Malaysian J Pathol 2014; 36(3) : 189 – 193

ORIGINAL ARTICLE

Morphometric analysis of epithelial thickness and blood vessels in different grades of oral submucous fibrosis

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Abstract

Background: Oral submucous fibrosis (OSF) is a common oral health problem in the Indian subcontinent. It is characterized by a juxtaepithelial inflammatory reaction followed by fibroelastic changes in the lamina propria. Traditionally, it is said to be associated with marked epidermal atrophy and decreased vasculature as the disease advances. *Objective:* To assess the changes in epidermal thickness and mucosal vasculature in various stages of the disease. *Material and methods:* Patients with histological diagnosis of OSF were included in the study. Demographic data and oral habits of each patient were collected. The severity of OSF was graded histologically according to Pindborg and Sirsat. Epithelial thickness and subepithelial blood vessel area, diameter and perimeter were measured and analysed using Image analysis software IMAGE PRO PLUS version 6.0. *Results:* Thirty-five patients with OSF were studied. 25 (71.4%) were males and 10 (28.6%) were females with a male to female ratio of 1.3:1. Most patients were in the 31–40 yrs age group. The majority of patients (40%) chewed areca nut/dohra. Each grade of the disease was found to display either hyperplastic or atrophic epithelial changes. The mean blood vessel area, diameter and perimeter did not show any sustained change with the increasing severity (grade) of the disease. *Conclusion***:** These findings question the role of ischaemia in the aetiopathogenesis of oral submucous fibrosis.

Keywords: Oral submucous fibrosis, morphometric analysis, epithelium, vessels, epidemiology

INTRODUCTION

Oral submucous fibrosis (OSF) was first reported by Schwartz[1] and defined by Pindborg *et al*.[2] It is a common oral health problem in the Indian subcontinent as well as in migrants to the Western world. It is characterized by a juxta-epithelial inflammatory reaction followed by fibroelastic changes in the lamina propria and associated epidermal atrophy. The disease affects most parts of the oral cavity as well as the upper third of the oesophagus and Eustachian tube.[3] The pathogenesis of OSF is not well established and is believed to be multifactorial. Aetiological factors include areca nut, chillies, spicy foods, tobacco chewing, vitamin B deficiency and protein malnutrition.[4,5] The chewing of betel quid (containing areca nut, tobacco and slaked lime) has been recognized as one of the most important risk factors for OSF.[6-8] Among the chemical constituents, alkaloids from areca nut is said to have the most important biological role. It is hypothesized that there is increased collagen synthesis or reduced collagen degradation in the disease.[3] Areca nut may induce the progression of the disease by increased levels of cytokines in the lamina propria.[9] Endothelin (ET-1) expression in the oral mucosa is also said to play a role in pathogenesis of OSF.[10] Disruption in the equilibrium between matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) has been postulated as another possible mechanism in the development of the disease.[11] Nitric oxide synthetase (iNOS) expression may explain the vasodilation reported in this disease.[12] Hypoxia inducible factor (HIF-1alpha) has also been found to be upregulated at both protein and mRNA levels in OSF.[13] Over the years, the incidence of OSF has increased manifold in various parts of the Indian subcontinent probably related to increasing popularity of areca nut in the form of cheaply and readily available gutka sachets.[14] OSF is a premalignant condition, with a higher occurrence in oral squamous cell

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carcinoma patients, histological diagnosis of cancer without any clinical suspicion, high frequency of epithelial dysplasia and higher prevalence of leukoplakia.[15] Clinically, the patients present with a burning sensation in the mouth, excessive salivation, xerostomia and decreased mouth opening (trismus). Traditionally, OSF is said to be associated with marked epidermal atrophy and decreased vasculature as the disease advances. This study was conducted to objectively assess the changes in epidermal thickness and mucosal vasculature in various grades of the disease.

MATERIAL AND METHODS

Thirty-five patients with OSF with histological diagnosis made in the Departments of Otorhinolayngology and Pathology, Moti Lal Nehru Medical College, Allahabad, India were studied. The study was approved by the institutional ethical committee.

Demographic and social information of each patient was collated - age, sex, occupation, dental hygiene, presenting complaints and habitual use of alcohol, areca nut, smoking, betel quid and tobacco.

All cases underwent lesional biopsy and were validated by histopathology. OSF findings were recorded according to the traditional grading by Pindborg and Sirsat (Table 1).[16] 10 controls were selected in a random fashion from a patient population attending the same Department but with unrelated lesions.

All histopathological slides were analyzed morphometrically by using Image analysis software IMAGE PRO PLUS version 6.0. Windows TM Media Cybernetics Inc. (Bethesda, MD). Epithelial thickness (Fig. 1) and numerous blood vessels (Fig. 2) were measured in different areas of the lesion. Blood vessel area, diameter and perimeter were recorded. An average measurement of each feature was used in the analysis. ANOVA test was used to test equality of several means without affecting Type I error.

RESULTS

Thirty-five patients with OSF were studied. 25 (71.4%) were males and 10(28.6%) females with a male to female ratio of 1.3:1. Most patients (37.1%) were in the 31–40 yrs age group.

According to their personal habits, the majority of patients (40%) chewed areca nut/dohra (Table 2). On morphometric analysis each grade of the disease was found to display either hyperplastic or atrophic epithelial changes. This difference was found to be statistically not significant. (Epidermis F=1.1, p= 0.32). The mean blood vessel area did not show any

Table 1: Histopathological grading of oral submucous fibrosis (Pindborg and Sirsat)

Very early stage (Grade I):	Early stage (Grade II):	Moderately advanced stage (Grade III):	Advanced stage (Grade IV):
• A finely fibrillar collagen, dispersed with marked oedema. • The fibroblastic response is strong. • The blood vessels are sometimes normal, but more often dilated and congested. • Inflammatory cells mainly polymorphonuclear leukocytes with an occasional eosinophil	• The juxta-epithelial area shows early hyalinization. • Plump young fibroblasts are present in moderate numbers. • The blood vessels are dilated and congested. • The inflammatory cells are mostly mononuclear lymphocytes, eosinophils and an occasional plasma cell.	• The collagen is moderately hyalinized. • The fibroblastic response is less marked, the cells present being mostly adult fibrocytes. • Blood vessels are normal or constricted. • The inflammatory exudates consist of lymphocytes and plasma cells, although an occasional eosinophil is seen.	• The collagen is completely hyalinized. • The hyalinized areas are devoid of fibroblasts. • Blood vessels are completely obliterated or narrowed. • The inflammatory cells are lymphocytes and plasma cells.

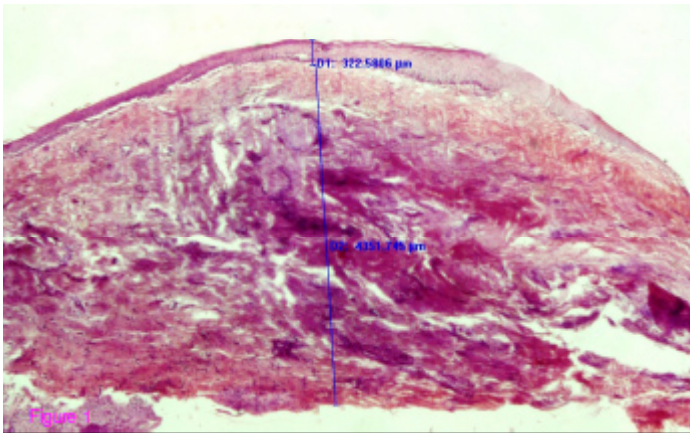

FIG. 1: Morphometric measurement of epithelial thickness in histopathological sections of OSF

sustained change with the increasing grades of the disease. The difference was found to be statistically not significant (Area F=0.69, p=0.59). The mean blood vessel diameter did not show any sustained change with the increasing grades of the disease and this was also found to be statistically not significant (Diameter F=0.35, p=0.83). The mean blood vessel perimeter did not show any sustained change with the increasing grades of the disease and the difference was found to be statistically non-significant (perimeter F=0.25, p=0.90) (Table 3).

DISCUSSION

In the present study, the male: female ratio was 1.3:1. Ahmad et al and Hazarey *et al* also reported a male preponderance for OSF[17,18] however Wahi *et al* observed a female predominance.[19] Shah *et al* observed oral submucous fibrosis in a younger age group.[20] Similarly, we observed the largest number of patients (37.1%) in the 31–40 yrs age group. However, Pindborg *et al* reported the maximum number of oral submucous fibrosis cases in a relatively older age group of 40-49 years.[21] This difference may be due to the increasing trend of using small, attractive and inexpensive sachets of betel quid substitutes by the younger generation which has become widely available in the last few decades.

In the present study, areca nut usage was the most frequently (26%) observed habit among patients with oral premalignant lesions. In the Allahabad region, consumption of a unique preparation called dohra is widespread. It is a wet preparation containing tobacco, slaked lime, areca nut, catechu (katha), peppermint and cardamom (illayachi).

The histopathological diagnosis of oral submcous fibrosis is based on the subjective evaluation of the morphological anomalies

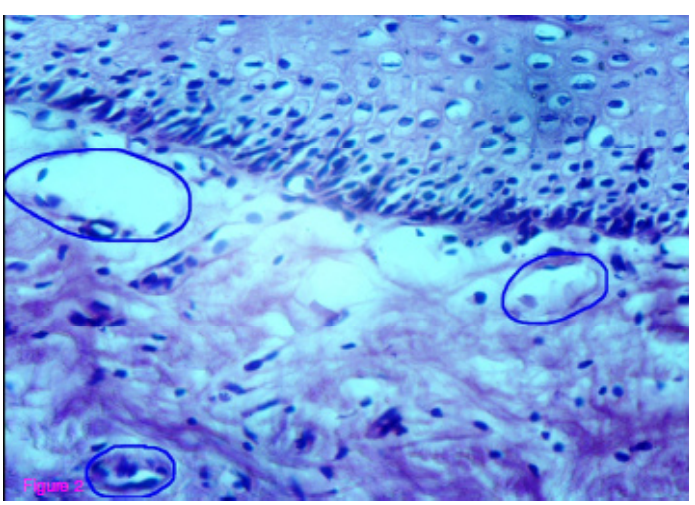

FIG. 2: Morphometric analysis of blood vessels in histopathological sections of OSF

Table 2: Distribution of patients based on their oral habits

Habit	Number of patients	Frequency per day	Duration (yrs)
Alcohol	06	0-1	10-20
Areca Nut	14	4-6	15-20
Smoking	10	2-3	10-15
Betel Quid	09	2-4	5-30
Tobacco	08	3-5	10-25

within the lesional tissue but there is a wide variation between different observers and there is a constant search for more objective means of evaluation.

Epithelial thickness: Epithelial atrophy is said to be one of the key features in OSF.[22,23] However, our study did not show consistent decrease in epithelial thickness with increasing grades of the disease. Each grade of the disease was found to display either hyperplastic or atrophic epithelial changes. (Epidermis F=1.1, p=0.32). Earlier, Pindborg *et al* and Vilmer *et al* also suggested that the overlying epithelium is either atrophic or hyperplastic and often hyperkeratotic.[2,24]

Vascularization: In the present study, the mean blood vessel area, diameter and perimeter did not showed consistent increase or decrease with different grades of OSF (Area F=0.69, p=0.59, diameter F=0.35, p=0.83, and perimeter F=0.25, p0.90). Sirsat *et al* and Fang *et al* suggested that dilatation and congestion of blood vessels occurred in the early stages and constriction to obliteration at the later stages.[25,26] In contrast, Rajendran *et al* found the mean vascular percentage area and the mean vascular luminal diameter had an increasing trend as the disease progresses (F = 8.63, p<0.01 and F = 34.1, p<0.001 respectively)[27] and Tilakaratne *et al* concluded that the mean vascular dilatation occurred as a result of adaptive response to

Table 3: Epidermal thickness, Blood vessel area, diameter and perimeter in different grades of disease

Epidermal thickness in different grades of disease							
Epithelium	**Control**	**OSF I**	**OSFII**	**OSF III**	**OSF IV**	**F**	**P**
Mean	1705	1497.42	1105.35	1075.11	938	1.19	.32
S.D>	550.87	1496.37	872.40	552.32	511.51		
Number of cases	10	7	14	9	5		
Blood vessel area in different grades of disease							
Area	**Control**	**OSF I**	**OSFII**	**OSF III**	**OSF IV**	**F**	**P**
Mean	3280.50	3257.23	3860.25	3153.06	2807.48		
S.D>	866.85	686.11	2074.09	1299.82	329.78		
Number of cases	10	7	14	9	5	0.69	0.55
Blood vessel diameter in different grades of disease							
Diameter	**Control**	**OSF I**	**OSF II**	**OSF III**	**OSF IV**	**F**	**P**
Mean	61.18	58.87	61.57	56.94	56.29		
S.D>	10.04	5.57	16.87	9.34	4.16	0.35	0.83
Number of cases	10	7	14	9	5		
Blood vessel perimeter in different grades of disease							
Perimeter	**Control**	**OSF I**	**OSFII**	**OSF III**	**OSF IV**	**F**	**P**
Mean	251.33	241.33	260.80	254.45	226.74		
S.D>	88.68	34.33	82.38	59.90	28.82	0.25	0.90
Number of cases	10	7	14	9	5		

compensate for tissue ischemia/hypoxia.[13] The diminished perfusion of the epithelium caused by defective vascularization of subjacent connective tissue due to physical constraints of fibrosis is generally considered to lead to epidermal atrophy as disease progresses. Histopathological changes in epidermis and blood vessels remain subjective. Aetiopathogenesis of epidermal atrophy based on a state of persistent ischemia need further objective elucidation with a larger number of patients, possibly in a multi-institutional setting, to help better define the clinical implications of these alterations.

Conclusion

Morphometric assessment of histological sections of the disease did not reveal consistent change in epidermal thickness, blood vessel area, diameter and perimeter in association with grade. Increasing epidermal atrophy may not necessarily be found in higher grades of the disease. Contrary to conventionally held views, ischemia may not play the most significant role in the aetiopathogenesis of oral submucous fibrosis.

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