ORIGINAL ARTICLE

Aggrandizing oral submucous fibrosis grading using an adjunct special stain: A pilot study

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ABSTRACT

Introduction: Oral submucous fibrosis (OSMF) is graded according to various histological factors which include the epithelial changes and the connective tissue changes. These features though could be identified in routine hematoxylin and eosin (H and E) staining; they could be better appreciated in special stains. This pilot study is an attempt to identify a single special stain that can act as an adjunct to H and E stain to help grade this potentially malignant disease. **Aims and Objectives:** To assess if special stains can improvise on differentiating the various histological changes seen in OSMF and to accordingly grade OSMF cases. **Materials and Methods:** Formalin-fixed paraffin-embedded tissue sections of OSMF-10 cases of each grade (n = 30). Three special stains: Van-Gieson, Mallory's trichrome and Masson trichrome. **Statistical Analysis:** The results obtained were tabulated and statistically analyzed using Chi-square test. **Observations and Results:** The thickness and degree of keratinization were best detected in Mallory's stain (100%) and were statistically significant; the subepithelial changes were better detected using special stains, especially Mallory's stain (100%). The changes in collagen fibers were better visualized in all three special stains but were not statistically significant. The changes in blood vessels were better detected in Van-Gieson's and Mallory's stain; the obtained results were statistically significant. The degree of fibrosis between muscle bundles could be detected in all the three special stains, but when compared the results were not statistically significant. The questionable areas of muscle degeneration, especially in deeper connective tissue were better detected in Mallory's (43%) and Masson's stain (43%) as compared to Van-Gieson stain (14%) and the results obtained were statistically significant. The inflammatory cells and dysplastic features are better visualized in routine H and E stains. **Conclusion:** Pathogenesis of OSMF is related to fibro-elastic and muscle degenerative changes in the connective tissue followed by secondary changes in epithelium. Routine H and E, stains all the connective tissue components in various shades of pink, use of special stains bestows contrast between different components of connective tissue, thus improvising grading of OSMF. Mallory's stain can be used as a single adjunct to H and E stain as both pattern of keratinization in the epithelium and changes in the superficial and deeper connective tissue could be ascertained.

Key words: Fibrosis, Mallory's stain, muscle degeneration, oral submucous fibrosis grading

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INTRODUCTION

Oral submucous fibrosis (OSMF), a chronic disease and a well-recognized potentially malignant condition is associated mainly with areca nut chewing habit. It is most prevalent among the people of South-Asian origin.[1,2]

Histologically, OSMF is characterized by inflammation and progressive fibrosis of lamina propria and deeper connective tissues[1] and atrophy or hyperplasia (along with keratinizing metaplasia) of the overlying epithelium that is likely to be a sequence of connective tissue changes.[3]

On the basis of the histopathological appearances in hematoxylin and eosin (H and E) stained sections, the cases of OSMF can be grouped into four grades according to the most widely accepted classification system given by Khanna and Andrade[4] as very early, early, moderately advanced and advanced stages. These grades are based on following criteria taken together: (a) The amount and nature of the subepithelial collagen, (b) presence or absence of edema and predominant cell type in the inflammatory exudates, (c) physical state of the mucosal collagen, (c) overall fibroblastic response (number of cells and age of individual cells), (d) state of the blood vessels (dilated or constricted) and changes in muscle tissue.[4,5]

H and E stained sections are a complete aid to diagnose and grade OSMF cases. A fair amount of justice could be carried out to the grading of epithelial dysplasia in H and E sections, but a better picture of connective tissue changes can be elusive in conventional staining. Previously, various special stains such as Van-Gieson, Masson's trichrome and phosphotungstic acid hematoxylin[6] were used to study these changes. We propose to study OSMF with 3 special stains, i.e. Van-Gieson, Masson's trichrome and Mallory's trichrome with an aim and objective to deduce on one best stain which can be used as an adjuvant to improvise on grading OSMF for better treatment options.

MATERIALS AND METHODS

OSMF cases that were previously diagnosed and graded on H and E stained sections using Khanna and Andrade classification system[4] were retrieved from our department. The histopathological features defined in each grade are described in Table 1.

In this study, only three grades were considered. Changes described in very early and early cases were taken as early cases followed by moderately advanced and advanced cases as described by Khanna and Andrade. This was because the treatment options do not vary much between very early and early cases.

Ten cases from each grade were considered for the study. Thus, a total of 30 cases were stained by the three special stains: Van-Gieson, Masson's trichrome and Mallory's trichrome and compared with each other and with H and E stain.

The staining procedure is described in Table 2.[7,8]

The following parameters were assessed and graded as follows:

The type of keratinization was identified as either no keratinization, parakeratinization or orthokeratnization. It was graded as follows:
- 1 = Normal keratinization
- 2a = Normal parakeratinization
- 2b = Increased parakeratinization
- 3a = Normal orthokeratinization
- 3b = Increased orthokeratinization.

The subepithelial changes whether edematous or hyalinized were graded as follows:
- Questionable hyalinization or edema = 1
- Edema = 2
- Hyalinization = 3.

The nature of collagen bundles was graded as follows:
- 1 = Predominantly fibrillar
- 2 = Predominantly bundled
- 3 = Predominantly hyalinized.

The changes in blood vessels were graded as follows:
- 1 = could not be appreciated
- 1a = Normal
- 2a = <10% of blood vessels dilated and congested
- 2b = 10–50% of blood vessels dilated and congested
- 2c = more than 50% of blood vessels dilated and congested.
- 3a = <10% of blood vessels constricted
- 3b = 10–50% of blood vessels constricted
- 3c = more than 50% of blood vessels constricted.

The fibrosis between skeletal muscle bundles (MBs) was staged as follows:[6]
- Stage 1: No change = 1
- Stage 2: Fibrosis involving superficial region of MB = 2
- Stage 3: Fibrosis involving deeper regions of MB = 3
- Stage 4: MB replaced by fibrosis = 4.

The degeneration of muscles bundles were categorized as follows:
- Normal = 1
- Superficial muscle degeneration = 2
- Superficial and deeper muscle degeneration = 3.

RESULTS AND OBSERVATIONS

The observations were accordingly tabulated for early, moderately advanced and advanced cases. These were analyzed using Chi-square test.

Table 1: Histological grading of oral submucous fibrosis given by Khanna and Andrade *et al*.

Groups	Epithelial changes	Nature of collagen	Blood vessels	Inflammatory cells	Muscle tissue changes
Group 1: Very early cases	Essentially normal and nonkeratinized with occasional hyperplasia	Fine fibrillar collagen network interspersed with marked edema. A large aggregate of plump young fibroblasts with abundant cytoplasm is evident	Dilated and congested	Consists mainly of polymorphonuclear leukocytes with a few eosinophils	No change
Group 2: Early cases	Flattening or shortening of the epithelial rete pegs with varying degrees of keratinization	Juxtaepithelial hyalinization, collagen present as thickened but separate bundles. Young fibroblasts seen in moderate numbers	Dilated and congested	Chronic inflammatory infiltrate consisting mainly of polymorphonuclear lymphocytes, eosinophils, and occasional plasma cells	No change
Group 3: Moderately advanced cases	Markedly atrophic, with total loss of rete pegs	Juxtaepithelial hyalinization. Thickened collagen bundles will be faintly discernible, separated by very slight, residual edema. Mature fibrocytes with scanty cytoplasm and spindle-shaped nuclei	Mostly constricted	Consisted mainly of lymphocytes and plasma cells	Muscle fibers are seen to be interspersed with thickened and dense collagen fibers. In certain areas, the muscle fibers also reveal the beginning of degeneration and irregularity of the striae
Group 4: Advanced cases	Total loss of epithelial rete pegs Group 4a: Without epithelial dysplasia Group 4b: With epithelial dysplasia	Hyalinized as a smooth sheet eliminating all evidence of individual bundles. Fibroblasts were markedly absent within the hyalinized zones, although an occasional thin, elongated cell was seen along the fiber bundles	Obliteration of mucosal blood vessels due to extensive fibrosis	Few chronic inflammatory cells, i.e., lymphocytes and plasma cells	Extensive degeneration of muscle fibers

Table 2: Staining procedure

Stains	Procedure	Results					
		Epithelium	Prekeratin and keratin	Collagen fibers	Muscle	RBC	Nuclei
For all the stains	Sections hydrated, stained with celestine-blue hematoxylin sequence - 5 min Acid alcohol differentiation Followed by						
Van Gieson	Acid fuchsin-picric acid solution - 4 min. Dehydrated, cleared and mounted	Yellowish-green	-	Red	Yellow		Blue-black
Masson's trichrome (Sections fixed in Bouin's fixative-overnight)	1% acid fuchsin - 5 min 1% phosphomolybdic acid - 5 min 1% methylene blue - 10 min Dehydrated, cleared and mounted	Shades of blue	-	Blue	Red	Red	Blue-black
Mallory's trichrome	1% acid fuchsin - 10 min Aniline blue-orange G - 10 min Dehydrated, cleared and mounted	Varying shades of blue	Shades of red to orange	Deep blue	Red	Red	Blue-black

RBC: Red blood cell

The predominant type of keratinization was only considered. The degree and unevenness in keratinization of the epithelium was readily evident in Mallory's stain [Figure 1a and b]. The layer of keratin stained in various shades of red and orange depending on the degree of maturation; prekeratin: red and keratin: orange. With the use of Mallory's stain, increased para or orthokeratnization could be better detected and the percentage of detection was 100% and was significant statistically [Table 3].

The subepithelial edema or hyalinization was better distinguished with special stains, especially Mallory's stain (100%) and was statistically significant [Figures 2a-c and 3a-c].

Table 3: Assessment of degree of keratinization by various stains in different grades of oral submucous fibrosis

Grades	Degree of Keratinization	Stains (%)				χ^2	*P*
		H&E stain	Mallory's stain	Masson's trichome	Van Gieson		
Early	Normal PK	29.4	11.8	29.4	29.4	25.588	0.002*
	Increased PK		100				
	Normal OK	31.3	6.3	31.3	31.3		
	Increased OK		100				
Moderately advanced	Normal PK	26.7	20	26.7	26.7	18.473	0.102
	Increased PK		100				
	Normal OK	27.3	18.2	27.3	27.3		
	Increased OK		100				
Advanced	Normal PK	29.4	11.8	29.4	29.4	13.746	0.132
	Increased PK		100				
	Normal OK	26.3	21.1	26.3	26.3		
	Increased OK		100				

*: Statistically significant; PK: parakeratinization; OK: orthokeratinization

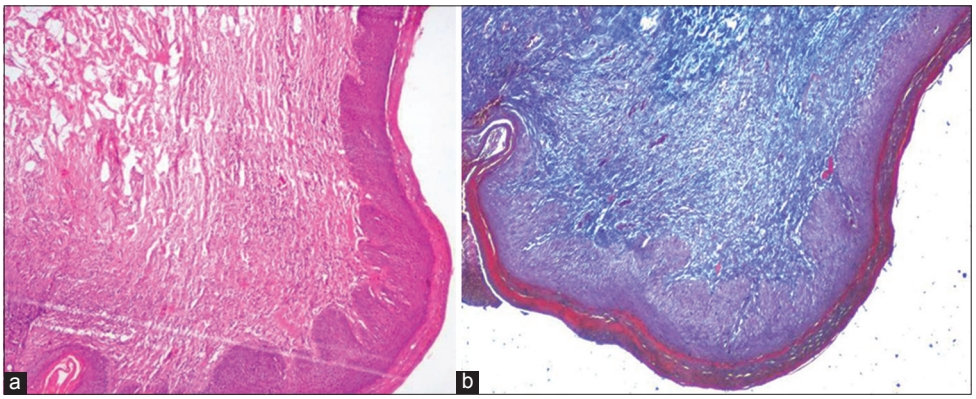

Figure 1: (a) Photomicrograph showing thick layer of keratin in the stratified squamous epithelium (H&E stain, ×100). (b) Photomicrograph showing variation in keratinizing pattern that is better visualized (Mallory's stain, ×100)

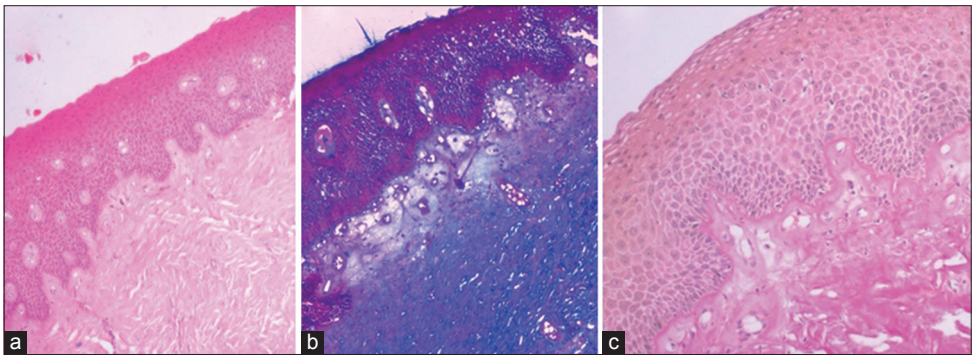

Figure 2: (a) Photomicrograph showing subepithelial homogenous eosinophilic zone (H&E stain, ×100). (b) Photomicrograph showing subepithelial edema better visualized (Mallory's stain, ×100), (c) Photomicrograph showing subepithelial edema (Van-Gieson stain, ×100)

The results obtained with various stains are tabulated in Table 4. Six cases showed edematous change and four cases showed juxta-epithelial hyalinization in early cases.

The nature of collagen bundles: Fine, fibrillar, bundled or homogenized was well-visualized in special stains, especially Van-Gieson's stain and Mallory's stain but the results were not statistically significant [Table 5, Figure 4a-c].

The predominant pattern seen regarding changes in blood vessels was only considered. Blood vessel changes such as congestion-dilation and constriction was clearer in special stains. Congestion was clear in Mallory's stain as the RBCs took a bright red color [Figure 5a and b]. The muscular coat of small arteries, veins and the capillaries were better delineated with Van-Gieson's stain [Figure 6], but changes seen in blood vessels could not be appreciated

Table 4: Assessment of sub-epithelial changes by various stains in different grades of oral submucous fibrosis

Grades	Sub-epithelial changes	Stains (%)				χ^2	*P*
		H&E stain	Mallory stain	Masson's Trichrome	Van Gieson		
Early	Hyalinizatin or edema	33		33	33	40.000	<0.001*
	Hyalinization		100				
	Edema		100				
Moderately advanced	Hyalinization	25.0	25.0	25.0	25.0	-	-
Advanced	Hyalinization	25.0	25.0	25.0	25.0	-	-

*: Statistically significant

Table 5: Assessment of nature of collagen bundles by various stains in different grades of oral submucous fibrosis

Grades	Nature of collagen bundles	Stains (%)				χ^2	*P*
		H&E stain	Mallory stain	Masson's trichome	Van Geision		
Early	Predominantly fibrillar	29.0	22.6	25.8	22.6	1.577	0.665
	Predominantly bundled	11.1	33.3	22.2	33.3		
Moderately advanced	Predominantly fibrillar	100.0				5.318	0.504
	Predominantly bundled	26.5	23.5	26.5	23.5		
	Predominantly hyalinized		40.0	20.0	40.0		
Advanced	Predominantly bundled	36.4	18.2	27.3	18.2	1.379	0.710
	Predominantly hyalinized	20.7	27.6	24.1	27.6		

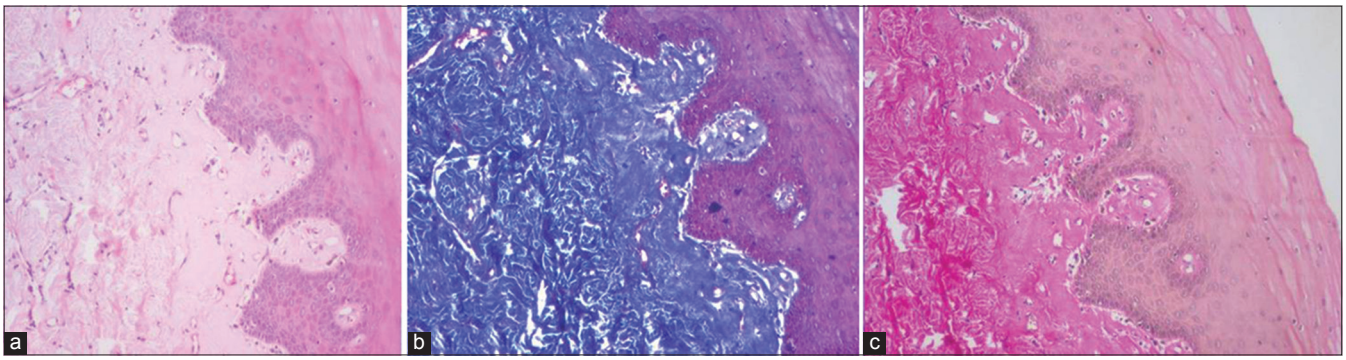

Figure 3: (a) Photomicrograph showing subepithelial homogenous eosinophilic zone (H&E stain, ×40), (b) photomicrograph showing subepithelial hyalinization better visualized (Mallory's stain, ×100), (c) photomicrograph showing subepithelial hyalinization (Van-Gieson stain, ×100)

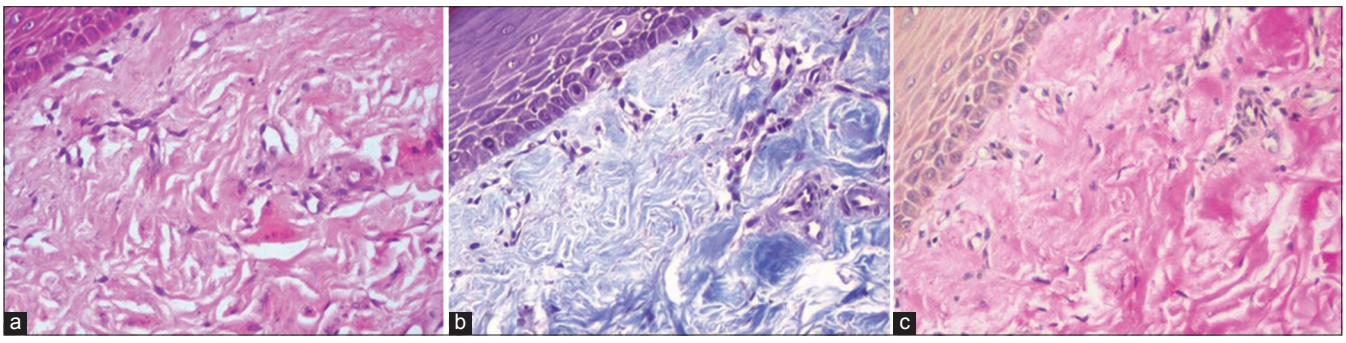

Figure 4: (a) Photomicrograph showing nature of collagen (H&E stain, ×100), (b) photomicrograph showing better visualized fibrillar and homogenous collagen (Mallory's stain, ×100), (c) photomicrograph showing fibrillar and homogenous collagen (Van-Gieson stain, ×100)

(100%) in most of the cases with Masson's trichrome stain [Table 6].

The changes in muscle tissue like increased fibrosis between MBs (superficial or deep) were better visualized in all the 3 special stains although the values were not statistically significant [Table 7, Figure 7a-d].

The questionable areas of degenerating MBs or areas of hyalinization were better distinguished with Masson's

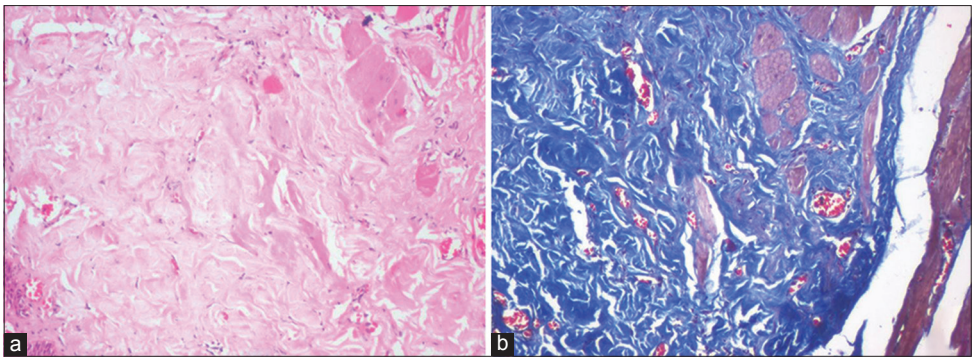

Figure 5: (a) Photomicrograph showing hardly observable congested blood vessels (H&E stain, ×100), (b) photomicrograph showing readily observable congested blood vessels as the RBCs take bright red color (Mallory's stain, ×100)

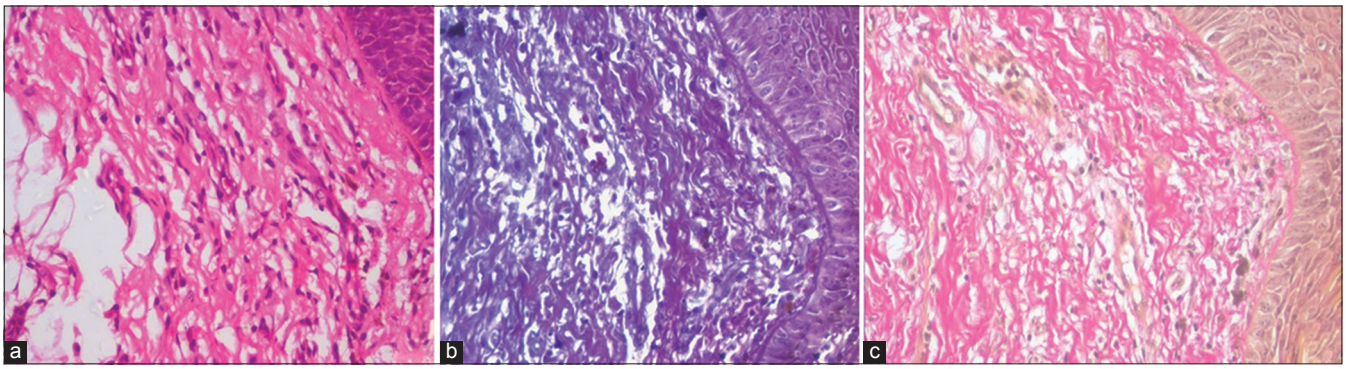

Figure 6: (a) Photomicrograph showing barely detectable constricted blood vessels (H&E stain, ×100), (b) photomicrograph showing barely detectable constricted blood vessels (Mallory's stain, ×100), (c) photomicrographs showing muscular coat of constricted blood vessels (Van-Gieson stain, ×100)

Table 6: Assessment of changes in blood vessels by various stains in different grades of oral submucous fibrosis

Grades	Blood vessels	Stains (%)				χ^2	*P*
		H&E stain	Mallory stain	Masson's Trichome	Van Geision		
Early	Not appreciable			100		42.087	<0.001*
	Normal		100				
	<10% BV dilated and congested	43.5	4.3	26.1	26.1		
	10-50% BV dilated and congested		50.0		50.0		
	>50% BV dilated and congested		50.0		50.0		
Moderately advanced	Not appreciable			100		33.939	0.001*
	Normal	66.7		33.3			
	<10% BV dilated and congested	50		50			
	10-50% BV dilated and congested		50		50		
	>50% BV dilated and congested	4.5	40.9	13.6	40.9		
Advanced	>50% BV dilated and congested	35.7	14.3	35.7	14.3	17.143	0.009*
	<10% BV constricted		50		50		
	10-50% BV constricted		50		50		

BV: Blood vessel, *: Statistically Significant

(43%) and Mallory's stain (43%), especially in deeper connective tissue and was statistically significant [Table 8, Figure 8a-d].

The morphology of inflammatory cell infiltrate was distinguished best with conventional H and E sections.

With the use of special stains, subtle histopathological changes such as edema or hyalinization, degree of bundling of collagen or homogenization, constricted blood vessels or dilated blood vessels and degree of fibrosis between MBs or degeneration could be readily appreciated. The degree of keratinization, subepithelial changes, changes seen in blood vessels, degeneration of MBs were distinguished appropriately using Mallory's stain and was statistically significant.

Few of the cases which were previously graded with H and E could now be better categorized as follows:

Table 7: Assessment of fibrosis between muscle bundles by various stains in different grades of oral submucous fibrosis

Grades	Fibrosis between muscle bundles	Stains (%)				χ^2	*P*
		H&E stain	Mallory stain	Masson's trichome	Van Geision		
Early	No change	32.3	22.6	22.6	22.6	3.871	0.276
	Fibrosis-superficial MB		33.3	33.3	33.3		
Moderate	Fibrosis-superficial MB	29.4	23.5	23.5	23.5	2.353	0.502
	Fibrosis-deeper MB		33.3	33.3	33.3		
Advanced	No change				100.0	7.048	0.316
	Fibrosis-superficial MB	38.1	19.0	23.8	19.0		
	Fibrosis-deeper MB	11.1	33.3	27.8	27.8		

Table 8: Assessment of degeneration of muscle bundle by various stains in different grades of oral submucous fibrosis

Grades	Degeneration of muscle bundle	Stains (%)				χ^2	*P*
		H&E, stain	Mallory stain	Masson's Trichome	Van Geision		
Early	Normal	25	25	25	25	-	-
Moderate	Normal	100				21.176	<0.001*
	Superficial muscle degeneration	12	29	29	29		
Advanced	Superficial muscle degeneration	39	15	15	31	11.868	0.008*
	Superficial and deep muscle degeneration		43	43	14		

*:Statistically significant

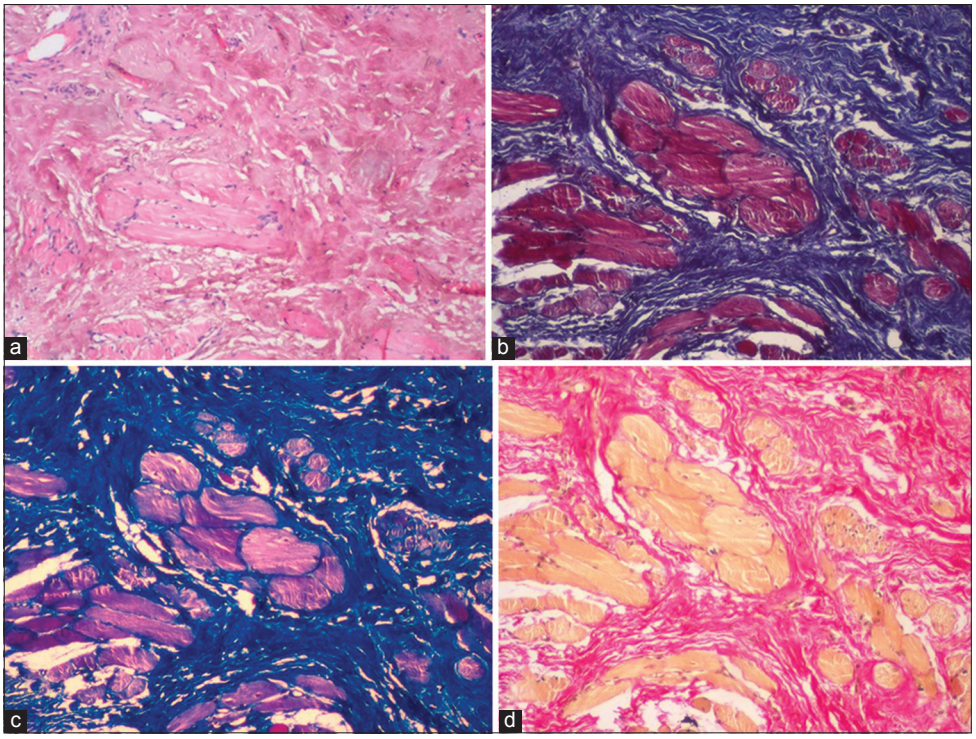

Figure 7: Photomicrograph showing fibrosis between muscle bundles (a) H&E stain, ×100, (b) Mallory's stain, ×100, (c) Masson's trichrome stain, x100 (d) Van-Gieson stain, ×100.

1. Based on subepithelial hyalinization or edema, the degree of bundling of collagen, number and nature of blood vessels and amount of fibrosis between MBs, three cases which were previously graded as early cases could be now grouped as the moderately advanced cases [Figure 9]
2. Based on the degree of bundling and amount of homogenization, number of constricted blood vessels, degree of fibrosis between MBs and the number of degenerating MBs, 2 cases which were previously categorized as moderately advanced could be grouped as advanced cases [Figure 10].

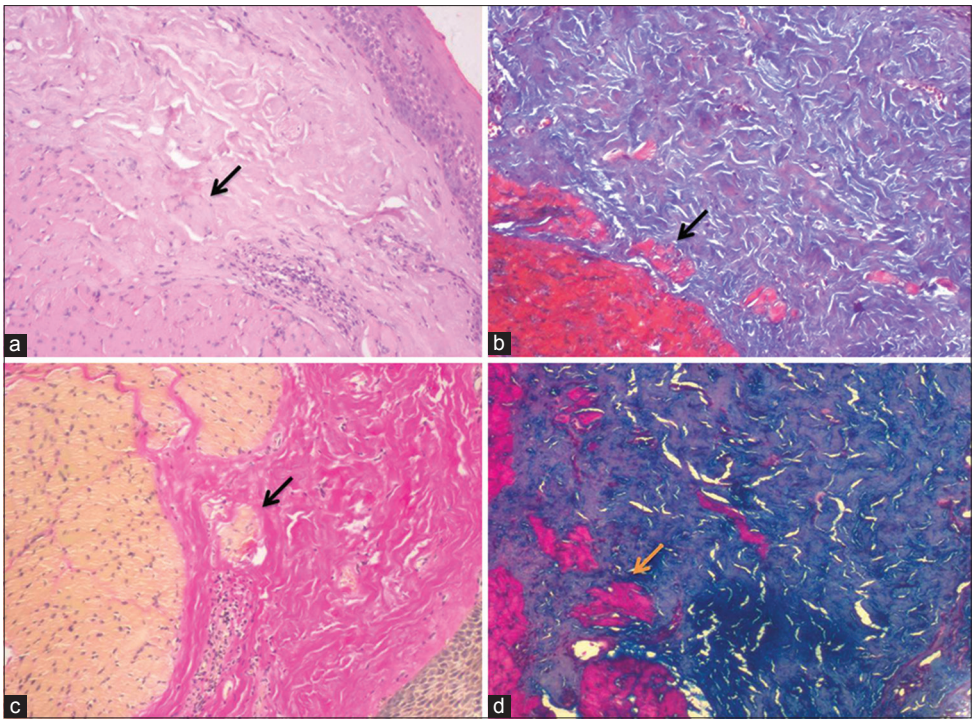

Figure 8: (a) Photomicrograph showing areas of muscle degeneration, arrow indicates a questionable area of muscle degeneration (H&E stain, ×200), area of muscle degeneration better visualized in, (b) Mallory stain, ×200, (c) Van-Gieson stain, ×200, (d) Masson's stain, ×200

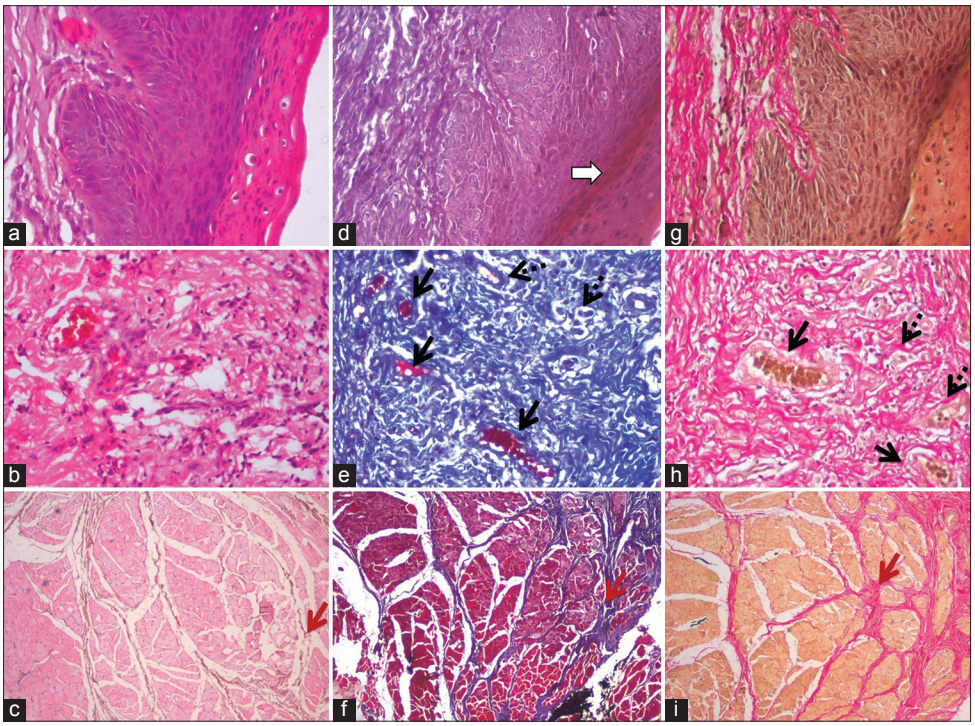

Figure 9: Photomicrographs of a case that was graded as early oral submucous fibrosis on H&E stained sections. (a) Photomicrograph showing epithelium with thick layer of keratin, (H&E stain, ×200), (d) corresponding image in Mallory stain showing variation in keratinization (thick white arrow, Mallory stain, ×200), (g) corresponding image in Van-Gieson stain, the pattern of keratinization shows no difference (Van-Gieson stain, ×200), (b) photomicrograph showing few congested blood vessels and predominantly fibrillar collagen (H&E stain, ×200), (e) corresponding image in Mallory stain showing more congested blood vessels (black arrow), few constricted blood vessels (dotted arrow), fibrillar and few bundled collagen (Mallory stain, ×200), (h) corresponding image in Van-Gieson stain showing congested blood vessels (black arrow), more constricted blood vessels (dotted arrow), fibrillar and bundled collagen (Van-Gieson stain, ×200), (c) photomicrograph showing normal collagen fibers between muscle bundles (H&E stain, ×40), (f) corresponding image in Mallory's stain showing increased fibrosis between muscle bundles (red arrow, Mallory's stain, ×40), (i) corresponding image in Van-Gieson stain showing increased fibrosis between muscle bundles (red arrow, Van-Gieson stain, ×40). Based on the enhanced features seen in special stain, this case could be graded as moderately advanced oral submucous fibrosis

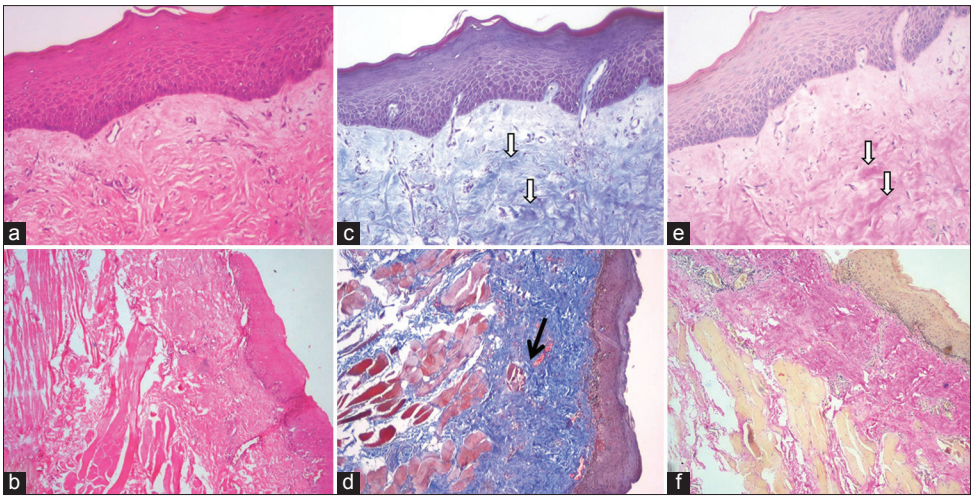

Figure 10: Photomicrographs of a case that was graded as moderately advanced oral submucous fibrosis on H&E stain. The bundling and hyalinization as visualized in H&E stain (a: ×100) was enhanced in special stains: (c) Mallory stain, ×100 (white arrows) and (e) Van-Gieson stain, ×100 (white arrows). The degeneration of muscle bundles which was not clear in H&E stain (b: ×40) was better visualized in special stains, (d) Mallory stain, ×40 (black arrow), (f) Van-Gieson stain, ×40). Based on increased hyalinization and muscle degeneration the cases could be graded as advanced oral submucous fibrosis

DISCUSSION

OSMF is a chronic debilitating disease and a premalignant condition of the oral cavity strongly associated with betel nut and gutkha chewing habit. It is characterized by juxtaepithelial inflammatory reaction followed by a fibro-elastic change of the lamina propria and epithelial atrophy leading to stiffness of the oral mucosa, causing trismus and inability to eat.[9]

OSMF has been classified based on various aspects, the important ones include Pindborg (1989), Rajendran (2003) and Kiran Kumar (2007) based on the clinical aspects; and Pindborg JJ and Sirsat SM (1966), Utsunomiya *et al*. (2005) based on histopathological features.[10] The most widely accepted classification is that of Khanna and Andrade who classified OSMF based on both clinical and histopathological features such that the patients can be managed appropriately.

To better describe the histological features to grade OSMF, many special stains have been used previously. These stains were mainly used to describe the connective tissue changes as they provided a better contrast between muscle and collagen fibers.[6]

In this pilot study, we used Van-Gieson, Masson's stain and Mallory's stain to select a stain which would help us to distinguish both epithelial and connective tissue changes effectively and act as an adjuvant to H and E stain in grading OSMF.

The epithelial changes, especially the amount and degree of keratinization whether even or uneven was better visualized using Mallory's trichrome. This trichrome, stains the keratin layer depending on the degree of keratinization. Here, prekeratin is stained red and mature keratin layer is stained orange. It is proven that OSMF is a premalignant condition where epithelium undergoes malignant transformation and its progression toward malignant transformation are as follows: The atrophic epithelium first becomes hyperkeratotic (clinically leukoplakic) and later intercellular edema and basal cell hyperplasia develop, eventually, it is followed by epithelial atypia with moderate epithelial hyperplasia.[3] Hence, detection of variation in keratinization marks the beginning of malignant transformation. However, even though, the dysplastic changes seen in the epithelium are better described in conventional H and E stain, special stains like Mallory's stain can detect abnormal pattern of keratinization which may mark the beginning of dysplastic changes.[3] According to Wahi *et al.*, high mitotic count in parakeratotic epithelia, which is more common with OSMF and OSMF associated with parakeratotic leukoplakia show enhanced predisposition to carcinoma.[4]

Subepithelial changes: OSMF is characterized by subepithelial inflammatory reaction followed by hyalinization. In the H and E stain both edema and homogenous hyalinization appear as eosinophilic bands.[11] On using a special stain, this distinction can be clearly visualized. Van-Gieson, Masson's and Mallory's stain could all distinguish subepithelial hyalinization and edema. The distinction was better delineated by Mallory's stain.

Most of the authors believe that OSMF is characterized by an inflammatory reaction followed by severe fibro-elastic changes.[12] In this context, if the homogenous area is due to edema then the disease would be in very early stage and based on the degree of subepithelial hyalinization, it would be categorized as either early or moderately advanced stage.

The changes in blood vessels are very well-detected using special stains. Mallory's stain could better detect congested vessels as the red blood cells took a bright red color as compared to the surrounding blue collagen. The muscular coat around the blood vessels could be visualized better with Van-Gieson stain as yellow color of muscle provided a better contrast to red-stained collagen. Hence, any constriction of the vessels was easily detected. The number of blood capillaries was also readily visible with the special stains, especially Van-Gieson's stain and Mallory's stain. Detection of these variations is important as the number and nature of blood vessel changes, as the disease progresses. The number decreases and they change from being congested and dilated to a stage of constriction as the disease advances.[5]

According to Kiran Kumar *et al*., the nature of collagen and degree of hyalinization as seen in OSMF are graded as: Grade I: loose, thick and thin fibers; Grade II: Loose or thick fibers with partial hyalinization; and Grade III: Complete hyalinization.[10] These features are better differentiated using the special stains, especially Van-Giesons and Mallory's trichrome.

The amount of fibroblasts and collagen fibers correlates with cellularity and maturity of the connective tissue. In initial stages of OSMF, the tissue consists of more number of plump fibroblasts and less of collagen fibers. As the disease progresses, the number of cells decrease and collagen fibers increase and mature. The ratio of collagen to fibroblasts could be best visualized in H and E section as was the morphology and type of inflammatory cells.

The best distinction that was made was between collagen fibers and MBs. All the three special stains provided a good contrast between skeletal muscle and collagen fibers. According to Rooban *et al*., the fibrosis between MBs increases as the disease progresses followed by degeneration of muscle fibers,[6] but Gollnick *et al.* state that glycogen consumption is physiologically related to the cellular activity. Overactivity of the muscle results in excessive glycogen consumption, leading to glycogen depletion. The increased muscle activity and diminished blood supply following connective tissue changes owing to extensive fibrosis lead to muscle degeneration.[5] Nevertheless, the degree of fibrosis between MBs and degeneration co-relate with disease progression. According to Khanna and Andrade, if only superficial muscles bundles are involved then the disease is moderately advanced, if the fibrosis and degeneration extend to deep MBs then the disease stage is more advanced.[5]

Although the questionable areas of degeneration of MBs was well-visualized using both Mallory's and Masson's as compared to Van-Gieson; Mallory's stains add an advantage as the procedure for this stain is less tedious as compared to Masson's trichrome (the sections have to be fixed in Bouin's fluid before staining).[7,8]

In this pilot study, the use of special stains helped to readily distinguish the various histological features and improvise on grading OSMF. Mallory's stain provided more advantages as most of the connective tissue changes, and epithelial keratinization was readily differentiated and the procedure was less tedious as compared to Masson's trichrome.

Mehrotra *et al*. have proposed that patients in Grade I and II be managed medically, whereas patients in Grade III and IV are to be treated surgically.[9]

The treatment regimes include cessation of habit, nutritional support (vitamins and minerals), use of immune-modulatory drugs (steroids, placental extracts, interferon gamma etc.) physiotherapy (mouth opening exercises, heat therapy, etc.,) local drug delivery (dexamethasone, hyaluronidase, placental extracts, chymotrypsin, etc.,) and finally surgical therapy (removal of fibrotic bands and placement of grafts). Depending on the stage of disease and extent of oral involvement, therapy mainly consists of a combination of the above-mentioned drugs and surgery.[2,4]

Revelation of individual connective tissue changes can help to provide a personalized treatment care. On detection of heavy edema and inflammation, more of anti-inflammatory drugs and steroids can be used. On detection of extensive hyalinization, fibrinolytic agents such as hyaluronidase and placental extracts can be tried. If the fibrosis is extensive then surgical excision can be carried out. On detection of reduced lamina propria due to progressive hyalinization a pedical graft can be placed to improvise the vascular supply.[11]

According to a study by Mittal *et al*., use of cocktail therapy would improve the condition of the patient. As per this study, the authors graded OSMF cases based on the clinical presentation and assessed the improvements in patients based on histopathological features. Hence, identifying histopathological changes not only helps to grade the patients but also helps to assess improvements in the treatment plan followed.[13]

CONCLUSION

OSMF is primarily a collagen disorder characterized by fibro-elastic changes in lamina propria, deeper connective tissue and blood vessels followed by degenerative changes in muscles with secondary changes in the epithelium. The extent of such change (involving superficial or deeper connective tissue) determines the advancement of the disease and thus the grading. The routine H and E procedure stains the various components of connective tissue in shades of pink which hinders the differentiation between nature of blood vessels, collagen fibers and MBs. Furthermore, the pattern of keratinization which is an initial manifestation of dysplastic change in epithelium helps to assess any premalignant change

in the epithelium. In this context, an adjuvant use of special stain helps to readily visualize the changes both in connective tissue and epithelium at a better level than seen in routine H and E stain. This could be appreciated in the present study with the use of Mallory's stain thus aggrandizing OSMF grading.

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Conflicts of interest

There are no conflicts of interest.

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