consequently, it could be argued that oral epithelial cells signify an ideal target site for early genotoxic events induced by carcinogenic agents entering the body via inhalation and ingestion. (1) In healthy individuals, epithelial cells of the buccal mucosa in the oral cavity are naturally exfoliated every day. Superficial cells of the oral mucosa are constantly being shed, as a result of constant abrasion of the surface tissue. However, it maintains its structural integrity because of cells in the basal layers, which act as stem cells. Any defects in this procedure can lead to developmental anomalies as well as cancerous growth. (2)

Chewing or holding of tobacco in the oral cavity allows the absorption of carcinogens present in tobacco through the oral mucosa and even orally absorbed these carcinogens remains for a longer period in the bloodstream. This leads to damage to the oral mucosa resulting in precancerous and cancerous conditions. All these pre-cancerous conditions may not develop into cancerous conditions, so WHO in 2005 stated that the term

"potentially malignant disorders (PMDs)" is more appropriate instead of precancerous. [3] The most common precancerous oral lesions are Erythroplakia, Leukoplakia, and Oral lichen planus.

In the beginning, leukoplakia looks like a little raised grayish-white plaque, which may be having a clear-cut demarcation or may be blended with the enclosing mucosa. Upon progression of the condition, it becomes whiter and thick and even in some cases, it looks like leather with fissures on the surface. [4] Erythroplakia looks like a soft red plaque with the velvety texture. In the majority of the cases this plaque is very well differentiated, but in a few cases, with time it blends with the enclosing mucosa. [5] At the site of the oral lichen planus white, wavy, parallel, non-elevated striae that do not crisscross appears. [6]

Exfoliated buccal cells are at the final stage of cell differentiation, and rarely display mitotic features. Cytomorphometric examination of these cells is one such priceless quantitative method to assess the influence of tobacco chewing on buccal mucosa. [7]

Micronuclei are extra nuclear cytoplasmic bodies, induced in cells by numerous genotoxic agents that damage the chromosomes. [8] The frequency of occurrence of micronuclei is a measure of chromosome breakage in early cell divisions, and the number of micronuclei is known to increase with carcinogenic stimuli, long before the development of clinical symptoms. The aim of the present study was to evaluate whether the micronuclei assay is a significant tool to differentiate cytomorphological changes between precancerous oral lesions and cancerous oral lesions.

Material and Method:

The present study was conducted on 36 patients with precancerous oral lesions and 24 patients suffering from oral carcinoma. The subjects chosen for the present study were the patients visiting to OPD of National Institute of Medical Sciences & Research and Hospital (Jaipur). The study was approved by the Institutional Ethical Committee. The subjects were informed about the study and a written consent was obtained from them.

To evaluate the effect of chewing habit at the cellular level micronuclei assay was performed, which may serve as an early biomarker. Micronuclei assay is a rapid, sensitive and easy method to assess the genotoxic damage, so it is used widely. Micronuclei are formed during mitosis by acentric chromosome or whole chromosome. During anaphase when the chromosomes are moving toward opposite poles; a part or whole chromosome lags behind, which results in one daughter cell having one nucleus and another is having one small nucleus (micronucleus) along with the main nucleus. The number of micronuclei increases with the amount of genotoxic damage, i.e. if the damage is more than the number of micronuclei will be more.

The methodology was as follows:

1. Before sample collection each subject was asked to rinse his/her mouth thoroughly with water to remove food particles and debris. The exfoliated buccal mucosal cells were collected from the oral mucosa of cheeks by gentle scraping from a wooden spatula. All precautions were taken to avoid infections of any type.

2. For making a cell suspension of buccal mucosal cells spatula were swirled into a 50ml conical centrifuge tube containing 15ml of buffer solution. Then centrifugation was done for 15 minutes at 1500rpm. After this, gentle pipetting of the cells was done in the buffer solution. Same step was repeated thrice for washing of buccal mucosal cells. The purpose of the washing is to inactivate the endogenous DNAases present in the oral cavity. Gentle pipetting of the cells reduces clumping and also lyse the broken cells.

3. The glass slides were cleaned and coded. 50-100µl of the cell suspension was laid and spread well on clean, pre-heated (37oC) slide. The slides thus prepared were air dried for 5-10 minutes.

4. Then the slides were fixed in methanol and stained with Giemsa. The purpose of using fixative is to maintain the cytomorphological characteristics and the diagnostically essential elements of the cell.

5. The stained slides were observed under the binocular microscope. Five areas of evenly spread cells were counted for the total number of normal cells, and the following nuclear changes were observed and recorded:

- Number of normal cells
- Number of micronucleated cells
- Total number of micronuclei
- Number of binucleated cells
- Number of pyknotic cells
- Number of cells with nuclear bud
- Number of karyorrhectic cells
- Number of karyolytic cells

Results

The present study was undertaken to know the effects of chewing tobacco on buccal mucosal cells of precancerous and cancerous patients. Micronuclei assay was used to see the cytomorphological changes in buccal epithelial cells of precancerous and cancerous patients.

Table 1. Distribution of subjects as per their clinical condition

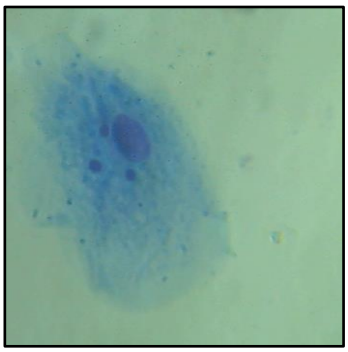
Clinical condition (CC)		No. of Subjects (50)
Precancerous Condition (36)	Erythroplakia	5
	Leukoplakia	20
	Oral Lichen Planus	11
Oral Squamous Cell Carcinoma		24

A total of 50 patients were included in the study. Among them 36 patients were having precancerous oral lesions. In the precancerous oral lesions 5 patients were having Erythroplakia, 20 were having Leukoplakia and 11 were having Lichen planus. Along with precancerous 24 patients included in the study was suffering from oral carcinoma.

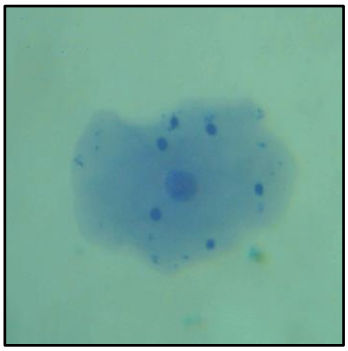
The data of the different nuclear anomalies in buccal mucosal cells was analyzed on the basis of precancerous and cancerous oral lesions. The mean ± SE of MNC and TMN in tobacco chewers with precancerous oral lesions was 2.35 ± 0.32 and 3.11 ± 0.36, respectively. The mean ± SE of MNC and TMN in tobacco chewers with cancerous oral lesions was 3.54 ± 0.60 and 4.62 ± 0.68, respectively and the difference among two was highly significant (P<0.001). The mean ± SE of other nuclear anomalies like number of bi-nucleated cells, pyknotic cells, karyorrhectic cells, karyolitic cells and cells with nuclear bud also shows that there is a highly significant difference between precancerous and cancerous patients. The cytomorphological changes were more evident in patients with precancerous oral lesions than cancerous oral lesions (Fig 1).

Table 2. Comparison of Mean ± SE of different nuclear anomalies (NA) among patients with Precancerous and Cancerous Oral Lesions

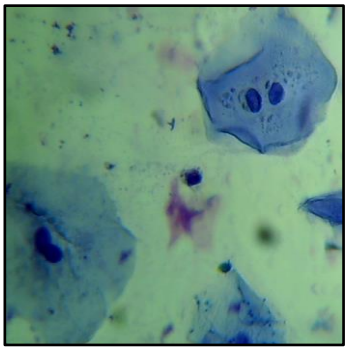
Nuclear Anomalies (NA)	Patients with Precancerous Oral Lesions	P value	Patients with Cancerous Oral Lesions	P value
Number of cells with micronuclei (MNC)	2.35 ± 0.32	<0.001 (HS)	3.54 ± 0.60	<0.001 (HS)
Total number of micronuclei (TMN)	3.11 ± 0.36	<0.001 (HS)	4.62 ± 0.68	<0.001 (HS)
Bi-nucleated cells (BN)	5.76 ± 0.89	<0.001 (HS)	6.58 ± 1.24	<0.001 (HS)
Pyknotic cells (P)	8.97 ± 1.34	<0.001 (HS)	10.33 ± 1.87	<0.001 (HS)
Cells with nuclear bud (NB)	0.26 ± 0.15	<0.001 (HS)	0.87 ± 0.17	<0.001 (HS)
Karyorrhectic cells (KR)	1.5 ± 0.25	<0.001 (HS)	2.70 ± 0.54	<0.001 (HS)
Karyolytic cells (KL)	3.67 ± 0.52	<0.001 (HS)	5 ± 0.73	<0.001 (HS)



A. Cell with 3 Micronuclei

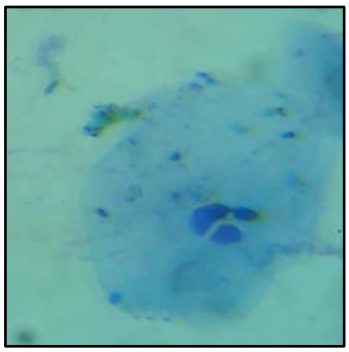


B. Cell with 6 Micronuclei

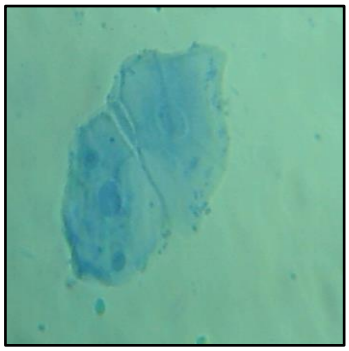


C. Binucleated cells

D. Karyorrhectic Cells and Cell with Nuclear bud



E. Cell with binucleation and nuclear bud



F. Cell with karyolysis and 2 micronuclei

Fig.1: Photographs (A-F) showing different nuclear anomalies present in buccal mucosal cells by Giemsa stain

Discusssion

Chewing of tobacco as a part of custom in rural India is followed for many centuries. However, use of tobacco has increased significantly because of large scale production and social marketing, which has even attracted the youth. The literature is replete with data which is thoroughly showing light on the genotoxic and carcinogenic potential of tobacco, but still major attention is diverted towards smoking as compared to chewing tobacco.

Different stains were used in different studies for scoring of micronuclei and other nuclear anomalies. In the majority of the studies Giemsa stain was used similar to our present study like Halder et al [9] and Adhikari et al. [10] Other stains used were Feulgen and fast green, acridine orange and Feulgen stain , PAP stain, acetoorecine and fast green and Schiff and fast green.

Presence of micronuclei and other nuclear anomalies shows the genotoxic damage. In the present study we found that the nuclear anomalies were more in patients with cancerous

oral lesions than the patients with precancerous oral lesions. Similar to our study, Casartelli et al. [11] also reported that micronuclei count was more in cancerous patients as compared to precancerous.

In the present study the difference between nuclear anomalies present among two groups was highly significant. Similar to our study, Devi et al. [12] reported that the difference in the micronuclei count in between precancerous patients and controls was statistically significant.

Conclusion

The data of the present study clearly revealed that chewing tobacco has deleterious effects. The cytomorphological changes were much more evident in patients with oral cancerous lesions in comparison to patients with precancerous oral lesions.

Contribution of each author

[1] Data collection

[2] Interpretation of data

[3] Study design and writing paper

[4] Study design and writing paper

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