

# Morphological Assessment of Epithelium and Nucleus in Oral Submucous Fibrosis Individuals

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## ABSTRACT

**Aim:** To evaluate histomorphological alterations present within nucleus and epithelium in oral submucous fibrosis subjects

**Methods:** This study recruited 50 clinically diagnosed cases of oral submucous fibrosis which were active chewers of areca nut and associated products. Oral mucosa tissue blocks were prepared and processed followed by staining with Hematoxylin & Eosin to observe morphological features. The findings were statistically analyzed through SPSS version 23

**Results:** Amongst the sample size of 50, 33(66%) of cases had pleomorphic nuclear structure out of which 21(91.3%) had prominent nucleoli. High statistical significance was appreciated relating to involvement of nuclear and nucleoli structure. In addition, epithelial demarcation was appreciated within epithelium present in disease. 37(74%) cases were histologically identified cases of fibrosed structure out of which 31(83.8%) of the subjects had hyperplasia in epithelial lining. Hyperplastic epithelium was significantly correlated with OSMF. However, strength of association was weak with a p value of 0.04

**Conclusion:** The results revealed pleomorphism in nuclei underlining a strong association of structural alteration with disease initiation and progression. Hyperplastic stratified squamous epithelium was found in abundance which could be attributed to adaptive response of epithelium to inflammatory alkaloids present in areca nut products. Such findings could serve as trigger factor for oral submucous fibrosis and should be considered significant for early diagnosis and treatment management.

**Keywords:** Oral submucous fibrosis, epithelium, nuclei, nucleoli, histology.

## INTRODUCTION

Diagnosis and management of oral submucous fibrosis is based on various architectural changes present within histological structure of oral mucosa.<sup>1</sup> Mucosal alteration at tissue and cellular level represent a risk of progression of up to 6-36%<sup>2</sup>, Nuclear morphometric parameters have shown measurable changes within cell structure correlating with malignant potential of oral submucous fibrosis<sup>3</sup>. Oral submucous fibrosis is preceded by vesicle formation occasionally but always associated with juxta epithelial inflammation leading to fibroelastic changes in lamina propria of oral mucous membrane. Main risk factor for initiating changes in histological structure is consumption of areca nut products. Chewing of areca nut is a practice dated to many years back in subcontinental region of Pakistan, India, Bangladesh, and Sri Lanka. It is a constituent of cultural, social, and religious rituals and everyday life and social practices which enjoys utmost acceptance in society. Areca nut is a mixture of carcinogenic compounds and carcinogen aiding products with ample clinical, epidemiological, and statistical evidence of involvement in pathogenesis of OSMF<sup>4</sup>.

The malignant potential of areca nut involves three main carcinogenic compounds namely, arecaidine, arecoline and safrole.<sup>5</sup> These compounds leach out harmful alkaloids facilitating fibroblastic changes in lamina propria and early epithelial hyperplasia which later leads to atrophy in epithelial structure.<sup>6</sup> One of the hallmark of malignant transformation is reflected as variable shape of nucleus in cell and uncontrollable growth rate of epithelium. Nuclear variability is considered as a most suitable biological marker of mitotic activity in disease.<sup>7</sup> Traditionally, Pathologic view of OSMF suggests a thinner epithelium than healthy. This refers to a state of atrophy.

Oral submucous fibrosis have been discovered from 70 years and yet the evidence regarding malignant potential of atrophic epithelium is debatable<sup>8</sup>. Dysplastic changes in epithelium can be validated to predict the malignant transformation risk and

treatment management of potentially malignant disorders of oral cavity. When dysplasia involves entire thickness of epithelium, the term carcinoma in situ could be used to define the presentation of tissue<sup>9</sup>.

The objective of this study was to evaluate pattern of nuclei and epithelial expression amongst oral submucous fibrosis cases.

## MATERIALS AND METHODS

A total of 50 clinically OSMF diagnosed cases were inducted in the study. Written, initialed and verbal consent was obtained from all participants of the research. Subjects with difficulty in mouth opening/trismus, blanched mucosa, ulcers, burning sensation and feeling of stiffness were included while subjects with any other system disease, TMJ disorders, diagnosed oral carcinoma, already being treated were excluded out of the study. A detailed questionnaire was filled to record history and examination protocols. Biopsy was taken from the buccal mucosal surface and formalin fixed tissue blocks were prepared followed by slide preparation using H & E stain. Data was categorically arranged in excel and statistically analyzed using SPSS. p value of  $\leq 0.05$  was considered as statistically significant. This study was approved by Institutional Review Board

## RESULTS

The hyperchromatic pleomorphic nuclear structure exhibited a high statistical value (p value 0.0001) while validating presence of prominent nucleoli between them. Statistical measures in study are elaborated in table 1. Chi square test confirmed significant association amongst categorical variables. Thus, there is increase in pleomorphism and hyperchromatism in nuclei of diseased patients as compared to the healthy oral mucosa. Interesting findings were revealed regarding epithelium associated with OSMF. Hyperplasia in epithelial structure showed high susceptibility to disease representing induction of local inflammation (p value 0.04). Integrity of oral mucosa normal epithelial structure was impaired in response to disease. Detailed descriptive statistics are depicted in table 2.

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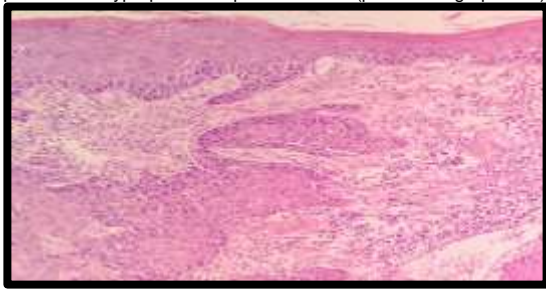
Table 1: Relationship of nuclei and nucleoli amongst OSMF cases

Nuclei	Nucleoli			Total	Sig. value
	Distant	Prominent	Unidentified		
Pleomorphic hyperchromatic nuclei	7(70%)	21(91.3%)	5(29.4%)	33(66%)	0.0001
Normal/round nucleus	3(30%)	2(8.7%)	12(70.6%)	17(34%)	
Total	10(100%)	23(100%)	17(100%)	50(100%)	

Table 2: Association of oral submucous fibrosis with epithelium

Fibrosis	Epithelium				Total	Sig. value
	Stratified squamous epithelium	Hyperplastic stratified squamous epithelium	Keratotic stratified squamous epithelium	Neoplastic Squamous epithelium		
Present	5(50%)	31(83.8%)	1(50%)	0%	37(74%)	0.041
Absent	5(50%)	6(16.2%)	1(50%)	1(100%)	13(26%)	
Total	10(100%)	37(100%)	2(100%)	1(100%)	50(100%)	

Figure 1: H & E-stained section of buccal mucous membrane of an OSMF patient with hyperplasia in epithelial tissue (photomicrograph 40X)



**DISCUSSION**

Asian population who consume areca nut and betel quid products are predominantly affected by OSMF<sup>10</sup>. It appears that fibrosis in epithelial and subepithelial connective tissue region signifies abnormal proliferative activity of cells in basal and suprabasal compartments of stratified squamous epithelium.<sup>9,10</sup>In the light of literature, OSMF represents a wound healing process of oral mucosa which has failed after chronic persistent injury.<sup>11</sup>Data available in literature recognize changes in epithelium of OSMF oral mucosa briefly. Most frequent findings reported are acanthosis and atrophied epithelium<sup>12</sup>.

This research shares its worthwhile experience of analyzing oral epithelium from subjects suffering from OSMF. Hyperchromasia in nucleus and cellular pleomorphism demonstrate relationship between OSMF and dysplastic epithelium in background of advancement in disease.<sup>13</sup>Our research showed highly significant results of variability in structural alteration of nucleus. This is indicative of high proliferative activity leading to hyperplasia rather than the conventional epithelial atrophy of oral mucosa in OSMF found in literature. Our findings of nuclear hyperchromatism and pleomorphism could also be correlated with molecular alterations for development of primary tumor cells<sup>9,13,14</sup>. This highlights procedure of molecular advancement in order to establish biomarkers and appreciate changes at genetic level which ultimately plays a vital role for OSMF transformation into OSCC<sup>15</sup>.

In this research we have compared epithelium of OSMF subjects to find histological feature to correlate with disease. Although the diagnosis of this condition is classically based on clinical manifestations, the commonly associated definition of OSMF is evidently on basis of its histological features. Therefore, through this research, novel findings of epithelial lining will be added to the database of literature. Since the gingivobuccal region is the most involved site for evaluating histomorphological modification, buccal mucosa is generally the biopsy site selected to isolate epithelial mucosal margins and compare with the disease<sup>16</sup>.

Our research showed significant association of hyperplastic stratified squamous epithelium. Desai, Mamatha, Khatri & Shetty<sup>17</sup> reported of contradictory results which associated traditional atrophic epithelium in fibrosis of oral cavity. Our study revealed of

increased epithelial proliferation rate causing cellular hyperplasia instead of conventional results of atrophied epithelium. Regarding this, Sarode & Sarode<sup>18</sup> believed that even though there is high exfoliation of mucosal cells due to insufficient nutrition from salivary mucous gel and recurrent trauma from alkaloids present in areca nut products, rapid proliferation of cells does not help to retain architectural structure of epithelium and atrophied epithelium is produced instead of hyperplasia. Veeravarmal, Austin, Siddavaram, Thiruneelakandan & Nassar<sup>19</sup> also reported of variability in results regarding epithelium in OSMF subjects elaborating that atrophy is an after effect of decreased cellularity and vascularity and can be called as ischemic atrophy while hyperplasia is justifiable as an adaptive response to recurrent inflammation to the oral mucous membrane<sup>19</sup>. This discrepancy of epithelial pattern might be because of variable sample size of research and different adaptation methods than current study.

Thus, it can be predicted that persistent stimulus to buccal mucosa can reduce blood supply eventually which allows carcinogens to penetrate the surface of mucosa and irritate it and reacts as hyperplasia in its cells and in longer period, decreased vascularity and reduced blood supply promotes atrophy of tissue.<sup>14,20</sup> We believe that histomorphological alterations in marginal tissue are closely associated to progression of OSMF into malignancy.

**CONCLUSION**

Emerging evidence suggests that hyperchromasia and pleomorphism is a crucial event to promote abnormal mitosis in nuclear structure along with epithelial cell hyperplasia. These could be high-risk histological changes for OSMF and will enable physicians to follow patients on priority basis for early management of the disease and prevent it from further malignant transformation into OSCC.

**Conflict of interest:** There was no conflict of interests.

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