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Variation of Epithelial and Nuclei Morphohistology in Oral Submucous Fibrosis Patients

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ABSTRACT

In the background of oral submucous fibrosis, oral cancer is the most commonly present malignant transformation in South Asian regions. Alteration of tissue architecture contributes significantly to determine the exact pathological state of the disease. **Objective:** To evaluate histological changes in structure of oral submucous fibrosis patients in context of textural properties of epithelium and morphology of nuclei. **Methods:** A total of 50 subjects were inducted in this cross-sectional study performed at dentistry department of Jinnah Postgraduate Medical Center (JPMC) in Karachi. Punch biopsy samples were taken from buccal mucosa followed by preparation of tissue blocks and slide preparation to analyze histology of premalignancy and record abnormal features present in normal oral tissue. Results were analyzed through SPSS version 23.0 and p value of ≤0.05 was considered as statistically significant. **Results:** 37 (74%) subjects had prominent Oral Submucous Fibrosis histologically out of which abnormal epithelial parameters such as stratified squamous, hyperplastic, keratotic, and neoplastic were noted in 5 (10%), 31 (62%), 10 (2%), 0 (0%) subject respectively. p value 0.041 was calculated using Chi square analysis for qualitative data which depicted relevance of hyperplastic epithelium in Oral Submucous Fibrosis. 29 (87.9%) subjects showed pleomorphism in nucleus and 8 (47.1%) showed normal/round nucleus in Oral Submucous Fibrosis subjects with a p value of 0.003. **Conclusions:** Histological analysis which is not routinely performed in oral submucous fibrosis patients could visualize accurate extent of disease through structural variability of epithelium and nucleus.

INTRODUCTION

In Asia and a few pacific Islands people consume Betel Quid (BQ) on a regular basis in some form which is clinically linked to rigid oral mucosa and pathologically characterized by inflammatory epithelial response and fibroelastic variations [1]. Development of Oral Submucous Fibrosis is strongly linked to causative factors of consuming Areca Nut [2]. Disruption of balance of extracellular matrix is due to flavonoids, alkaloids and copper present in Areca nut therefore it is certainly a prime factor in genesis of Oral Submucous Fibrosis as Flavonoids (tannins and catechins) block collagenase and stabilize fibers of collagen recruiting mucosal inflammation [3]. During chewing mechanism of areca nut products there is chemical stimulation by toxic components leading to trauma to the underlying tissues through physical frictional damage. These factors affect oral mucosal epithelium which in reaction responds and becomes hyperplastic in contrast to the conventional findings of atrophy in epithelium. Epithelial cells which survive the harsh trauma exhibit heterogenicity, variable nucleus and cytoplasmic ratio, abnormal mitosis and

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cellular irregularities [4]. Cytokines are predominantly associated to contribute to pathogenesis of OSMF such as increased expression of Tissue Growth Factor (TGF- β). Cytotoxic effects of areca nut products lead to epithelial cell apoptosis and promote avascularity. This pathomechanism yet needs to be further elucidated [5, 6]. OSMF is a chronic form of oral disorder which produces scarring of tissue through its fibrosis potential. Oral Submucous fibrosis is classified as a precancerous lesion by WHO having a highest rate of transformation into Oral Squamous Cell Carcinoma (OSCC). This premalignancy is prevalent most in Southeast Asia with relevant burden of disease in China, India, Pakistan and Taiwan while in USA and other European population, only a few cases have been documented that too because of migratory Asians [7]. The premalignant nature of OSMF also characterizes high frequency of epithelial dysplasia in OSMF [4]. Various risk factors are attributed to bear causative role in OSMF etiology. This includes malnourishment, genetic predisposition, alteration of salivary composition, chewing areca nut, intake of chilies, collagen defects and autoimmune disorder [5, 6]. Areca nut is the 4th ranked social drug following ethanol, nicotine, and caffeine. Hence, therefore it is accepted as the prime risk factor for OSMF. Betel quid and areca nut usage is a native habit in subcontinent and most prevalent risk factor having a significant contribution for OSMF development [5]. Overconsumption of oral mucosal irritants induce inflammatory reaction to initiate OSMF which further leads to chronic inflammation in the form of abundant deposition of collagen in connective tissue and muscular degeneration along with reduced vascularization of blood vessels present in connective tissue and dense hyalinized area [8]. As a result, there is increased synthesis of collagen as a principle underlying factor contributing to OSMF [7, 9]. Xu HQ *et al.*, states that pathological changes in connective tissue of OSMF are likely to affect overlying epithelium [10]. The conventional image of OSMF pathology suggests that epithelial surface becomes thin and occupies state of epithelial atrophy where as in seventy years' history of OSMF pathology, facts related to malignancy conversion of atrophic epithelium is contestable and contrary to the classic pathology, oral mucous membrane subjected to trauma by chemical exposure of betel quid showed physical trauma locally which is later compensated by hyperplastic epithelial tissue in early phase of OSMF [11].

The aim of this research was to have an insight through histological features present in epithelium and nucleus of oral submucous fibrosis patients to elucidate effective understanding of pathogenesis, diagnosis and management strategies for it.

METHODS

This cross-sectional study was conducted for data collection at the dentistry department of Jinnah Postgraduate Medical Center, Karachi after approval from the scientific ethical review board of the institute, No.F.2-81/2020-GENL/48908/JPMC and ethical review committee of Bahria University Medical and Dental College Karachi, No:FRC-BUMDC-13/2020-Ana-113.Data was systematically collected from January to August 2021. Subjects who gave written consent were included. Sample size was calculated using open epi software online by using prevalence 4.47% of oral submucous fibrosis worldwide [12]. The confidence interval was kept at 95% and precision was set at 6%. The sample size calculated was 46 but 50 patients were enrolled. Informed written consent was obtained from all subjects of the research. Data were systematically collected for 6 months using a detailed questionnaire proforma to confirm cases of Oral Submucous Fibrosis and consideration for histological analysis of nucleus and epithelium. Subjects with restricted mouth opening, burning sensation, white fibrotic bands, ulceration, and pain were included in the research. Subjects already undergoing treatment, Temporomandibular Joint disorders, any other systemic disease or carcinoma were constituted as the exclusive criteria. Paraffin embedded tissue blocks were prepared after incisional biopsy taken from buccal mucosa of clinically identified oral submucous fibrosis patients. Collected data was organized in Microsoft excel and processed with SPSS 23.0. Fisher Exact and Chi square analysis was utilized for comparison of gender, epithelium, and nuclei with Oral Submucous Fibrosis. All p values of ≤0.05 were considered to indicate statistical significance.

RESULTS

Current results has estimated high preponderance of male gender which includes 73% of total sample size but with non-significant statistical association using Chi square analysis. In total, 37 patients had active oral submucous fibrosis out of which 27% population was female as shown in table 1.

Table 1: Association of OSMF with Gender (n=50)

Gender	Count (%)	Fibrosis N (%)		Total N (%)	p-value
		Present	Absent		
Male	Within Fibrosis	27 (73.0%)	7 (53.8%)	34 (68%)	0.301
Female		10 (27.0%)	6 (46.2%)	16 (32.0%)	
Total		37 (100%)	13 (100%)	50 (100%)	

While analyzing the statistical association of epithelium, 5 (10%) symptomatic OSMF subjects showed presence of stratified squamous epithelium, 1 (2%) of subjects showed keratotic epithelium, 0 (0%) subject showed neoplastic stratified squamous epithelium while most of the sample 31 (62%) of participants were found to have hyperplastic

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stratified squamous epithelium as shown in Figure 1A. A significant correlation amongst variables was found (p value = 0.041) however the strength of statistical association was weak between epithelial variety and the disease. Chi square test was performed as shown in table 2.

Table 2: Epithelium Correlation amongst Cases of Submucous Fibrosis (n=50)

Epithelium						
Fibrosis	**Stratified Squamous N (%)**	**Hyperplastic Stratified Squamous N (%)**	**Keratotic Stratified Squamous N (%)**	**Neoplastic Squamous N (%)**	**Total N (%)**	**p-Value**
Present	5 (10%)	31 (62%)	1 (2%)	0 (0%)	37 (74%)	0.041
Absent	5 (10%)	6 (12%)	1 (2%)	1 (2%)	13 (26%)	
Total	10 (100%)	37 (100%)	2 (100%)	1 (100%)	50 (100%)	

The cells were assessed through histopathological evaluation after treatment with H and E staining techniques. The results showed that most cells 29 (87.9%) had pleomorphic hyperchromatic nuclei indicating cytotoxic cells amongst OSMF. Figure 1B showed distribution of nuclei amongst OSMF cases based on morphology of oral mucosal cells. Fisher Exact test was applied to test the difference between nuclei morphology (p = 0.003). This showed that nuclei of symptomatic OSMF cases were affected to adapt to an alteration in normal morphology (Table 3). p value ≤0.05 was considered statistically significant.

Table 3: Nuclei Morpho-Histology Distribution (n=50)

Nuclei				
Fibrosis	**Pleomorphic Hyperchromatic Nuclei N (%)**	**Normal/Round NucleusN (%)**	**Total N (%)**	**p-Value**
Present	29 (87.9%)	8 (47.1%)	37 (74%)	0.003
Absent	4 (12.1%)	9 (52.9%)	13 (26%)	
Total	10 (100%)	37 (100%)	50 (100%)	

In Figure 1A a 5µm thick H and E-stained section of OSMF oral mucosa showing hyperplastic stratified squamous epithelium due to basal cell hyperplasia overlying connective tissue core (photomicrograph 40X). while in figure 1B H and E-stained section image showing pleomorphic hyperchromatic nuclei (red circle) arranged around widespread cytoplasm of cells in an irregular pattern with multiple shaped nuclei due to wrinkling of nuclear membrane (photomicrograph 100X).

Figure 1 A: A 5µm thick H and E-stained section of OSMF oral mucosa showing hyperplastic stratified squamous epithelium due to basal cell hyperplasia overlying connective tissue core (photomicrograph 40X)

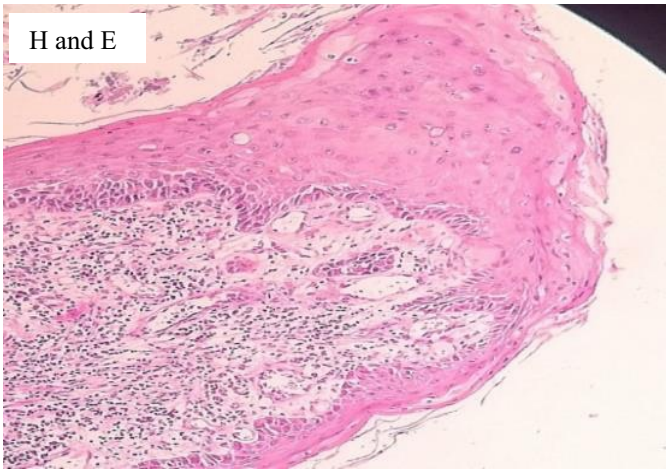
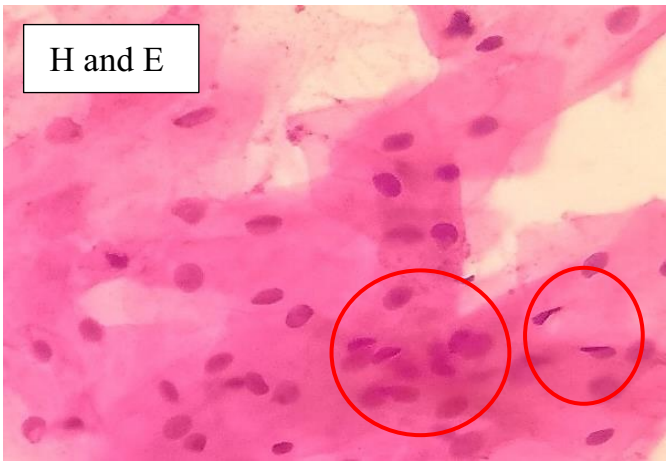


Figure 1 B: H and E-stained section image showing pleomorphic hyperchromatic nuclei (red circle) arranged around widespread cytoplasm of cells in an irregular pattern with multiple shaped nuclei due to wrinkling of nuclear membrane (photomicrograph 100X)



DISCUSSION

This research showed a non-significant statistical association of gender distribution between OSMF with greater incidence amongst male. In contradiction to these findings study by Fauzi and fellows showed 92.6% male prevalence of OSMF and while correlating gender with disease, 0.00 p value was the statistical significance which indicated that males are much likely to be affected by this disease than females [12]. These results were also inconsistent with findings of research by Sowmya and Sangavi, which revealed that majorly males are involved to have OSMF. Male predominance in OSMF could be validated due to social and traditional practices to relieve stress, freshen breath or as a stimulant [13]. Normal oral mucosa has a regenerating cell reservoir of basal stem layer for clonogenicity and self-renewal. Interruption in this cell turnover is antecedent to promote disease in the form of Oral Potentially Malignant Disorders (OPMD) and Oral

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Squamous Cell Carcinoma (OSCC) [14]. Deficiency of clonogenicity in basal stem cell surface activates epithelial atrophy which undergoes through hyperplasia amongst cells as a biological consequence [15]. Carcinogens diffuse gradually below epithelial surface. Hence, changes in epithelium are expressed and distinguished over a prolonged period of duration [16]. Present study suggests that epithelial hyperplasia, perhaps is an alternative adaptive response to local irritants to yield a greater degree of protection to the tissue present beneath. A similar study by Adhane YB *et al.*, postulated in depth observation regarding sequential adaptations in epithelial, muscular and connective tissue during gradual progressive stages of disease. To improve survival rate, it is significant to observe and identify early changes which include subepithelial inflammatory response occurring due to mucosal trauma by areca nut compounds. This inflammatory reaction consists of epithelial cell inflammatory infiltrate which is in the form of epithelial hyperplasia along with polymorphonuclear lymphocytes and plasma cells further manifesting vesicle formation and erosion [17]. Study by Jian X *et al.*, also states that OSMF pathological traits include oral epithelial tissue as either atrophic or hypertrophic while dysplastic characteristics are acquired by nucleus which becomes hyperchromatic and pleomorphic. In advanced stage of dysplasia, cellular atypia and irregularly arranged mitotic figures are present [18]. Additionally, various studies have demonstrated that OSMF transforms into malignancy through gradual changes of dysplasia amongst epithelial tissue [19]. Multifactorial etiological analysis is essential to establish corresponding therapeutic approach and integrate multidisciplinary modalities [20]. The present study is a small effort to bridge the gap between conventional concepts regarding OSMF and novel findings regarding malignisation.

CONCLUSIONS

Almost half of oral cancer conditions are diagnosed as stage III or IV during the time of first diagnosis eventually causing bad prognosis as only visual grading based on clinical parameters is insufficient and a time-consuming process. Through this study it is suggested that increased hyperplasia in epithelial layer and nuclear pleomorphism are high risk histological features of Oral Submucous Fibrosis and once these histological variations are detected, a quick strategical response to prevent oncostatic effects can be initiated timely.

Authors Contribution

Conceptualization: RRA
Methodology: MFQ
Formal analysis: SB, ZA
Writing, review and editing: AZ, RH, MH

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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