Noninvasive Diagnostic Techniques in Oral Submucous Fibrosis

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Contents

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13.1 Introduction

The clinical diagnosis of oral submucous fibrosis (OSF) is relatively simple and based on presenting signs and symptoms. The potential utility of noninvasive diagnostic techniques in OSF includes early detection of OSF, assessment and monitoring of OSF severity (and response to treatment), and risk stratification or monitoring of OSF patients for malignant transformation (i.e., to oral squamous cell carcinoma (OSCC)). The detection of early-stage OSF before irreversible fibrosis or malignant transformation has occurred may provide opportunities to intervene (such as areca nut cessation) and prevent worsening of the disease and associated morbidity and mortality. Noninvasive diagnostic techniques that offer frontline clinicians in primary care settings more efficient ways to identify patients with early OSF may facilitate earlier interventions. In secondary care settings, such techniques might help expert clinicians to assess disease severity or monitor patients during treatment. The most important parameter is the OSF patient's propensity for malignant transformation necessitating long-term surveillance. OSF patients are reluctant to undergo serial invasive tissue biopsies, and therefore the use of noninvasive diagnostic adjunctive techniques to facilitate the risk stratification for malignant progression would seem reasonable.

This chapter will report on the utility of several noninvasive diagnostic adjunctive techniques and their utility in patients with OSF.

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Learning Goals

Readers will be able to:

- Appreciate the different noninvasive diagnostic aids that have been tested on patients with oral submucous fibrosis (OSF)
- Identify the utility and limitations of such aids for screening (primary care setting), determination of disease severity, or assessment of risk for malignant transformation of OSF

13.2 Measurement of Mouth Opening

The interincisal distance, measured from the maxillary central incisor to the corresponding mandibular central incisor, is simple, reproduceable, and widely employed to assess disease severity in patients with OSF. The mean value of the interincisal distance in normal Nepalese population was 46.8 mm for males and 47.3 mm for females [1], and for Indian population, it was 47.5 mm for males and 44.6 mm for females [2]. A study investigated the maximal mouth opening (MMO) measured by paraclinical workers in patients with OSF compared with normal subjects in Nepal [1]. In this study, the minimum limit for a normal oral opening was determined to be 34 mm among healthy Nepalese adults, and 10 of 13 patients with OSF (histologically confirmed) had a maximum oral opening of less than 34 mm. The authors concluded that reduction of the oral opening as a single screening test for OSF has a sensitivity of 77% and detected only advanced cases. In Taiwan, a study was conducted to develop a scoring system for the early detection of OSF (betel quid users) based on clinical symptoms collected by a self-administered questionnaire [3]. The results showed that a scoring system that included MMO measurement (>35 mm of cutoff value) achieved 82% sensitivity and 85.8% specificity to detect OSF (histologically confirmed).

Measurements of MMO are correlated to the degree of fibrosis, but it is an indirect procedure. Therefore, it does not provide information about the actual condition of oral mucosa [4]. However, measuring MMO may be useful as a screening method in countries where the prevalence of OSF is high, medical resources are scarce, access to care is limited, and a detailed visual and tactile oral examination is not otherwise performed. In such situations, a community worker could simply measure mouth opening with the exclusion of other oral and maxillofacial diseases associated with trismus (i.e., temporomandibular joint disorders, injury to the masticatory muscle, arthritis, and chronic dental infections).

13.3 Optical Instruments

13.3.1 Tissue Autofluorescence

Evaluating tissue autofluorescence (AF) of oral mucosal sites can inform architectural and metabolic perturbations associated with the presence of oral dysplasia and neoplasia [5]. In vivo adjunctive techniques, both fiber-optic spectroscopic systems measuring the specific spectral signatures of fluorophores and direct visualization devices revealing changes in AF (i.e., retained versus loss of fluorescence visualization (FVR: fluorescence visualization retention vs. FVL: fluorescence visualization loss)), have been tested in patients with OSF. Spectroscopy using a 330 nm light wavelength for excitation demonstrated an AF pattern with two distinct spectral emission bands in patients with OSF compared to those with normal mucosa: at 380 nm (an increased fluorescence compared to normal oral mucosa) dictated by the increased collagen deposition associated with OSF, and at 460 nm (a reduced fluorescence) related to lower epithelial NADH content associated with an atrophic mucosa [6, 7]. This pattern was not deemed sensitive enough to predict the severity of OSF and

was attenuated in OSF patients with both concomitant leukoplakia (with or without dysplasia) and benign keratoses. 76% of 88 OSF patients were correctly diagnosed using this pattern from a mixed patient cohort with OSF, leukoplakia, lichen planus, OSCC, and otherwise normal mucosa [8]. However, AF spectroscopy alone has not convincingly been demonstrated to help distinguish between OSF with and without dysplasia or malignancy. Low-level evidence from one study on 40 patients with OSF undergoing AF spectroscopy, coupled with a pre-rinsing with 5-aminolevulinic acid (ALA) to harness PpIX fluorescence, showed that OSF patients with epithelial hyperplasia or dysplasia may be discriminated from OSF patients without epithelial changes [9]. AF spectroscopy requires expensive equipment and therefore has limited applicability in a primary care setting. There is only one study reporting the use of a direct visualization device (VELscope, LED Inc., Vancouver, Canada) in 12 patients with OSF, 11 testing "positive" (i.e., loss of fluorescence) of which 10 demonstrated mild dysplasia, and one OSF patient without dysplasia tested "negative" [10]. Overall, the utility of AF as a noninvasive diagnostic adjunctive technique is limited.

13.3.2 Ultrasonography

13.3.2.1 Colored Doppler Ultrasonography

Ultrasonography (USG) is a noninvasive, reproducible, and time-saving examination test method that offers high cost-benefit in medical diagnosis. Ultrasound, a sound energy, is in the form of waves with frequencies of >20 KHz. In medical diagnostics, ultrasound uses frequencies of 2–10 MHz and only the longitudinal mode of ultrasonic vibration. Colored Doppler, combining USG and the Doppler system, is a color-coded representation of blood flow velocity of the reflecting tissue. USG quantitatively provides information about the nature of the lesion and the adjacent normal structure and quantitatively assesses the lesion size, distance from the skin or mucosal surface, and relative proximity to the skin or mucosal surfaces [11].

Clinical applications of USG as diagnostic aids or for evaluation of the treatment for OSF have been carried out exclusively in India [12–23]. Studies measured the submucosal thickness of the buccal and labial mucosal sites and reported that as the severity of the disease increased, the submucosal tissue thickness increased and the vascularity of OSF lesions decreased compared to controls. ◘ Figure 13.1 shows the submucosal thickness of buccal mucosa on USG. A systematic review of 12 studies [12–23] investigated the role of USG in evaluating OSF [24]. Although the results did not provide clear evidence of the clinical value of USG for the early diagnosis of OSF, USG is a safe and conventional modality to assess muscular and submucosal tissue thickness, especially in terms of availability and cost factors. The authors indicated that to precisely evaluate OSF using USG, a highly accurate intraoral probe should be used.

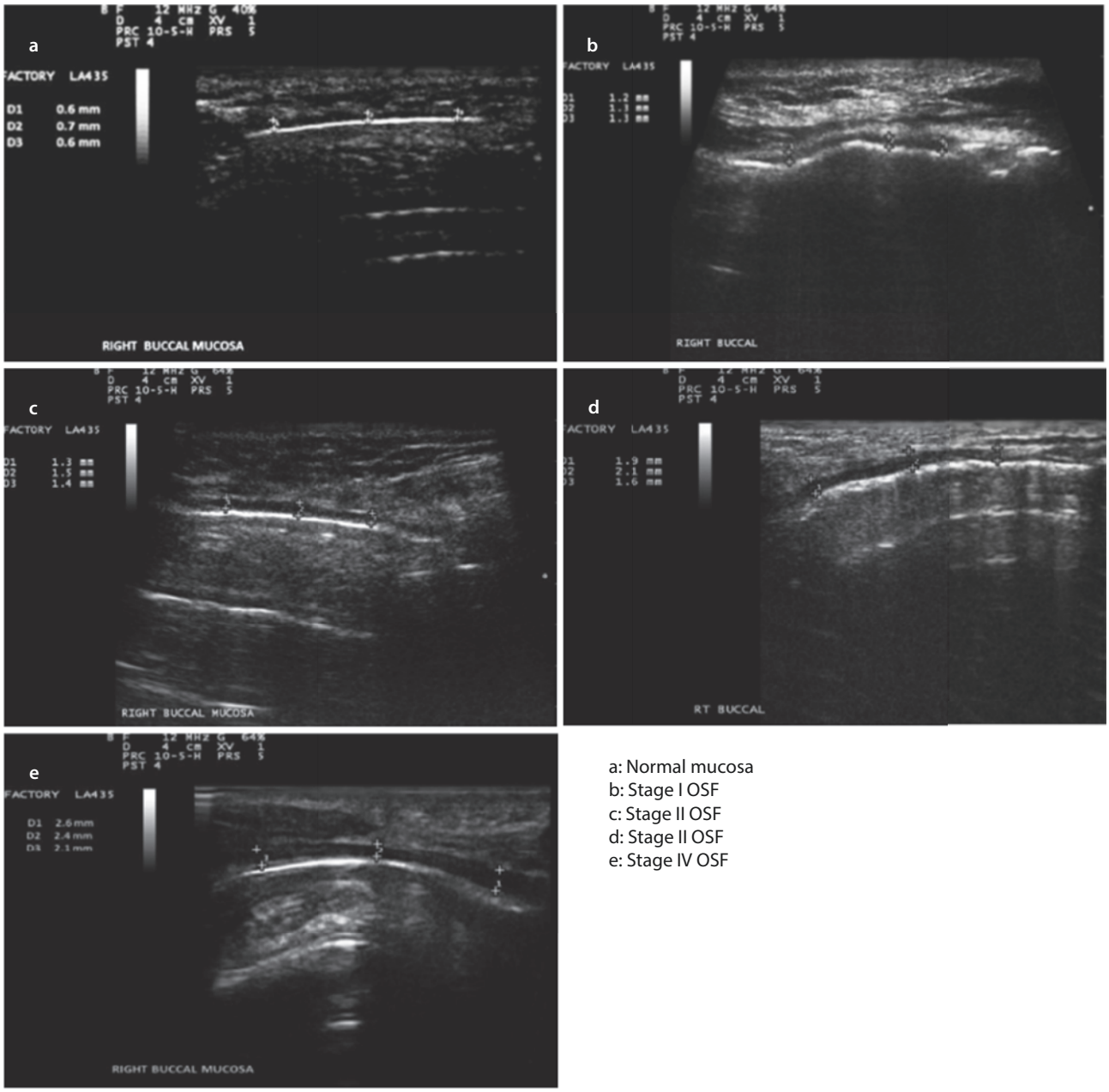
These findings were also useful for examining masseteric hypertrophy in OSF during the initial diagnosis or evaluation of treatment. Kamala et al. conducted a preliminary study to measure the masseter muscle thickness both at rest and at maximum clenching state by USG in patients with OSF and showed that the masseter muscle thickness increased as the duration and frequency of areca nut use increased and as the disease progressed clinically and histologically [12].

Peak systolic velocity (PSV) is measured by colored Doppler USG to assess vascular distribution of subcutaneous or submucosal tissues (◘ Fig. 13.2). Manjunath et al. conducted a study to elucidate the usefulness of USG (two-dimensional [2D] and duplex Doppler including color flow imaging) in the buccal mucosa for patients with OSF. This was assessed by measuring the PSV ratio before and after the medical treatment [13]. The study indicated that OSF tissues showed increased hyperechoic areas representing fibrous bands or diffuse fibrosis and reduced vascularity and PSV.

Dupare and Dhole [20] conducted a study to evaluate the role of USG in OSF patients (grades I–IV). They examined submucosal thickness and vascularity by PSV, bilaterally on buccal and labial mucosae. The results showed a decrease in PSV with the progression of OSF severity, and in ultrasonographic diagnosis of OSF, the reported submucosal thickness had a sensitivity, specificity, PPV, NPV, and accuracy of 80%, 100%, 100%, 71.4%, and 87%, respectively, but PSV was unable to classify lesions.

A biopsy of an OSF patient is not 100% representative of OSF disease severity because OSF is a diffuse disease and may vary in severity across oral subsites in the same patient [14]. Multiple or serial biopsies to detect dysplastic or malignant changes are difficult for patients. USG may facilitate both diagnosis and monitoring of OSF patients chairside and may also be used to evaluate treatment outcomes without patient discomfort. For clear evidence of USG efficacy in patients with OSF, the correlation of USG assessment to clinical grading and histopathological findings should be examined [21]. More well-designed clinical trials are needed to elucidate the effectiveness of USG on OSF.

Overall, the utility of ultrasonography as a noninvasive diagnostic adjunctive technique is limited in patients with OSF. Diagnostic performance varies depending on the objectives and needs standardized image interpretation skills.



a: Normal mucosa
b: Stage I OSF
c: Stage II OSF
d: Stage II OSF
e: Stage IV OSF

Fig. 13.1 Submucosal thickness of buccal mucosa on ultrasonography (By courtesy of Dr. Aditya Dupare)
Submucosal thickness measurements in millimeters (mm) are taken at three points: the anterior (D1), middle (D2), and posterior (D3) for buccal mucosa
As the severity of the disease increased from grade I b**B** to IV e the thickness of the submucosal tissue increased compared to the normal control **a** point D1 (**a**–**e** 0.6 mm, followed by 1.2, 1.3, 1.9, and 2.6 mm, respectively), point D2 (**a**–**e** 0.7 mm, followed by 1.3, 1.5, 2.1, and 2.4 mm, respectively), and point D3 (**a**–**e** 0.6 mm, followed by 1.3, 1.4, 1.6, and 2.1 mm, respectively)

13.3.3 ATR-FTIR Spectroscopy

Fourier transform infrared (FTIR) spectroscopic imaging is used for the analysis of biochemical components (e.g., proteins, carbohydrates, and nucleic acids) and has been proposed as an adjunct to current histopathological techniques [25]. FTIR imaging provides a nondestructive image of the sample and does not require staining. Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) has been used to diagnose cancer in biofluids, such as the serum, plasma, urine, and saliva; however, its clinical translation is still under development [26, 27]. Shaikh et al. used ATR-FTIR spectroscopy to measure total protein estimation in saliva to discriminate patients with OSF from healthy controls [28] (Table 13.1).

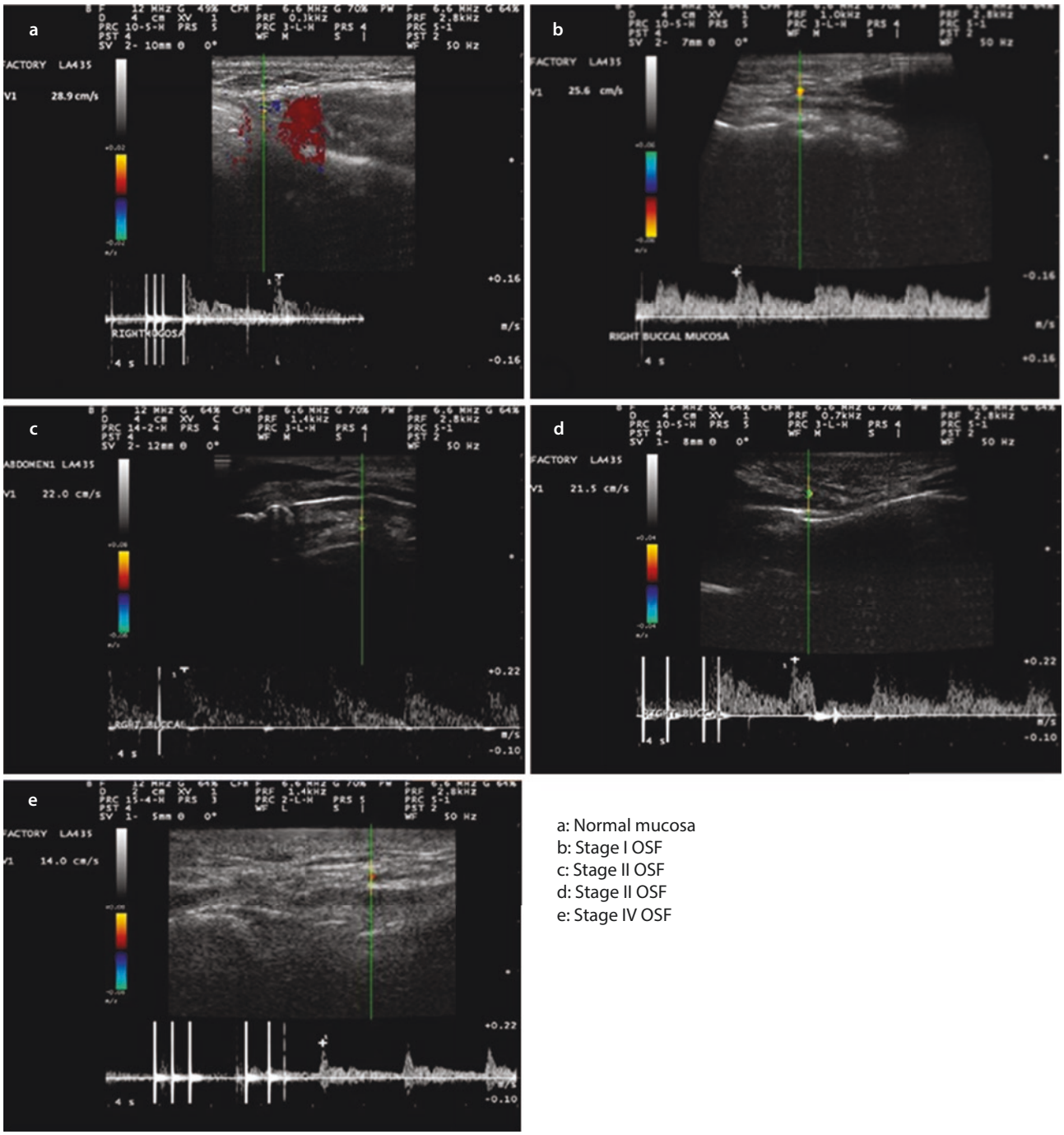


Fig. 13.2 Peak systolic velocity (PSV) on colored Doppler ultrasonography (By courtesy of Dr. Aditya Dupare)
Vascular distribution by peak systolic velocity (PSV) measured on colored Doppler USG of right buccal mucosa shows a decrease as the severity of OSF progresses from grade I b to IV **E** e-compared to normal control **a** (**a**–**e** 28.9 cm/s, followed by 28.9, 22.0, 21.5, and 14.0 cm/s, respectively)

The study showed that the specific infrared spectrum of patients with OSF could be distinguished from the healthy controls based on the spectral shift of proteins/amide II, carbohydrates, and nucleic acids using a principal component analysis and hierarchical cluster analysis. However, there were study limitations due to the small sample size. Therefore, further studies should be conducted to assess the ATR-FTIR using saliva for the assessment as a screening tool to support early diagnostic aid of OSF.

Table 13.1 Noninvasive diagnostic techniques in oral submucous fibrosis

Instrument/ method		Test objectives	Outcomes	Time value of test	Ease of introduction to the chairside of clinics[a]	Maintaining test accuracy	Diagnostic performance
Measurement of mouth opening		OSF diagnosis degree of severity Monitoring therapeutic effect	Functional staging by mouth-opening grade: severity of OSF	Real time	High	Easy measurement but needs standardization	High but indirect procedure
Optical instrument	Autofluorescence spectroscopy optical coherence tomography Contact endoscopy ultrasonography	Early diagnosis Monitoring therapeutic effect Degree of severity Monitoring susceptibility to cancer	Optical inspection images (unclear threshold): detection of malignancies, blood flow pattern, presence of fibrotic bands	Real time	Low to high	Needs standardized image interpretation skill	Low to moderate (depending on the objectives) insufficient evidence
Biomarkers in saliva	Lactate dehydrogenase (LDH) Trace elements oxidative stress markers Micronutrients predictive tumor markers	OSF diagnosis degree of severity Monitoring susceptibility to cancer Therapeutic effect and prognosis	Biological or genetic evaluation (unclear threshold): detection of malignancies, grading of OSF	Real time but time consuming depending on instruments	Low	Standardized	Insufficient evidence Not specific to OSF
X-ray (lateral cephalometric analysis)		OSF diagnosis Degree of severity	Morphological change images of lateral soft palate and uvula: severity of OSF, comorbidities in patients with OSF such as sleep apnea syndrome, dysphagia, and other disorders	Moderate time	High: conventional methods in dentistry	Standardized and needs X-ray interpretation skill	High for late stage but not for early stage

[a]In low- and middle-income countries

13.3.4 Optical Coherence Tomography

Optical coherence tomography (OCT) is a high-resolution cross-sectional imaging modality analogous to ultrasound imaging except that it uses light instead of sound [29]. In OCT, distance and microstructure measurements are performed by backscattering and backscattering light waves from various microstructure features within a material or tissue [30]. Imaging depths are not as deep as ultrasound, up to 2–3 mm deep; however, the OCT resolution is >10–100 times finer than ultrasound [31]. A study was conducted to elucidate the possibility of using OCT to identify differences in oral and oropharyngeal mucosal and submucosal tissue in patients with benign and malignant processes in the oral cavity and oropharynx [32], and the results showed that disruption of tissue structure was observed as the pathological tissues invade healthy areas, clearly demonstrating the transition from normal epithelium with an intact basement membrane to invasive tumors. Tsai et al. [33] developed a handheld OCT system for in vivo oral cavity imaging, enabling the identification of the different structures, epithelium, lamina propria layers, fungiform papilla, and salivary gland, and the observation of the microcirculation patterns across various oral mucosal types, including non-keratinized, keratinized (masticatory), and specialized mucosae.

According to clinical scans of a swept source OCT (SS-OCT) system, the following parameters were identified to facilitate the clinical diagnosis of oral lesions [34]: epithelial (EP) thickness, standard deviation (SD) of A-mode scan intensity profile of the EP layer, and decay constants of the spatial domain spectrum of the A-mode scan profile. Lee et al. conducted a study in Taiwan to elucidate the effectiveness of the SS-OCT system for clinical diagnosis of OSF [4]. The results of the study showed that the EP layer was thinner and the SD of the A-mode scan intensity in the lamina propria (LP) layer was reduced in OSF compared to healthy controls (Fig. 13.3, Table 13.1). The EP thickness cutoff values of 350 and 400 μm achieved 100% sensitivity and specificity, and the SD value of 0.21 in the LP layer achieved 90.9% sensitivity and 84.1% specificity for OSF. On the other hand, as the lesion begins to progress from epithelial hyperplasia to dysplasia, the EP layer thickens if the EP/LP boundary is still identified. When early OSCC coexists with OSF, the surface features are unclear, and variable EP thickness can make distinguishing between healthy tissues, other OPMDs, OSCC, and OSF difficult. The researchers categorized OSF patients into three groups based on the maximum mouth opening (MMO) and showed that EP thickness and average LP SD in the LP layer may be a more effective OSF diagnostic method than measuring MMO [4] (Fig. 13.4).

Some limitations of OCT include the following: (1) pathologists should interpret and evaluate the acquired live histology images; (2) images do not provide quantitative information and require subjective visual assessment; and (3) due to the small size of the OCT probe, only a very small area can be inspected at a time [35]. Overall, although OCT is good at diagnosing OSF, clinical application of OCT remains challenging in patients with OSF in terms of early detection of malignant changes.

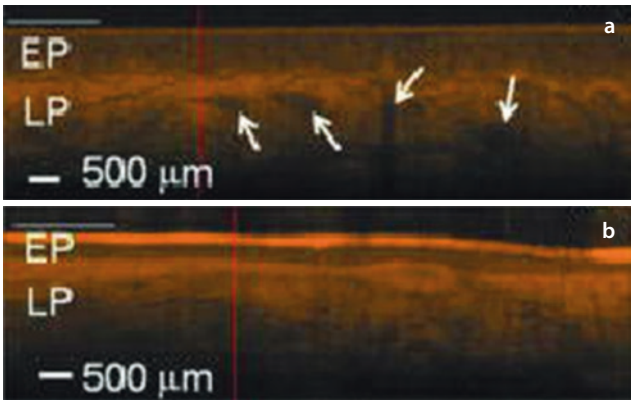


Fig. 13.3 Swept source OCT (SS-OCT) scanning images: **a** healthy mucosa, **b** OSF mucosa
In healthy mucosa, glands or blood vessels can be observed as black spots as indicated by arrows. *EP* epithelial, *LP* lamina propria (Reproduced from Lee et al. Diagnosis of oral submucous fibrosis with optical coherence tomography. J Biomed Opt. 2009; 14:054008 [4])

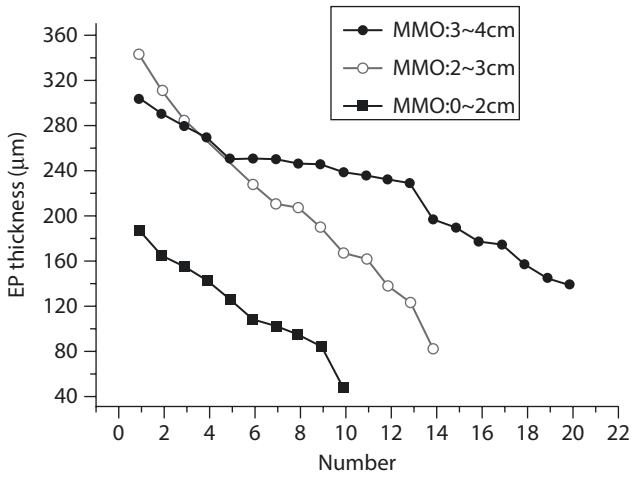


Fig. 13.4 EP thickness data points of OSF samples grouped according to different MMO ranges
EP thickness is related to MMO. (Reproduced from Lee et al. Diagnosis of oral submucous fibrosis with optical coherence tomography. J Biomed Opt. 2009; 14:054008 [4])

13.3.5 Contact Endoscopy

Contact endoscopy (CE) is a noninvasive optical detection technique first used in minimally invasive gynecologic surgery [36] and has subsequently been applied in urology, bronchoscopy, arthroscopy, and otolaryngol-

ogy. This technique enables real-time evaluation of the superficial cell layers of the epithelium and magnifies the images. Mishra et al. examined the diagnostic accuracy of CE in the detection of oral mucosal lesions and OSCC, including OSF [37] (Table 13.1). CE showed 84.2% sensitivity and 94.4% specificity for the diagnosis of malignancies of oral mucosal lesions compared to histopathological diagnosis; however, no malignancy was detected in OSF due to the small sample size ($n = 7$). The results did not include CE images indicating OSF.

One of the types of contact endoscopy, colposcopy, is designed as a gynecological diagnostic technique to evaluate changes in mucosal surface topography and vascular patterns of the cervix. Parameters include vascular pattern, intercapillary distance, surface pattern, color tone, opacity, and clarity of the mucosal lesion demarcation [38]. This technique has been applied to patients with OPMDs and OSCC to distinguish between benign, dysplastic, and malignant diagnoses and to assist in biopsy site selection [39–42]. Ujwara et al. used this technique to diagnose OSCC in 90 cases of OPMDs, including 30 patients with OSF, following the application of acetic acid and Lugol's iodine, and showed that colposcopy was useful in outlining the most suspicious lesion for histopathological diagnosis by biopsy [42] (Table 13.1). However, colposcopy did not help reveal clinical patterns of dysplastic or malignant changes in OSF, and the iodine application did not show uniform uptake even in dysplastic lesions. Overall, colposcopy may not be useful in defining early-stage malignancy in patients with OSF.

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13.4 Biomarkers in Saliva

Saliva contains a wide range of proteins/peptides, nucleic acids, electrolytes, and hormones from multiple local and systemic sources [43] and may be useful for multiplexed assays developed as point-of-care devices [44]. Saliva may be reflective of the serum or contain biomarkers that are shed from the surface of the oral mucosa. Several studies have been conducted using unstimulated saliva for OSF diagnosis, monitoring of treatment, and surveillance of patients for progression and malignant transformation [45–62] (Table 13.2).

13.4.1 Lactate Dehydrogenase

Lactate dehydrogenase (LDH) is an enzyme found in the cytoplasm of a number of body tissues [58, 63], which converts lactate to pyruvate. It is also a nonspecific marker of tissue turnover, a normal metabolic process. It's increase can serve as a prognostic marker for the progression of different types of cancer [63]. In a meta-analysis [64], 2 out of 13 studies [58, 59] evaluated salivary LDH in patients with OSF and showed that standardized mean difference (SMD) of salivary LDH levels was higher in the OSF group than in healthy control, but this was not statistically significant (SMD 25.83; 95% CI: −1.74 to 53.40).

13.4.2 Trace Elements

Among trace elements, tissue copper levels were determined to be elevated in patients with OSF [65–67]. Copper acts as an initiating factor for OSF and plays a role in stimulating fibrosis by upregulating lysyl oxidase activity [65]. A meta-analysis showed a significant increase in the copper levels and a significant decrease in zinc and iron levels in patients with OSF [68]. A significant difference was observed in the mean salivary copper [45, 46, 48], zinc [46, 47], iron [45–47], and copper/zinc levels [46, 47] of OSF patients when compared to the normal controls (Table 13.2). However, there is a limitation in that the influence of dietary intake was not investigated in most studies.

13.4.3 Oxidative Stress/Micronutrients

Saliva serves as the primary defense against free radicals generated in the oral cavity during various physiological processes, and several oxidative markers have been analyzed in unstimulated saliva from both OSF and control groups (Table 13.2). Serum and saliva ascorbic acid (vitamin C) levels consistently decreased with the increased severity of OSF [49, 53, 69]. A case-control study was conducted in India to determine the correlation between oxidative stress marker levels and OSF severity as defined according to the mouth-opening grade, fibrotic bands, and histopathological grades [53]. The results showed that vitamins A, C, and E levels and salivary superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were significantly lower in OSF patients than in controls. These changes were significantly correlated with increased histopathological grades of OSF and clinical staging of mouth opening, reflecting increased oxidative stress as the disease progressed.

Salivary malondialdehyde, one of the final by-products of lipid peroxidation, levels were significantly higher in OSF patients than in controls and increased as the clinical stage [54] as well as the histopathological grade of OSF worsened [56].

The level of salivary 8-hydroxy-2-deoxyguanosine (8-OHdG), one of the reactive oxygen species and a potential DNA damage biomarker, is significantly higher in patients with OSCC or OSF than that in the control group [55, 57]. Salivary lipid peroxide, conjugated diene, hydroxyl radicals, and superoxide dismutase levels were higher, and

Table 13.2 Biomarkers in saliva for diagnostic aids of oral submucous fibrosis

Purpose	Biomarkers		Age range or mean, SD	Outcome values (mean, SD)		Unit	Number (OSF, control)	Significance (p-value)	Diagnostic confirmation of OSF	Note	Year	References
				OSF	Healthy control							
OFS diagnosis	Salivary lactate dehydrogenase (LDH)		18-	606.83, 60.01	80.73, 12.06	U/L	30, 30	<0.001	Clinicopathological		2015	Sivaramakrishnan [58]
			20–70	608.28, 30.22	182.21, 34.85	Unstated	25, 10	<0.001	Clinical		2016	Kallalli [79]
			28.63, 10.39	1057.30, 640.12	668.25, 498.45	µg/dl	20, 20	0.04	Clinical		2018	Mishra [80]
			18–70	350.43, 5.90	86.12, 7.05	IU/L	30, 30	Not indicated	Clinicopathological	Grade I-IV OSF	2019	Mantri [81]
			–	631.67, 7.67	140.62, 8.87	Unstated	40, 40	<0.001	Clinical		2020	Panda [82]
Monitoring susceptibility to cancer	Salivary trace elements	Cu	30–50	0.13, 0.015	0.15, 0.016	ppm/L	35, 35	<0.05	Clinicopathological		2012	Chitra [45]
			–	87.45, 2.67	46.07, 4.56	µg/dl	50, 50	0.001	Clinicopathological		2015	Shetty [46]
			16–60	0.087, 0.162	0.051, 0.06	ppm/L	30, 30	NS	Clinical		2015	Okade [47]
			20–63	27.023, 14.498	8.393, 2.256	µg/dl	30, 30	0.005	Clinicopathological	Grade I-III OSF	2015	Mohammed [48]
		Zn	35–50	0.15, 0.017	0.13, 0.014	ppm/L	35, 35	NS	Clinicopathological		2012	Chitra [45]
			–	24.67, 4.86	35.78, 3.97	µg/dl	50, 50	0.001	Clinicopathological		2015	Shetty [45]
			16–60	0.100, 0.214	0.100, 0.295	ppm/L	30, 30	<0.05	Clinical		2015	Okade [47]

(continued)

Table 13.2 (continued)

Purpose	Biomarkers		Age range or mean, SD	Outcome values (mean, SD)		Unit	Number (OSF, control)	Significance (*p*-value)	Diagnostic confirmation of OSF	Note	Year	References
		Cu/Zn ratio	35–50	1	1		35, 35	NS	Clinicopathological		2012	Chitra [45]
			–	3.53	1.28	µg/dl	50, 50	0.001	Clinicopathological		2015	Shetty [46]
			16–60	3.78, 4.13	10.91, 15.89	ppm/L	30, 30	<0.05	Clinical		2015	Okade [47]
		Fe	35–50	0.4, 0.05	0.10, 0.02	ppm/L	35, 35	<0.05	Clinicopathological		2012	Chitra [45]
			20–40	107.448, 28.932	119.898, 31.519	Unstated	65, 21	NS	Clinicopathological		2012a	Shetty [49]
			16–60	0.149, 0.242	0.524, 0.143	ppm/L	30, 30	<0.05	Clinical		2015	Okade [47]
			–	35.67, 1.34	76.07, 3.65	µg/dl	50, 50	0.001	Clinicopathological		2015	Shetty [46]
	Salivary predictive tumor markers	S100A7	28.2 (mean) for OSF, 26.9 for control	0.28, 0.9	0.19, 0.03	ng/ml	30, 30	<0.001	Clinical		2019	Raffat [50]
		MMP-12	33.27, 12.43	12.53, 3.2	0.82, 0.45	ng/ml	30, 30	<0.001	Clinical		2021	Saleem [51]
		microRNAs	34.1, 13.4	14.31, 23.33	0.50, 0.30		61, 63	<0.001	Clinical	miRNA expression fold	2020	Prasad [52]
Monitoring therapeutic effect and prognosis	Salivary oxidative stress markers	LPO	20–70	197.22, 64.5	15.86, 4.63	µM of MDA/ml	63, 63	<0.001	Clinicopathological	Grade I-IV OSF	2018	Divyambika [53]
		GSH		464.51, 84.15	474.6, 47.2	µM/ml		NS				
		SOD		0.72, 0.22	1.42, 0.28	U/100 mg protein		<0.001				

		GPX		0.85, 0.33	1.41, 0.38	mM of GSH reduced/ min/mg protein		<0.001			
		Malondi-aldehyde	20–40	0.434	0.224	nmol/mg	65, 21	<0.001	Clinicopath-ological		2012b	Shetty [54]
			41–60	0.43, 0.07	0.08, 0.07	µM/ml	40, 40	<0.005	Clinicopath-ological		2016	Kaur [55]
			Unstated	0.359, 0.06	0.179, 0.04	nmol/mg	40, 40	<0.05	Clinicopath-ological	Grade I-III OSF	2021	Ganta [56]
		8-OHdG	41–60	0.49, 0.08	0.07, 0.07	ng/dl	40, 40	<0.005	Clinicopath-ological		2016	Kaur [55]
			31–60	13.89, 1.96	6.59, 1.47	ng/dl	30, 30	<0.0001	Clinicopath-ological		2020	Nandaku-mar [57]
	Salivary micronutri-ents	Ascorbic acid (vitamin C)	20–70	226.91, 77.34	302.65, 95.32	µg/ml	63, 63	<0.01	Clinicopath-ological	Grade I-IV OSF	2018	Divyam-bika [53]
			20–40	0.665, 0.282	0.936, 0.274	Unstated	65, 21	<0.05	Clinicopath-ological		2012a	Shetty [49]
			41–60	0.53, 0.12	1.2, 0.6	µg/L	40, 40	<0.005	Clinicopath-ological		2016	Kaur [55]

LPO oxidative markers of lipid peroxides, *GSH* reduced glutathione, *SOD* superoxide dismutase, *GPx* glutathione peroxidase, *8-OHdG* 8-hydroxy-2-deoxyguanosine, *NS* not significant

hydrogen peroxide and sodium levels were lower in patients with OSF than those in normal healthy controls [45]. Measuring these markers may help predict the severity of oral diseases, but it is not specific to OSF patients.

13.4.4 Predictive Tumor Markers

S100A7 (Psoriasin) is a signaling molecule that regulates cell function and is highly expressed in hyperproliferative skin conditions [70]. High S100A7, which expresses in the oral mucosa, is found in dysplasia associated with a high risk of cancer development among OPMDs including OSF patients [71]. Salivary S100A7 in OSF was examined in Pakistan and showed a significant positive association between salivary S100A7 levels and duration of gutkha use and mouth opening [50]. However, no sensitivity and specificity were calculated because no histological examination was performed, and the threshold for diagnostic S100A7 levels in OSF remains unclear.

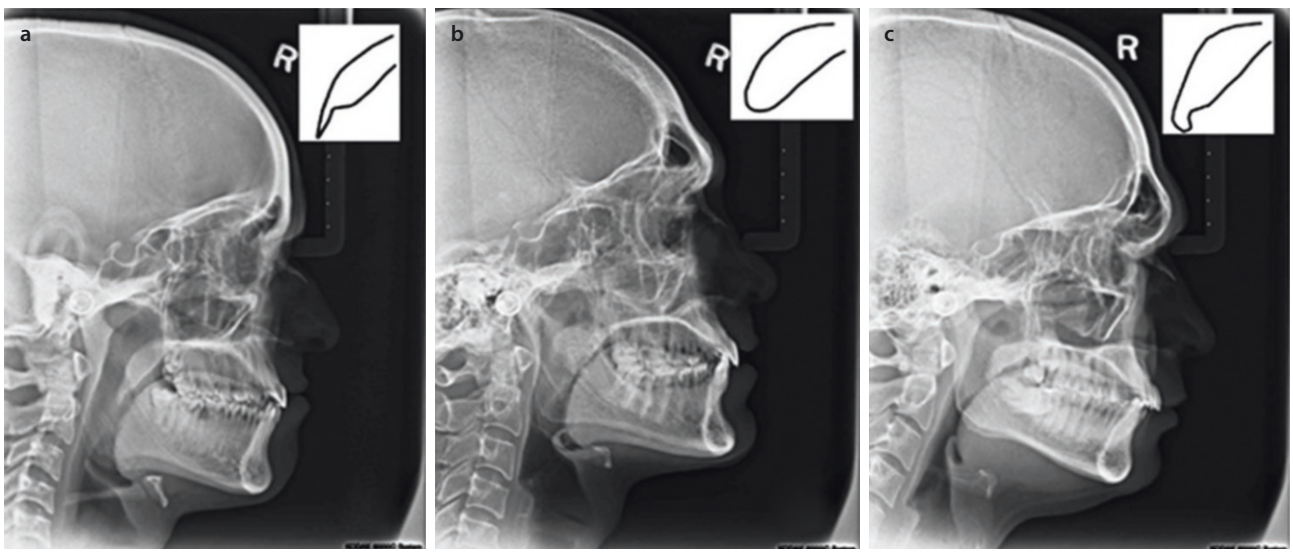
Matrix metalloproteinases-12 (MMP-12) has been shown to have important sensitivity and specificity to qualify as a diagnostic biomarker for OSCC [72]. Salleem et al. showed that salivary MMP-12 expression was higher in patients with OSF or OSCC than that in the healthy controls [51] (◘ Tables 13.1 and 13.2). They also showed that salivary MMP-12 in OSF was significantly lower than that of OSCC, explaining that MMP-12 increases as OSF progresses to OSCC.

Salivary microRNAs have been explored as possible predictive biomarkers for malignant transformation of OPMDs [73]. miRNA-21 overexpression in OSF has been suggested to be due to areca nut stimulation mediated by the TGF-β pathway [74]. A recent study analyzed the salivary miRNA-21 in areca nut users with OSF compared to areca nut users without OSF. They showed that miRNA-21 was overexpressed in the OSF patients; however, expression levels were not significantly associated with disease severity [52].

Although salivary biomarkers can be used as sensitive diagnostic and disease progression markers for OSF, it should be noted that they are not OSF specific. Furthermore, analyte concentration in saliva can be affected by stimulating or non-stimulating sampling procedures, fluid intake, and ingestion of some drugs [75]. Overall, evidence on the utility of biomarkers in saliva was insufficient for the diagnosis of OSF. In the future, saliva tests hold promise both as a screening tool and as a marker for predicting the progression of malignant transformation in OSF.

13.5 X-Ray (Lateral Cephalometric Analysis)

OSF can also cause changes in the morphology and function of the soft palate. The morphology of the soft palate in normal individuals according to digital cephalometric studies falls into six types [76]. Studies evaluating the morphology of soft palate in patients with OSF using a digital lateral cephalogram showed that the soft palate in areca nut users changes from an elongated to a short and thick morphology as the disease progresses [77–80] (◘ Fig. 13.5). These changes are due to fibro-



◘ **Fig. 13.5** Lateral ► cephalogram for morphology of the soft palate of OSF
a: type 1 (leaf-shaped), **b**: type 3 (butt shaped), and **c**: type 6 (crook shaped) soft palates (Reproduced from Shankar et al. Morphometric evaluation of soft palate in oral submucous fibrosis--a digital cephalometric study. J Craniomaxillofac Surg. 2014; 42:48–52 [77])

sis in the soft palate and uvula [81], which functionally impairs speech, respiratory, and auditory function. The uvula shrinks and hooks up, exhibiting a shape known as the "hockey-stick uvula" [82], which is confirmed not only by visual examination but also by lateral cephalogram (as type 6 by Shankar et al. [77]). Verification of soft palate cephalometric findings helps identify the likelihood of developing comorbidities in patients with OSF, including obstructive sleep apnea syndrome, dysphagia, and other disorders. The X-ray examinations by lateral cephalometric analysis of OSF patients may be morphologically and functionally useful in the late stages, but not in the early stages. X-ray interpretation skills and standardization of the tests are needed for this analysis. Radiation exposure is a limitation of repeated examinations.

13.6 Discussion

The key objectives of using noninvasive diagnostic techniques for patients with OSF include early detection of OSF, assessment and monitoring of OSF severity (and response to treatment), and risk stratification or monitoring of OSF patients for malignant transformation (i.e., OSCC). Point-of-care diagnostic techniques for OSF must demonstrate utility, accuracy, and ease to deploy in both primary and secondary settings (i.e., in rural areas).

Most studies have evaluated the effectiveness of adjunctive diagnostic aids in patients with OSF in cohort studies with heterogeneous populations including OSCC and other OPMDs rather than specifically addressing OSF patients alone. The diagnostic adjunctive techniques presented in this chapter are based on the results of non-randomized controlled trials (mostly case-control) with small sample size, suggesting that the evidence of their effectiveness is low (i.e., due to the high risk of biases, such as selection bias in normal control, and the case group (diagnosis of OSF) itself). Nevertheless, some promising adjunctive diagnostic methods have been reported for OSF.

Early diagnosis of OSF is challenging but critical given that some children use areca nut (Chap. 5). Change in clinical features of the oral mucosa is an important sign of early exposure to areca nut products. A simple history and clinical examination with standardized measuring MMO remain the most important way to detect OSF. There is insufficient evidence that other noninvasive adjunctive diagnostic techniques can play a role here.

Optical instruments and X-rays used chairside can help patients visually understand the nature of the disease without harm or adverse events, although these diagnostic techniques add little overall value to the current assessment, risk stratification, and monitoring of patients with OSF. Perturbations in various salivary markers have been identified from patients with OSF; however, cutoff values are not well defined, and the current body of evidence to support their use is low. In addition, the use of time-consuming and costly diagnostic techniques is also unsupported by the evidence, particularly in lower resource countries where betel quid and areca nut use are a social problem and the prevalence of OSF is high.

Summary

The potential utility of noninvasive diagnostic techniques in oral submucous fibrosis (OSF) includes early detection, assessment of OSF severity (and response to treatment), and monitoring of OSF patients for malignant transformation. Numerous adjunct techniques, including optical techniques, ultrasonography, and salivary biomarkers, have been evaluated but have demonstrated limited utility in primary and secondary settings. Many adjunctive techniques require expensive equipment or laboratory testing. The development of low-cost point-of-care testing for monitoring patients with OSF is needed in low- and middle-income countries where the use of areca nut is widespread.

References

1. Cox SC, Walker DM. Establishing a normal range for mouth opening: its use in screening for oral submucous fibrosis. Br J Oral Maxillofac Surg. 1997;35:40–2.
2. Ranganathan K, Davi U, Joshua E, Bhardwaj A, Rooban T, Viswanathan R. Mouth opening, cheek flexibility and tongue protrusion. Parameters of 800 normal patients in Chennai. South India J Indian Dent Assoc. 2001;7:78–80.
3. Chiu CJ, Lee WC, Chiang CP, Hahn LJ, Kuo YS, Chen CJ. A scoring system for the early detection of oral submucous fibrosis based on a self-administered questionnaire. J Public Health Dent. 2002;62:28–31.
4. Lee CK, Tsai MT, Lee HC, Chen HM, Chiang CP, Wang YM, et al. Diagnosis of oral submucous fibrosis with optical coherence tomography. J Biomed Opt. 2009;14:054008.
5. Gillenwater A, Jacob R, Ganeshappa R, Kemp B, El-Naggar AK, Palmer JL, et al. Noninvasive diagnosis of oral neoplasia based on fluorescence spectroscopy and native tissue autofluorescence. Arch Otolaryngol Head Neck Surg. 1998;124(11):1251–8.
6. Chen HM, Wang CY, Chen CT, Yang H, Kuo YS, Lan WH, et al. Auto-fluorescence spectra of oral submucous fibrosis. J Oral Pathol Med. 2003;32(6):337–43.
7. Haris PS, Balan A, Jayasree RS, Gupta AK. Autofluorescence spectroscopy for the in vivo evaluation of oral submucous fibrosis. Photomed Laser Surg. 2009;27(5):757–61.
8. Chaturvedi P, Majumder SK, Krishna H, Muttagi S, Gupta PK. Fluorescence spectroscopy for noninvasive early diagnosis of oral mucosal malignant and potentially malignant lesions. J Cancer Res Ther. 2010;6:497–502.

9. Wang CY, Tsai T, Chiang CP, Chen HM, Chen CT. Improved diagnosis of oral premalignant lesions in submucous fibrosis patients with 5-aminolevulinic acid induced PpIX fluorescence. J Biomed Opt. 2009;14(4):044026.
10. Shah S, Waknis P, Saha A, Setiya S, Ratra T, Vaswani V. The use of Velscope to assess cellular changes occurring in oral premalignancy. J Oral Biol Craniofac Res. 2020;10(2):99–103.
11. Rangaiah P, Annigeri RG, Lingappa A. Transcutaneous ultrasonographic assessment of oral submucous fibrosis: a preliminary study. Int J Oral Med Sci. 2010;9:137–47.
12. Kamala KA, Annigeri RG, Ashok L. Ultrasonic diagnosis of masseteric hypertrophy in oral submucous fibrosis: a preliminary study. J Indian Acad Oral Med Radiol. 2010;22:197–200.
13. Manjunath K, Rajaram PC, Saraswathi TR, Sivapathasundharam B, Sabarinath B, Koteeswaran D, et al. Evaluation of oral submucous fibrosis using ultrasonographic technique: a new diagnostic tool. Indian J Dent Res. 2011;22:530–6.
14. Krithika C, Ramanathan S, Koteeswaran D, Sridhar C, Satheesh Krishna J, et al. Ultrasonographic evaluation of oral submucous fibrosis in habitual areca nut chewers. Dentomaxillofac Radiol. 2013;42:20120319.
15. Devathambi JR, Aswath N. Ultrasonographic evaluation of oral submucous fibrosis and masseteric hypertrophy. J Clin Imaging Sci. 2013;3(Suppl. 1):12.
16. Chakarvarty A, Panat SR, Sangamesh NC, Aggarwal A, Jha PC. Evaluation of masseter muscle hypertrophy in oral submucous fibrosis patients -an ultrasonographic study. J Clin Diagn Res. 2014;8:ZC45–ZC47.
17. Kant P, Bhowate RR, Sharda N. Assessment of cross-sectional thickness and activity of masseter, anterior temporalis and orbicularis oris muscles in oral submucous fibrosis patients and healthy controls: an ultrasonography and electromyography study. Dentomaxillofac Radiol. 2014;43:20130016.
18. Agarwal RK, Hebbale M, Mhapuskar A, Tepan M. Correlation of ultrasonographic measurements, histopathological grading, and clinical staging in oral submucous fibrosis. Indian J Dent Res. 2017;28:476–81.
19. Tiwari M, Deoghare A, Sharma A, Saha S, Poptani R. Evaluation of OSMF with ultrasonography. Int J Oral Health Dent. 2017;3:169–74.
20. Dupare A, Dhole A. Ultrasonographic evaluation of submucosal thickness in oral submucous fibrosis patients: a cross-sectional study. Pol J Radiol. 2018;83:e280–8.
21. Nadendla LK, Tatikonda VK, Bangi BB, Bhayya H, Devulapally RV, Pokala A. Sonographic imaging of fibrosis of oral mucosa and its correlation with clinical staging in oral submucous fibrosis. J Cancer Res Ther. 2018;14:394–7.
22. Mukul SK, Kumar S, Pandey S, Mokhtar EA, Kumar A. Ultrasound elastography as a potential diagnostic aid in oral submucous fibrosis. Natl J Maxillofac Surg. 2019;10:129–33.
23. Sadaksharam J, Mahalingam S. Evaluation of oral pentoxifylline in the management of oral submucous fibrosis—an ultrasonographic study. Contemp Clin Dent. 2017;8(2):200–4.
24. Patil SR, Maragathavalli G, Ramesh DNSV, Rao KA, Bandela V, Alam MK. Role of ultrasonography in evaluation of oral submucous fibrosis patients: a systematic review. Int Med J. 2021;28:254–7.
25. Walsh MJ, Kajdacsy-Balla A, Holton SE, Bhargava R. Attenuated total reflectance Fourier-transform infrared spectroscopic imaging for breast histopathology. Vib Spectrosc. 2012;60:23–8.
26. Elkins KM. Rapid presumptive "fingerprinting" of body fluids and materials by ATR FT-IR spectroscopy. J Forensic Sci. 2011;56:1580–7.
27. Sala A, Anderson DJ, Brennan PM, Butler HJ, Cameron JM, Jenkinson MD, et al. Biofluid diagnostics by FTIR spectroscopy: a platform technology for cancer detection. Cancer Lett. 2020;477:122–30.
28. Shaikh S, Yadav DK, Rawal R. Saliva based non invasive screening of Oral submucous fibrosis using ATR-FTIR spectroscopy. J Pharm Biomed Anal. 2021;203:114202.
29. Fujimoto JG, Pitris C, Boppart SA, Brezinski ME. Optical coherence tomography: an emerging technology for biomedical imaging and optical biopsy. Neoplasia. 2000;2:9–25.
30. Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, et al. Optical coherence tomography. Science. 1991;254(5035):1178–81.
31. Fujimoto JG. Optical coherence tomography for ultrahigh resolution in vivo imaging. Nat Biotechnol. 2003;21:1361–7.
32. Ridgway JM, Armstrong WB, Guo S, Mahmood U, Su J, Jackson RP, et al. In vivo optical coherence tomography of the human oral cavity and oropharynx. Arch Otolaryngol Head Neck Surg. 2006;132:1074–81.
33. Tsai MT, Chen Y, Lee CY, Huang BH, Trung NH, Lee YJ, et al. Noninvasive structural and microvascular anatomy of oral mucosae using handheld optical coherence tomography. Biomed Opt Express. 2017;8:5001–12.
34. Tsai MT, Lee HC, Lee CK, Yu CH, Chen HM, Chiang CP, et al. Effective indicators for diagnosis of oral cancer using optical coherence tomography. Opt Express. 2008;16:15847–62.
35. Olivo M, Bhuvaneswari R, Keogh I. Advances in bio-optical imaging for the diagnosis of early oral cancer. Pharmaceutics. 2011;3:354–78.
36. Hamou JE. Microhysteroscopy Acta Endosc. 1980;10:415–22.
37. Mishra AK, Nilakantan A, Sahai K, Datta R, Malik A. Contact endoscopy of mucosal lesions of oral cavity—preliminary experience. Med J Armed Forces India. 2014;70:257–63.
38. Kolstad P. Terminology and definitions. In: Kolstad P, editor. Atlas of colposcopy. 3rd ed. London: Churchill Livingstone; 1982. p. 21–31.
39. Gynther GW, Rozell B, Heimdahl A. Direct oral microscopy and its value in diagnosing mucosal lesions: a pilot study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;90:164–70.
40. Kumar A, Shankar YU, Prakash S, Radhika B, Fatima N. Colposcopy—a novel diagnostic technique for oral mucosal lesions. J Clin Diagn Res. 2014;8:ZE25-8.
41. Nayyar AS, Khan M, Bafna UD, Siddique A, Gayitri HC. Colposcopy: gynecological vision in viewing oral lesions. Indian J Pathol Microbiol. 2014;57:223–30.
42. Ujwala N, Singh NA, Milind N, Prafulla P, Vidhya P, Bhushan B, et al. Colposcopy in pre-malignant lesions and oral squamous cell carcinoma: linking threads of clinical, histopathological and colposcopic inferences. J Cancer Res Ther. 2016;12:295–301.
43. Chianeh YR, Prabhu K. Biochemical markers in saliva of patients with oral squamous cell carcinoma. Asian Pac J Trop Dis. 2014;4(Suppl. 1):S33–40.
44. Malamud D. Saliva as a diagnostic fluid. Dent Clin N Am. 2011;55:159–78.
45. Chitra S, Balasubramaniam M, Hazra J. Effect of α-tocopherol on salivary reactive oxygen species and trace elements in oral submucous fibrosis. Ann Clin Biochem. 2012;49:262–5.
46. Shetty SR, Babu S, Kumari S, Shetty P, Hegde S, Karikal A. Status of trace elements in saliva of oral precancer and oral cancer patients. J Cancer Res Ther. 2015;11:146–9.
47. Okade AR, Hallikeri KS, Trivedi DJ. Salivary estimation of copper, iron, zinc and manganese in oral submucous fibrosis patients: a case-control study. Clin Cancer Invest J. 2015;4:302–3016.
48. Mohammed F, Manohar V, Jose M, Thapasum AF, Mohamed S, Shamaz BH, et al. Estimation of copper in saliva and areca

nut products and its correlation with histological grades of oral submucous fibrosis. J Oral Pathol Med. 2015;44:208–13.
49. Shetty SR, Babu S, Kumari S, Shetty P, Vijay R, Karikal A. Evaluation of micronutrient status in serum and saliva of oral submucous fibrosis patients: a clinicopathological study. Indian J Med Paediatr Oncol. 2012a;33(4):224–6.
50. Raffat MA, Hadi NI, Hosein M, Zubairi AM, Ikram S, Akram Z. Differential expression of salivary S100A7 in oral submucous fibrosis. Saudi Dent J. 2019;31:39–44.
51. Saleem Z, Shaikh AH, Zaman U, Ahmed S, Majeed MM, Kazmi A, et al. Estimation of salivary matrix metalloproteinases- 12 (MMP- 12) levels among patients presenting with oral submucous fibrosis and oral squamous cell carcinoma. BMC Oral Health. 2021;21:205.
52. Prasad SR, Pai A, Shyamala K, Yaji A. Expression of salivary miRNA 21 in Oral submucous fibrosis (OSMF): an observational study. Microrna. 2020;9:295–302.
53. Divyambika CV, Sathasivasubramanian S, Vani G, Vanishree AJ, Malathi N. Correlation of clinical and histopathological grades in oral submucous fibrosis patients with oxidative stress markers in saliva. Indian J Clin Biochem. 2018;33: 348–55.
54. Shetty SR, Babu SG, Kumari S, Rao V, Vijay R, Karikal A. Malondialdehyde levels in oral sub mucous fibrosis: a clinicopathological and biochemical study. N Am J Med Sci. 2012b;4:125–8.
55. Kaur J, Politis C, Jacobs R. Salivary 8-hydroxy-2-deoxyguanosine, malondialdehyde, vitamin C, and vitamin E in oral pre-cancer and cancer: diagnostic value and free radical mechanism of action. Clin Oral Investig. 2016;20:315–9.
56. Ganta SR, Chittemsetti S, Sravya T, Guttikonda VR. Salivary malondialdehyde in oral submucous fibrosis—a marker for oxidative damage. J Oral Maxillofac Pathol. 2021;25:82–7.
57. Nandakumar A, Nataraj P, James A, Krishnan R, Mahesh KM. Estimation of salivary 8-Hydroxydeoxyguanosine (8-OHdG) as a potential biomarker in assessing progression towards malignancy: a case-control study. Asian Pac J Cancer Prev. 2020;21:2325–9.
58. Sivaramakrishnan M, Sivapathasundharam B, Jananni M. Evaluation of lactate dehydrogenase enzyme activity in saliva and serum of oral submucous fibrosis patients. J Oral Pathol Med. 2015;44:449–52.
59. Kallalli BN, Rawson K, Muzammil SA, Awati MA, Shivhare P. Lactate dehydrogenase as a biomarker in oral cancer and oral submucous fibrosis. J Oral Pathol Med. 2016;45:687–90.
60. Mishra S, Kritika C, Bajoria AA, Choudhury P, Sahoo SK, Sangamesh NC. Estimation of salivary and serum lactate dehydrogenase in oral submucous fibrosis. J Int Soc Prev Commun Dent. 2018;8:289–95.
61. Mantri T, Thete SG, Male V, Yadav R, Grover I, Adsure GR, et al. Study of the role of salivary lactate dehydrogenase in habitual tobacco chewers, oral submucous fibrosis and oral cancer as a biomarker. J Contemp Dent Pract. 2019;20:970–3.
62. Panda A, Behura SS, Bhuyan L, Dash KC, Kumar V, Mukherjee S. Assessment of lactate dehydrogenase enzyme levels in saliva and serum of oral submucous fibrosis and leukoplakia patients. J Oral Maxillofac Pathol. 2020;24(1):183.
63. Farhana A, Lappin SL (2021). Biochemistry, lactate dehydrogenase. 2021 May 7. In: StatPearls. Treasure. Island, FL: StatPearls Publishing
64. Iglesias-Velázquez Ó, López-Pintor RM, González-Serrano J, Casañas E, Torres J, Hernández G. Salivary LDH in oral cancer and potentially malignant disorders: a systematic review and meta-analysis. Oral Dis. 2020; https://doi.org/10.1111/odi.13630.
65. Trivedy CR, Warnakulasuriya KA, Peters TJ, Senkus R, Hazarey VK, Johnson NW. Raised tissue copper levels in oral submucous fibrosis. J Oral Pathol Med. 2000;29:241–8.
66. Ayinampudi BK, Narsimhan M. Salivary copper and zinc levels in oral pre-malignant and malignant lesions. J Oral Maxillofac Pathol. 2012;16:178–82.
67. Kode MA, Karjodkar FR. Estimation of the serum and the salivary trace elements in OSMF patients. J Clin Diagn Res. 2013;7:1215–8.
68. Sachdev PK, Freeland-Graves J, Beretvas SN, Sanjeevi N. Zinc, copper, and iron in oral submucous fibrosis: a meta-analysis. Int J Dent. 2018;2018:3472087.
69. Bhat S, Babu SG, Bhat SK, Castelino RL, Rao K, Madi M. Status of serum and salivary ascorbic acid in oral potentially malignant disorders and oral cancer. Indian J Med Paediatr Oncol. 2017;38:306–10.
70. Son ED, Kim HJ, Kim KH, Bin BH, Bae IH, Lim KM, et al. S100A7 (psoriasin) inhibits human epidermal differentiation by enhanced IL-6 secretion through IκB/NF-κB signalling. Exp Dermatol. 2016;25:636–41.
71. Kaur J, Matta A, Kak I, Srivastava G, Assi J, Leong I, et al. S100A7 overexpression is a predictive marker for high risk of malignant transformation in oral dysplasia. Int J Cancer. 2014;134:1379–88.
72. Choudhry N, Sarmad S, Waheed NUA, Gondal AJ. Estimation of serum matrix metalloproteinases among patients of oral squamous cell carcinoma. Pak J Med Sci. 2019;35:252–6.
73. Maheswari TNU, Venugopal A, Sureshbabu NM, Ramani P. Salivary micro RNA as a potential biomarker in oral potentially malignant disorders: a systematic review. Ci Ji Yi Xue Za Zhi. 2018;30:55–60.
74. Yang HW, Yu CC, Hsieh PL, Liao YW, Chu PM, Yu CH, et al. Arecoline enhances miR-21 to promote buccal mucosal fibroblasts activation. J Formos Med Assoc. 2021;120:1108–13.
75. Khoubnasabjafari M, Ansarin K, Vaez-Gharamaleki J, Jouyban A. Comments on "salivary 8-hydroxy-2-deoxyguanosine, malondialdehyde, vitamin C, and vitamin E in oral pre-cancer and cancer: diagnostic value and free radical mechanism of action". Clin Oral Investig. 2016;20(2):395–6.
76. You M, Li X, Wang H, Zhang J, Wu H, Liu Y, et al. Morphological variety of the soft palate in normal individuals: a digital cephalometric study. Dentomaxillofac Radiol. 2008;37:344–9.
77. Shankar VN, Hegde K, Ashwini NS, Praveena V, Ravi Prakash SM. Morphometric evaluation of soft palate in oral submucous fibrosis—a digital cephalometric study. J Craniomaxillofac Surg. 2014;42:48–52.
78. Raja Lakshmi C, Ayesha Thabusum D, Bhavana SM. An innovative approach to evaluate the morphological patterns of soft palate in oral submucous fibrosis patients: a digital cephalometric study. Int J Chronic Dis. 2016;2016:5428581.
79. Kaur Domir S, Gargava A, Deoghare A, Agrawal R. Morphometric evaluation of soft palate in OSMF patients using cephalometrics. Indian J Otolaryngol Head Neck Surg. 2019;71(Suppl. 1):1018–22.
80. Tripathy M, Anekar J, Ac R, Sandeepa NC, Napplli D, Lokanath P, Alharbi AN, Alsobil FM, et al. A digital cephalometric study on the morphometric evaluation of soft palate in oral submucous fibrosis. Asian Pac J Cancer Prev. 2020;21:2169–76.
81. Chaturvedi P. Uvular abnormalities in patients with submucous fibrosis. Oral Dis. 2009;15:516.
82. O'Riordan BC. Oral submucous fibrosis. Proc Royal Soc Medicine. 1974;67:876–8.