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Brief Communication

Proliferative pool analysis in gutkha chewers

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Gutkha chewing is a widely prevalent habit in South and Southeast Asia [1]. Chronic exposure to gutkha constituents affects the epithelial proliferative pool [2]. This study investigates the proliferative pools and nuclear diameters in oral submucous fibrosis (OSMF), oral epithelial hyperkeratosis and low-grade epithelial dysplasia to elucidate a hypothesis regarding their potentially malignant progression.

1. Materials and methods

1.1. Tissue material

Forty-five formalin-fixed, paraffin-embedded tissue samples were obtained from histopathologically proven cases of OSMF, oral epithelial hyperkeratosis and low-grade epithelial dysplasia ($n = 15$, each). Study cohort was age-matched between 18 and 39 years and had a chronic habit history of minimum 5 years. All subjects were males and the biopsy site selected was buccal mucosa. Institutional ethical clearance was obtained. Immunohistochemistry was performed using mouse antihuman Ki-67 (BGX-297 clone, Biogenex Lab, Cherlapalli, Secunderabad, Andhra Pradesh, India).

1.2. Mean Ki-67 (labeling) proliferation index

Five areas were randomly selected along the length of the epithelium. Basal and suprabasal layers were selected and proliferation index was calculated by counting the total number of Ki-67-positive nuclei divided by total number of areas counted under 40× (Fig. 1a–c).

1.3. Nuclear diameter

An ocular grid was standardized at 40× magnification.

40 divisions of stage micrometer = 10 divisions of ocular grid; therefore, 1 division = 0.25 mm.

2. Results and discussion

The current study design included three groups aimed at comparing values obtained after employing Student's *t*-test. Group I comprised OSMF, group II hyperkeratosis and group III low-grade epithelial dysplasia, respectively. For correlation, comparisons were made between groups. The proportions of mean Ki-67-positive cells were 2.1 ± 0.1 (group I), 3.2 ± 0.2 (group II) and 4.1 ± 2 (group III).

One-way ANOVA and Student's *t*-test were used for statistical analysis. No significant correlation (Table 1) was obtained in Ki-67 labeling indices on group comparison (OSMF and hyperkeratosis: $P = 0.240$; OSMF and low-grade epithelial dysplasia: $P = 0.113$; hyperkeratosis and low-grade epithelial dysplasia: $P = 0.672$). Hence, it can be deduced that the Ki-67 labeling index cannot be used to predict any correlation between these potentially malignant disorders, specifically in deducing the aggressive nature of OSMF.

Mean nuclear diameters in groups I, II and III were 2.1 ± 1, 3.26 ± 2 and 4 ± 1, respectively. On comparing nuclear diameters,

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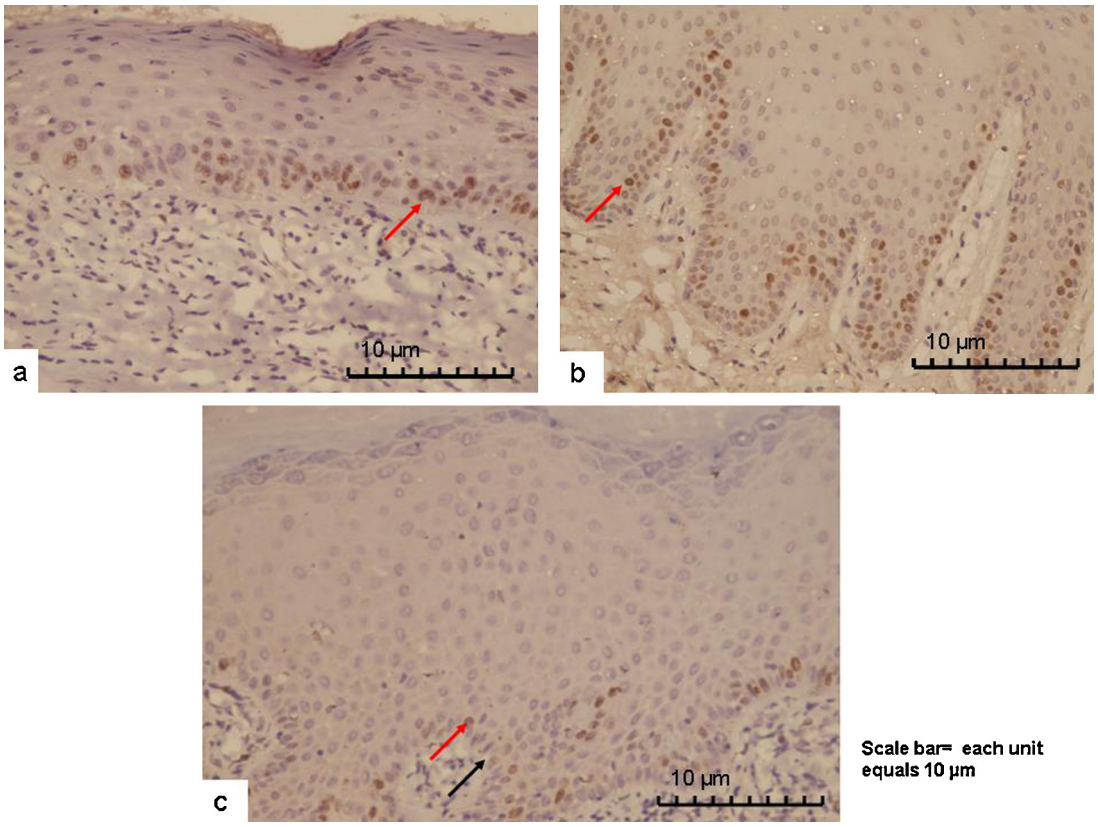

Fig. 1. Photomicrograph depicting Ki-67-positive basal and parabasal cells in (a) oral submucous fibrosis (40×), scale bar indicating area chosen for morphometric measurement wherein the red arrow indicates strongly immunopositive nuclei with moderate distribution; (b) low-grade epithelial dysplasia (40×), scale bar indicating area chosen for morphometric measurement where the red arrow indicates strong immunopositivity of nuclei for Ki-67 with greater distribution; and (c) hyperkeratosis (40×), scale bar indicating area chosen for morphometric measurement where the red arrow indicates weak immunoreactivity and the black arrow shows negative or weak Ki-67 uptake. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Comparison between proliferation indices and nuclear diameters.

	Oral submucous fibrosis ($n=15$) and hyperkeratosis ($n=15$)	Oral submucous fibrosis ($n=15$) and low-grade epithelial dysplasias ($n=15$)	Hyperkeratosis ($n=15$) and low-grade epithelial dysplasias ($n=15$)
Comparison among Ki-67 labeling indices (proliferation index)			
T	0.76	0.67	0.14
P value	0.240	0.113	0.672
Comparison among nuclear diameters			
T	0.74	0.40	1.03
P value	0.028	0.476	0.005

significant values were obtained between OSMF and hyperkeratosis and hyperkeratosis and low-grade epithelial dysplasia ($P=0.028$; $P=0.005$, respectively; Table 1).

Ki-67 is a proliferation marker indicated for 'growth fraction' determination. Its expression reflects upon cellular kinetics [3]. Determination of epithelial proliferative activity can be useful for investigating biological behavior. The present study results showed no correlation in Ki-67 labeling (proliferation) indices between values obtained from any of the groups (Table 1). Therefore, it can be validated that counting of Ki-67-positive cells cannot be used as a progressive or prognostic criterion in this particular disease spectrum. Nuclear diameters, however, showed a significant correlation between groups I and III ($P=0.028$ and 0.005, respectively). Therefore, nuclear diameters are indicative of a progressive spectrum of potentially malignant disorders. However, the Ki-67 labeling index cannot be used for predicting the aggressive nature of OSMF. Based upon statistical findings obtained in this study, an explainable hypothesis regarding the pathogenesis of malignancy from OSMF can thus be elucidated. Gutkha chewing is an addiction afflicting a large population. This smokeless tobacco form contains various constituents, in particular, areca nut. N-nitroso compounds cause genotoxic damage to the proliferative compartment, thereby initiating a progressive spectrum of disease usually terminating in a malignancy [4].

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