RESEARCH

Ultrasonographic evaluation of oral submucous fibrosis in habitual areca nut chewers

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Objectives: To characterize the sonographic features of the buccal mucosa in patients with oral submucous fibrosis (OSF).

Methods: Three groups (controls with areca-related habits, controls without areca-related habits and clinically diagnosed OSF cases), each comprising 30 subjects, were included in the study. After a thorough clinical examination, transcutaneous B-mode ultrasonography was performed with a multifrequency linear transducer (5–10 MHz) for anterior and posterior buccal mucosa bilaterally. Both clinical and ultrasound findings were recorded by three independent observers. One-way analysis of variance and Tukey's honestly significant difference post-hoc tests were used for statistical comparisons between groups and Pearson χ^2 tests to compare the proportions. Kappa statistics was used to determine the interobserver agreement.

Results: The submucosa that appeared hypoechoic in the control groups had significantly increased echogenicity in the case group (hypo- to isoechoic in 46.7% and isoechoic in 53.3%). The differentiation between the submucosa and the muscle layer appeared distinct in the control groups while it was not clear in the case group (indistinct in 50% and completely lost in 50%). The number of sites found positive on the ultrasound was significantly greater than the number of clinically positive sites. There was a very good interobserver consistency in clinical and ultrasound findings.

Conclusions: Ultrasonography of the buccal mucosa demonstrates increased submucosal echogenicity and reduced echo differentiation between submucosa and muscle layer in OSF cases. Hence, it can be used as a non-invasive imaging modality to assess the disease extent and severity across the entire buccal mucosa to supplement clinical evaluation.

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Introduction

Oral submucous fibrosis (OSF) is a well-recognized "potentially malignant" disorder that carries an increased risk of oral cancer.¹ Conclusive evidence

now exists implicating the use of areca nut products in the causation of OSF.² The disease is common in India and other South Asian countries, where betel quid is chewed with areca nut as a customary practice; however, it is also seen in South Asian immigrants in other countries.³ The increasing popularity of newer industrially manufactured areca nut products (gutkha, pan masala,

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etc.) is a cause for concern, especially because their attractive packaging and aggressive marketing primarily target adolescents and young adults.⁴ OSF is clinically characterized by a burning sensation in the mouth, intolerance to spicy foods, blanching and stomatitis in the early stages. Later, fibrous bands become palpable in the buccal mucosa as well as in other parts of the oral cavity. This results in progressive reduction of interincisal mouth opening.⁵ Histologically, OSF is characterized by fibrosis of the lamina propria, which progresses to involve the submucosa and deeper tissues, including the muscles of the oral cavity, resulting in loss of fibro elasticity.² The blood vessels get obliterated owing to fibrosis, resulting in a reduction of vascularity and epithelial atrophy. Various studies have shown a poor correlation between the clinical and histological grading of the disease, the discrepancy attributable to the difference in the extent of fibrosis in various parts of the oral cavity.^{6,7}

Clinical examination alone may be subjective and is probably insufficient to characterize the disease severity adequately. Interincisal mouth opening could be influenced by the anatomical site of fibrosis (*e.g.* limitation of mouth opening can be more pronounced when the pterygomandibular raphae region is involved, rather than the more anterior part of the buccal mucosa, irrespective of the amount of fibrosis) and, therefore, cannot be relied upon as a sole objective indicator of the general disease severity. Biopsy, being a minor surgical procedure, is often associated with some discomfort to the patient (pain, local anesthesia, etc.) and is time consuming (sectioning, staining, etc.). Besides, biopsy does have its limitations in a diffuse disease such as OSF, where the extent of fibrosis differs in the various parts of the oral mucosa, and the tissue sample obtained from a single site may not be representative of the true extent or severity of the disease. This problem is compounded in OSF because restricted mouth opening limits access to the posterior parts of the buccal mucosa, which would otherwise serve as a more representative site for biopsy. Moreover, biopsy cannot be performed repeatedly for following the progress of the lesion or to assess the response to treatment. As pointed out in a Cochrane systematic review, extraoral imaging systems could offer a more sensitive measurement tool to assess the severity of the disease.⁸ Ultrasonography is a safe, readily available, non-invasive, non-ionizing and cost-effective modality that can be used to image superficial tissues. Real-time imaging allows assessment of the *in vivo* status of tissues at any given point of time instantaneously. Because of its non-invasive nature and safety, it has better patient acceptance. In addition, because of the wider area imaged, ultrasound can be a valuable tool to determine the extent and severity of the disease as well as to monitor the response to treatment, thus supplementing clinical and histological details.

The use of ultrasonography in the evaluation of OSF draws inspiration from its application in scleroderma, where ultrasound studies have been used for diagnosis as well as follow-up, based on the alteration in the thickness and the echogenicity of the dermis.^{9,10} OSF is

similar in many aspects to scleroderma, where continuous and uncontrolled fibrosis accompanies a chronic inflammatory reaction.¹¹ Although the cause, distribution and prognosis of scleroderma and OSF differ, it is likely that both diseases share a defect of collagen maturation, which gives rise to a clinically and histologically similar lesion.¹² The fibrosis that occurs in OSF is a dynamic process, much like scleroderma and, therefore, needs periodic monitoring of the disease activity, to assess the response to cessation of habit or other medical interventions. Currently, laboratory markers or other validated clinical measures are not available to assess the disease activity or to grade the severity. Buccal mucosa is involved in almost all cases of OSF and also lends itself well to ultrasonographic examination because of its superficial location and absence of underlying bone. Despite these facts, very few studies have reported the use of ultrasonography in the evaluation of OSF.^{13,14} It is with this background that we chose to undertake this preliminary study on ultrasonographic evaluation of OSF to characterize the sonographic features of the buccal mucosa in OSF patients, compared with normal individuals and habitual areca chewers without oral lesions.

Materials and methods

The study sample consisted of 90 subjects recruited from the outpatient Department of Oral Medicine and Radiology in a teaching hospital. They were divided into three groups matched for age and sex as follows: Group I (controls without habits): 30 subjects with a negative history of areca use and clinically normal oral mucosa; Group II (controls with habits): 30 subjects with a positive history of habitual use of areca nut products and clinically normal mucosa; Group III (cases): 30 subjects with a positive history of habitual use of areca nut products and a clinical diagnosis of OSF. The criterion used for the clinical diagnosis of OSF was the presence of at least one of the following features in the buccal mucosa of a habitual chewer:

- (a) blanching (with or without erythema, ulcers, vesicles or pigmentation changes)
- (b) unilateral or bilateral fibrotic bands in the buccal mucosa (with or without restricted mouth opening).

This study recruited only male patients aged between 20 and 50 years to avoid any possible age- and gender-related variation in mucosal echo texture that could influence the results. In each group, 30 consecutive subjects who satisfied the inclusion criteria were recruited into the study. A written informed consent was obtained from all subjects participating in the study. The study was approved by the institutional ethics committee.

The exclusion criteria were:

- (1) trismus owing to causes other than OSF
- (2) a history of skin or other systemic diseases

- (3) a history of radiation therapy to the head and neck region
- (4) oral cancer
- (5) previous biopsy or treatment for OSF
- (6) other co-existing oral mucosal lesions.

A detailed history was elicited from all subjects with specific reference to the use of areca nut products (betel nut, pan masala, gutkha, mawa, etc.). A thorough clinical intraoral examination was performed by three independent observers, two of whom (Authors 1 and 3) were dental surgeons with master's degrees in oral medicine and the other was a general physician (author 4) with a master's degree in internal medicine. The presence or absence of palpable fibrous bands was noted in each of the four regions—right anterior buccal mucosa (RAB), right posterior buccal mucosa (RPB), left anterior buccal mucosa (LAB) and left posterior buccal mucosa (LPB)—to permit site-specific correlation with the ultrasound findings later. The documentation of the presence or absence of fibrous bands was determined by the majority opinion among the three observers. The other clinical parameters recorded were mouth opening and cheek flexibility, as per the technique described by Reddy *et al.*¹⁵ The mean of the measurements given by the three observers was taken as the recorded value for both these parameters.

Ultrasonographic procedure

Transcutaneous B-mode ultrasonography of both cheeks was performed for all the subjects by an experienced medical radiologist. The equipment used for the scan was a medical ultrasonographic unit (Model Acuson X 300™; Siemens, Munich, Germany). A multifrequency linear transducer (4.5 × 1 cm in size) was used, whose frequency ranged from 5–10 MHz. The technical parameters of the scan, like depth, gain, focus and time-gain compensation, were kept constant throughout the study to avoid potential bias in the assessment of echo texture. The scan was performed with the patients in the supine position and in subdued lighting. A glove finger filled with water was placed in contact with the buccal mucosa intraorally, which served as a water path and improved the contrast between the mucosa and the empty space of the oral cavity. A coupling gel was applied to the transducer, and the scan was performed for the anterior and posterior buccal mucosa separately on each side. To obtain the image of the buccal mucosa, the transducer was positioned on the cheek extraorally along the line joining the angle of the mouth to the tragus of the ear (Figure 1). For imaging the anterior buccal mucosa, the anterior end of the probe was placed at the angle of the mouth and for the posterior buccal mucosa, the posterior end of the probe was placed at the anterior border of the masseter muscle. The transducer as well as the water path was placed in gentle contact with the tissues to avoid undue compression. The

Figure 1 The scan procedure—the glove finger filled with water served as the water path. The linear transducer was placed on the cheek along the line joining the angle of the mouth to the tragus of the ear

entire thickness of the cheek could be imaged, consisting of all the layers starting with (from below upwards) the mucosal lining, the submucosa, the buccinator muscle, the buccal pad of fat, the superficial muscles of facial expression, subcutaneous tissue and the skin of the cheek, as described in earlier studies.^{13,16} The ultrasound images were interpreted by three independent medical radiologists with master's degrees in radiodiagnosis (Authors 2, 5 and 6), who were blinded to the clinical details. All the ultrasonographic findings were recorded separately for the four regions—RAB, RPB, LAB and LPB. Firstly, the echo texture of the submucosa was noted in relation to the muscle layer. It was recorded as being (1) hypoechoic when it appeared uniformly dark in relation to the muscle layer; (2) hypo to isoechoic when it had an intermediate echotexture; and (3) isoechoic when it had the same echotexture as the overlying muscle layer. Secondly, the differentiation between the submucosa and the muscle layer was recorded as being distinct, indistinct or as having been completely lost. The majority opinion among the three observers was documented for both these sonographic parameters.

Statistical analysis

The data collected was analysed using SPSS® software (v. 18.0; SPSS Inc, Chicago, IL). Simple descriptive statistics including mean, standard deviation and percentages were used to describe the distribution of data collected. One-way analysis of variance was used to compare the mean values between groups. Tukey's honestly significant difference post-hoc tests were used for multiple pair-wise comparisons between groups. Pearson χ^2 tests were used to compare the proportions. To assess the interobserver agreement, kappa statistics was used for categorical data (clinical and sonographic assessment) and Dahlberg's measurement error was used for continuous data (clinical measurements).

Results

General sonographic appearance

The sonographic appearance of the normal buccal mucosa in Group I subjects (controls without habits) (Figure 2) was as follows.

The oral mucosal lining (epithelium and connective tissue together) appeared as a single hyperechoic line (white arrow) just adjacent to the water path (WP), which appeared anechoic. Just lateral to the mucosa (above the mucosal lining in cross-sectional ultrasound images), the submucosa appeared as a uniformly hypoechoic zone of varying thickness (SM). Just above the submucosa, the muscle layer (comprising predominantly of buccinator) appeared as an echogenic band (MU). Above this zone, the hypoechoic buccal pad of fat (BFP), superficial muscles of facial expression (SFM), subcutaneous tissue (SCT) and skin (black arrow) were seen in that order. The boundary between the submucosa and the muscle layer was clear and distinct. The layers could be differentiated well from each other because of their differences in echo texture.

The sonographic appearance of the buccal mucosa in Group II subjects (controls with habits) was the same as that of Group I, except in one patient where the submucosa appeared hypo- to isoechoic and the differentiation with the muscle layer appeared indistinct.

The sonographic appearance of the buccal mucosa in Group III subjects (cases) was as follows: there was a variable but definite increase in the echogenicity of the submucosal layer. In some cases, the submucosa appeared hypo- to isoechoic and the submucosa–muscle

layer junction appeared indistinct (Figure 3). In the remaining cases, the submucosa appeared isoechoic with the muscle layer, and the differentiation between those two layers was totally lost (Figure 4). These two findings were observed in at least one region, out of the four regions evaluated (RAB, RPB, LAB and LPB) in all the OSF cases.

Clinical parameters

The mean age of Group I subjects was 30.47 (± 9.43), that of Group II subjects was 29.2 (± 6.54) and that of Group III subjects was 30.17 (± 9.02). There was no statistically significant difference in the mean age between the three groups. The mean mouth opening (in millimetres) was 50.43 (± 4.95) in Group I subjects, 48.17 (± 3.23) in Group II subjects and 34.07 (± 9.82) in Group III subjects. The difference in the mean mouth opening was statistically highly significant between Group I and Group III subjects ($p < 0.001$) and also between Group II and Group III subjects ($p < 0.001$). However, the difference in the mean mouth opening between the Group I and Group II subjects was not statistically significant ($p = 0.39$). The mean cheek flexibility (in millimetres) was 10.13 (± 1.33), 9.83 (± 1.5) and 2.62 (± 1.23) for Groups I, II and III, respectively. The difference between Groups I and III and the difference between Groups II and III were statistically highly significant ($p < 0.001$). However, the difference in the mean cheek flexibility between Groups I and II was not statistically significant ($p = 0.671$).

Ultrasonographic parameters

Echotexture of submucosa: The echo texture of the submucosa was assessed in all the four sites (RAB, RPB, LAB and LPB) in all the subjects. For statistical correlation of the sonographic findings with the clinical parameters, the worst affected site was considered in

Figure 2 Normal sonographic appearance of the buccal mucosa in the control group—the layers of the cheek is seen above the anechoic water path (WP). They are (from below upwards) the hyperechoic oral mucosal lining (white arrow), the hypoechoic submucosa (SM), the echogenic buccinator muscle layer (MU), the hypoechoic buccal pad of fat (BFP), the superficial muscles of facial expression (SFM), the subcutaneous tissue (SCT) and the skin (black arrow). Please note the distinct differentiation between the submucosa and the muscle layer

Figure 3 Hypo- to isoechoic submucosa (SM) in oral submucous fibrosis cases. Please note that the echogenicity of the SM is increased compared with Figure 2. The junction between the SM and the muscle layer (MU) appears indistinct

Figure 4 Isoechoic submucosa (SM) in oral submucous fibrosis cases. Please note the marked increase in the echogenicity of the SM to approximate that of the muscle layer (MU) and the total loss of echo-differentiation between the two layers

each case; hypoechoic being the mildest grade, hypo- to isoechoic being the intermediate grade and isoechoic being the most severe grade on the ultrasound images. All Group I subjects (100%) demonstrated a hypoechoic submucosa. Of the Group II subjects, 29 (96.7%) showed a hypoechoic appearance similar to Group I, while only one subject (3.3%) showed a hypo- to isoechoic (intermediate) appearance. Of the Group III subjects, 14 subjects (46.7%) showed a hypo- to isoechoic appearance while 16 subjects (53.3%) showed an isoechoic appearance where the echo texture of the submucosa was similar to that of the muscle layer. χ^2 tests revealed a statistically significant difference in the echo texture of the submucosa between cases and Group I controls as well as cases and Group II controls ($p < 0.001$). However, there was no statistically significant difference between Group I and Group II controls (Table 1).

Differentiation between submucosa and muscle layer: All Group I subjects (100%) demonstrated a distinct differentiation between the submucosa and the muscle layer. Of the Group II subjects, 29 (96.7%) showed this distinct differentiation, while in one subject (3.3%), the junction appeared indistinct. In Group III subjects

(cases), the differentiation between the submucosa and the muscle layer appeared indistinct in 50% while it was totally lost in the remaining 50% of cases. χ^2 tests revealed a statistically significant difference in the echo differentiation of submucosa and muscle layer between cases and Group I controls as well as cases and Group II controls ($p < 0.001$). However, there was no statistically significant difference between Group I and Group II controls (Table 1).

Relationship between clinical measurements and sonographic findings in the case group: There was no statistically significant difference in the mean mouth opening ($p = 0.14$) or cheek flexibility ($p = 0.26$) between the cases that had a hypo- to isoechoic submucosa and those that had an isoechoic submucosa (Table 2). Similarly, there was no statistically significant difference in the mouth opening ($p = 0.29$) or cheek flexibility ($p = 0.34$) between the cases in which the submucosa–muscle junction appeared indistinct and those in which this differentiation was totally lost (Table 3).

Site-specific comparison between clinical and ultrasonographic findings in the case group: The clinical finding of presence or absence of fibrous bands in each of the four sites bilaterally was correlated with the ultrasound findings of increased submucosal echogenicity and reduced echo differentiation of the submucosa–muscle layer junction in the case group. The clinical and ultrasonographic findings were compared for a total of 120 sites (4 in each case *i.e.* RAB, RPB, LAB, LPB). It was found that 97% of the clinically positive sites were also positive on the ultrasound images. 71.7% of clinically negative sites were found to be positive on the ultrasound. Of the 79 sites that appeared hypo- to isoechoic on the ultrasound images, 43 (54.4%) were clinically positive. Of the 24 sites that appeared isoechoic on the ultrasound, 22 (91.6%) were clinically positive. The number of sites found positive on the ultrasound was significantly greater than the number of clinically positive sites both in the anterior and posterior parts of the buccal mucosa ($p \leq 0.05$) (Table 4).

Interobserver agreement: There was very good consistency in the assessment of clinical and ultrasonographic findings among the three observers. The kappa values for the interobserver variability in clinical findings were 0.94

Table 1 Ultrasonographic findings in the different groups

Ultrasonographic parameter	Appearance	Group		Controls without habits		Controls with habits		Cases		Total	
		n	%	n	%	n	%	n	%	n	%
Echo texture of submucosa:	Hypoechoic	30	100.0	29	96.7	0	0.0	59	65.6		
	Hypo to isoechoic	0	0.0	1	3.3	14	46.7	15	16.7		
	Isoechoic	0	0.0	0	0.0	16	53.3	16	17.8		
Differentiation between submucosa and muscle layer:	Distinct	30	100.0	29	96.7	0	0.0	59	65.6		
	Indistinct	0	0.0	1	3.3	15	50.0	16	17.8		
	Lost	0	0.0	0	0.0	15	50.0	15	16.7		
Total		30	100.0	30	100.0	30	100.0	90	100.0		

Table 2 Relationship between the submucosal echo texture and clinical measurements in the case group

Clinical measurement	Submucosal echo texture	n	Mean	Standard deviation	Minimum	Maximum	p-value
Mouth opening (mm)	Hypo- to isoechoic	14	36.93	10.004	18	52	0.138
	Isoechoic	16	31.56	9.252	18	49	
	Total	30	34.07	9.826	18	52	
Cheek flexibility (mm)	Hypo- to isoechoic	14	2.89	1.243	1	5	0.257
	Isoechoic	16	2.38	1.204	1	6	
	Total	30	2.62	1.230	1	6	

(between Observer 1 and 2), 0.94 (between Observer 2 and 3) and 0.93 (between Observer 1 and 3). The Dahlberg's measurement errors for clinical measurements were 0.98 (between Observer 1 and 2), 1.03 (between Observer 2 and 3) and 0.97 (between Observer 1 and 3). The kappa values for the interobserver variability in the ultrasonographic findings were 0.93 (between Observer 1 and 2), 0.90 (between Observer 2 and 3) and 0.91 (between Observer 1 and 3). All the kappa values were statistically significant ($p < 0.05$).

Discussion

OSF is characterized by the presence of fibrous bands, most commonly in the buccal mucosa.¹⁷ Hence, imaging the buccal mucosa in OSF could offer a third dimension to the assessment of disease severity by noninvasive means, in addition to clinical and histological evaluation. The age group chosen for our study was 20–50 years, the rationale being the relative constancy of the tissue architecture in this adult population.¹⁸ To document the specific ultrasonographic findings in OSF, we excluded patients with other systemic diseases and oral mucosal lesions, since fibrosis results in a non-specific increase in echogenicity of tissues, irrespective of the cause.¹⁰ Although an intraoral approach has been described in some studies,¹⁴ we preferred transcutaneous ultrasonography because OSF is a disease that causes restriction of mouth opening, hence it may not have been feasible to place a relatively large endocavitary transducer intraorally in all the cases. The normal echo architecture of the layers of the cheek from the skin to the mucosa, as determined by transcutaneous ultrasonography in our study, was consistent with that described in earlier studies.^{13,16} Although the mouth opening and cheek flexibility measurements showed a decreasing trend as we moved along the three groups, the absence of statistically significant difference in these measurements between controls with habits and controls without habits suggests that significant reduction in clinically measurable parameters occurs only in advanced disease.

All Group I controls (100%) demonstrated a hypoechoic submucosa with a clear, distinct differentiation from the muscle layer. Therefore, it is reasonable to assume that this is the normal sonographic appearance of the buccal mucosa in the healthy adult population. Since ours is one of the first studies of its kind, it is limited by the inclusion of only adult males in the 18–50 years age group. Further studies are required involving larger samples with appropriate gender distribution and age stratification to document the age-related changes in the mucosal echotexture. In controls with habits, only one subject showed a hypo- to isoechoic submucosa and the remaining 29 (96.3%) showed a hypoechoic submucosa similar to controls without habits. Since that patient was not willing to undergo biopsy, owing to the absence of clinical signs and symptoms, we could not ascertain if this finding was representative of a sub-clinical disease. Studies should be undertaken in future to determine whether ultrasonography could be used as a screening tool for detection of early OSF in high-risk groups. In the case group, nearly half (43.3%) showed a hypo- to isoechoic appearance while the rest (56.6%) showed an isoechoic appearance where the echotexture of the submucosa was similar to that of the muscle layer. No OSF case showed a completely hypoechoic appearance in all the sites, which was a finding that suggested that all clinically detectable OSF cases were also detectable ultrasonographically.

The junction between the submucosa and the muscle layer was distinct in all the controls without habits, a large majority of the controls with habits (96.3%) and in none of the cases. The junction appeared indistinct in half of the cases, and the differentiation was totally lost in the remaining half. This total loss of differentiation was not seen in both the control groups, suggesting that complete loss of echo differentiation between the submucosa and the muscle layer is highly indicative of well-established OSF.

As far as thickness of the submucosa is concerned, Rangaiah *et al*¹³ reported an increase in the thickness of the submucosa in OSF patients, compared with

Table 3 Relationship between the echo differentiation at the submucosa–muscle layer junction and clinical measurements in the case group

Clinical measurement	Echo differentiation	n	Mean	Standard deviation	Minimum	Maximum	p-value
Mouth opening (mm)	Indistinct	15	36.00	10.413	18	52	0.289
	Lost	15	32.13	9.141	18	49	
	Total	30	34.07	9.826	18	52	
Cheek flexibility (mm)	Indistinct	15	2.83	1.220	1	5	0.343
	Lost	15	2.40	1.242	1	6	
	Total	30	2.62	1.230	1	6	

Table 4 Site-specific comparison between clinical and ultrasonographic evaluation in the case group

Part			Clinical evaluation						χ^2 p-value
			Negative		Positive		Total		
			n	%	n	%	n	%	
Anterior buccal mucosa	Echo texture of submucosa	Hypoechoic	13	27.7	0	0.0	13	21.7	p-value 0.050
		Hypo- to isoechoic	32	68.1	9	69.2	41	68.3	
		Isoechoic	2	4.3	4	30.8	6	10.0	
	Differentiation between submucosa and muscle layer	Distinct	13	27.7	0	0.0	13	21.7	p-value 0.050
		Indistinct	32	68.1	9	69.2	41	68.3	
		Lost	2	4.3	4	30.8	6	10.0	
	Total		47	100.0	13	100.0	60	100.0	
Posterior buccal mucosa	Echo texture of submucosa	Hypoechoic	2	33.3	2	3.7	4	6.7	p-value 0.010
		Hypo- to isoechoic	4	66.7	34	63.0	38	63.3	
		Isoechoic	0	0.0	18	33.3	18	30.0	
	Differentiation between submucosa and muscle layer	Distinct	2	33.3	2	3.7	4	6.7	p-value 0.011
		Indistinct	4	66.7	35	64.8	39	65.0	
		Lost	0	0.0	17	31.5	17	28.3	
	Total		6	100.0	54	100.0	60	100.0	

controls. In our study, however, we were unable to measure the thickness of the submucosa accurately in those cases where the submucosa appeared isoechoic with the muscle layer and the differentiation between the two layers was completely lost. Therefore, in our study, we chose to exclude the measurement of the submucosa, because it would not have been accurate in cases where this junction was not clearly defined and comparison with controls would not have been possible. Ultrasonographic equipments with much higher resolution or higher frequency could help overcome this problem by showing the submucosa-muscle junction in greater detail.

The echo texture of the submucosa or the echo differentiation between the submucosa and the muscle layer seemed to have no relationship to the mouth opening or cheek flexibility in the OSF cases. The probable reason could be that these clinical measurements may be dependent more on the anatomical site of involvement rather than the degree of fibrosis (increase in echogenicity), as has been demonstrated in various clinicopathologic studies.^{7,15} Another interesting observation was that ultrasonography demonstrated an increase in echogenicity of the submucosa in 71.7% of the sites that were clinically negative. As the echogenicity of the submucosa progressively increased to approach the isoechoic appearance, the clinical detection rate of those sites also improved. Based on this, although it can be hypothesized that ultrasound could pick up a significantly greater number of sites affected by OSF than clinical examination alone, it is unfortunate that neither clinical nor ultrasonographic evaluation can be considered as the gold standard and therefore the sensitivity, specificity, etc. could not be assessed. It may not be feasible or ethical to perform a biopsy from all the four

sites in all the patients to enable histopathological evaluation. This is particularly true in our study, which is one of the first of its kind where ultrasonography has been used in OSF, and the results of which need to be validated in larger samples across different age groups. Besides, biopsy may not be 100% representative of the disease severity in a diffuse disease. Although it can be considered as the gold standard for the diagnosis of OSF, its role as a gold standard to measure disease severity across the entire buccal mucosa is questionable.

In conclusion, it can be said that a diffuse disease such as OSF, in which the severity varies from site to site in the same patient, requires a multimodal approach for effective assessment, management and follow-up. In addition to the established methods of clinical and histopathological evaluation, ultrasonography provides a whole new third dimension by permitting tissue characterization over the entire buccal mucosa and indicating the depth of fibrosis in the tissue, which are not possible by other methods. Going by the applications of ultrasonography in scleroderma and the results of our own study, we hypothesize that the degree of increase in the echogenicity of the submucosa and the decrease in the echo-differentiation of the submucosa-muscle junction could directly correlate with increasing fibrosis and therefore increasing disease severity. Multicentric studies involving larger sample sizes are necessary to validate our results. We also firmly believe that ultrasonographic transducers of much greater frequency and resolution could throw more light onto the tissue characterization in OSF across the entire oral mucosa, which will help in the assessment of disease severity and in monitoring the response to treatment.

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