Correlation of Ultrasonographic Measurements, Histopathological Grading, and Clinical Staging in Oral Submucous Fibrosis

Abstract
Aims and Objectives: To evaluate the presence and thickness of submucosal fibrosis in oral submucous fibrosis (OSMF) patients ultrasonographically and to correlate these findings with the clinical stage and histological grade of OSMF. Materials and Methods: Forty participants (twenty cases and twenty controls) were included in the study. The patients diagnosed clinically as having OSMF and consented for biopsy were included in the cases, and completely healthy individuals with no habit history or oral lesions were included in the control group after matching the body mass index. After clinically staging, the patients’ transcutaneous ultrasonography (USG) was performed and after that punch biopsy was taken and the specimen was graded histopathologically. Results: The data were statistically analyzed using Mann–Whitney test and Spearman’s rank correlation. The cases showed increased submucosal thickness as compared to the controls. The USG measurements statistically correlated with the clinical stage and histopathological grade of OSMF. Conclusion: USG proves to be a valuable adjunctive modality in diagnosing, staging and also evaluating the prognosis of OSMF.

Keywords: Correlation, diagnosis, high frequency, oral submucous fibrosis, ultrasonography

Introduction
In 1952, J. Schwartz coined the term atrophia idiopathica mucosa oris to describe an oral fibrosing disease he discovered in five Indian women from Kenya. S.G. Joshi subsequently coined the term oral submucous fibrosis (OSMF) for the condition in 1953. Pindborg and Sirsat have defined OSMF as “an insidious chronic disease of unknown etiology affecting the mucosa of any part of the oral cavity, occasionally extending to pharynx, esophagus, and rarely to larynx. The condition is sometimes preceded by vesicle formation and is always associated with juxta-epithelial inflammatory reaction followed by a fibroelastic change of the lamina propria with epithelial atrophy, leading to stiffness of oral mucosa causing trismus and inability to eat.”[1]

Since then, numerous researches have been carried out for early diagnosis and treatment of this condition. Diagnosis of OSMF is given by a clinical and histopathological correlation. Clinically, the disease presents with blanching of the mucosa, fibrous bands, restricted mouth opening, sunken cheeks, difficulty in cheek blowing, and tongue movements. Accordingly, different classifications have been made to denote the severity of this condition. However, these clinical findings are subjective to the clinician, and also the severity of the mouth opening depends on fibrosis present in the pterygomandibular raphae region which may be misleading the severity of the disease. Histopathology is the gold standard in diagnosing the disease. OSMF is a diffuse condition which poses a problem regarding the selection of the biopsy site for histopathological grading of the disease. In severe stages where the mouth opening is minimal, it is difficult to take a biopsy and it is not feasible to take biopsies repeatedly to determine the progression of the disease and also to check for the improvement after the treatment employed. Ultrasonography (USG) is a noninvasive, nonionizing, safe, readily available, and cost-effective modality to image superficial tissues in real time. As it facilitates the imaging of the wider area, it can be a valuable tool to determine the extent and severity of this diffuse disease as well as to monitor the response to treatment, thus supplementing clinical and histological details.[2]

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The use of USG in the evaluation of OSMF 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.\(^3\)

Keeping this in mind the aim of the study was to evaluate the presence and thickness of submucosal fibrosis in OSMF patients ultrasonographically and to correlate these findings with the clinical stage and histological grade of OSMF.

**Materials and Methods**

After obtaining clearance from the Institutional Ethical Committee, the study was conducted in the Department of Oral Medicine and Radiology. Forty patients (divided into two groups of twenty each) visiting the outpatient department were included as participants in this study. Among these, twenty patients who were clinically diagnosed with OSMF and consented for biopsy constituted the study group and twenty patients who were healthy with age, gender, and body mass index matched without any habit or intraoral lesions constituted the control group.

Clinical examination was done, and mouth opening was measured using a digital vernier caliper scale from the incisal edge of maxillary central incisor to the incisal edge of mandibular central incisor and recorded in millimeters. Accordingly, the patients were classified in different clinical stages according to Khanna and Andrade\(^4\) as follows:

**Group I: Very early cases**

Common symptom is burning sensation in the mouth. Acute ulceration and recurrent stomatitis not associated with mouth opening limitation.

**Group II: Early cases**

Buccal mucosa appears mottled and marble like. Widespread sheets of fibrosis palpable. Patients with an interincisal distance of 26–35 mm.

**Group III: Moderately advanced cases**

Trismus evident with an interincisal distance of 15–25 mm. Buccal mucosa appears pale and firmly attached to underlying tissues. Atrophy of vermilion border. Vertical fibrous bands palpable at the soft palate, pterygomandibular raphe, and anterior faucial pillars.

**Group IVA: Advanced cases**

Trismus is severe with an interincisal distance of <15 mm. The fauces is thickened, shortened, and firm to palpation. Uvula is shrunken and appears as small, fibrous bud. Tongue movements are limited. On palpation of lips, circular band felt around entire mouth.

**Group IVB: Advanced cases with premalignant and malignant changes**

Hyperkeratosis, leukoplakia, or squamous cell carcinoma can be seen.

After this, USG measurements of submucosal thickness were performed for forty participants comprising twenty each of OSMF patients (study group) and controls. Scanning was performed with the patient in supine position. Transcutaneous imaging was done using Philips iU22 with multifrequency linear transducer with a frequency ranging from 3 to 12 MHz which was connected to the scanner. A real-time imaging of buccal mucosa and labial mucosae was performed. For the purpose of standardization of transcutaneous imaging of buccal mucosa, an imaginary line was drawn joining the corner of the mouth and the inferior point of the tragus of the ear. The patient was asked to clench the teeth, and anterior border of masseter muscle was palpated which depicted the boundary between the posterior buccal mucosa (PBM) and the anterior buccal mucosa (ABM) [Figure 1]. Imaging was done from an extraoral approach by placing the transducer parallel to the lower border of mandible using the blown cheek method (cheek signature) as given by Bharat et al. [Figure 2].\(^5\)

For imaging upper labial mucosa (ULM) and lower labial mucosa (LLM), the transducer probe was placed in the mid-region of the philtrum and mentalis region, respectively. Since it is difficult to contour the rigid transducer probe onto the flexible upper and lower lip, patients were prior instructed to indicate the mucosa by placing the forefinger inside the mouth against the mucosa to delineate the lining mucosa and empty space of the oral cavity as stated by Wilson et al. [Figure 3].\(^6\)

The real-time imaging of submucosa of buccal and labial mucosae was carried out, and multiple measurements were made, and an average of the readings was considered [Figures 4 and 5].

Punch biopsy was performed after the procedure of USG. The specimens were preserved in 10% formalin and sent for further laboratory procedures.
Histological grading was done according to Pindborg and Sirsat\textsuperscript{[7]} as follows:

**Very early stage**

It is characterized by finely fibrillar collagen, dispersed marked edema, and strong fibroblastic response with plump young fibroblast abundant in cytoplasm. The blood vessels are sometimes normal, but more often they are dilated and congested. Inflammatory cells, mainly polymorphonuclear leukocytes with an occasional eosinophil are present.

**Early stage**

The juxta-epithelial area shows early hyalinization. The collagen is still seen as separate bundles, which are thickened. Plump young fibroblasts are present in moderate numbers. The blood vessels are often dilated and congested. The inflammatory cells are mononuclear lymphocytes, eosinophils, and occasional plasma cells.

**Moderately advanced stage**

The collagen is moderately hyalinized, the amorphous change starting from the juxta-epithelial basement membrane. Occasionally, thickened collagen bundles are still seen separated by slight residual edema. The fibroblastic response is less marked; the cells present being mostly adult fibrocytes with elongated spindle-shaped nuclei and scanty cytoplasm. Blood vessels are either normal or constricted as a result of increased surrounding fibrous tissue. The inflammatory exudate consists of lymphocytes and plasma cells and occasional eosinophils are seen.

**Advanced stage**

The collagen is completely hyalinized and is seen as a smooth sheet, with no separate bundles discernible. Edema is absent. The hyalinized areas are devoid of fibroblasts although a thin, elongated cell or vestigial nucleus is seen at rare intervals along the fiber bundle. Blood vessels are completely obliterated or narrowed. The inflammatory cells observed are lymphocytes and plasma cells.

The entire data entered in the case pro forma approved by the committee were tabulated, and statistical analysis was performed.

**Statistical analysis**

Correlation of USG measurements between the study and control group was analyzed using Mann–Whitney test. Clinical and histopathological correlation with USG measurements was analyzed using Spearman’s rank correlation.

**Results**

**Age and gender distribution**

In the total study population enrolled (20), two were females and 18 were males showing a male predominance.
of OSMF. Out of these, maximum were in the age range of 21–30 years, i.e., 45%. This was followed by 40% in the age range of 31–40 years. Their mean age (standard deviation) was 33.6 (8.9), minimum age was 23 and maximum was 59 years of age. Thus, in the present study, the prevalence of OSMF was more in the third and fourth decades (i.e., 21–40 years).

Clinical stage distribution

Out of twenty, three participants (15%) had stage I, six (30%) had stage II, seven (35%) had stage III, and four (20%) had stage IV of the clinical stage of Khanna et al. classification of OSMF.

Comparison of ultrasonographic measurements in study and control group

The study showed high statistically significant difference between the submucosal thickness of study and control group ($P < 0.001$, by Mann–Whitney test) [Table 1 and Graph 1].

Correlation of clinical stage and ultrasonographic measurements

Table 2 and Graph 2 show the mean USG measurements in different clinical stages of OSMF. It is observed that, as the clinical stage increases, the thickness of ABM, PBM, ULM, and LLM also increases.

Table 3 shows the statistical correlation between the clinical stage and the USG measurements. We observed the following:

1. Higher ABM related to higher clinical stage (Spearman’s rank correlation coefficient was 0.860, $P = 0.000$ and <0.001) means highly significant direct association

2. Higher PBM related to higher clinical stage (Spearman’s rank correlation coefficient was 0.960, $P = 0.000$ and <0.001) means highly significant direct association

3. Higher ULM related to higher clinical stage (Spearman’s rank correlation coefficient was 0.881, $P = 0.000$ and <0.001) means highly significant direct association

4. Higher LLM related to higher clinical stage (Spearman’s rank correlation coefficient was 0.841, $P = 0.000$ and <0.001) means highly significant direct association.

Correlation of histopathological grade with ultrasonographic measurements

Table 4 shows the statistical correlation between the histological grade and the USG measurements. We observed the following:

1. Higher ABM related to higher histological stage (Spearman’s rank correlation coefficient was 0.633, $P = 0.001$ and <0.01) means highly significant direct association

2. Higher PBM related to higher histological stage (Spearman’s rank correlation coefficient was 0.786,
P = 0.000 and <0.001) means highly significant direct association

3. Higher ULM related to higher histological stage (Spearman’s rank correlation coefficient was 0.684, P = 0.000 and <0.001) means highly significant direct association

4. Higher LLM related to higher histological stage (Spearman’s rank correlation coefficient was 0.528, P = 0.017 and <0.05) means significant direct association.

Discussion

USG of all the twenty study cases showed a significant increase in the submucosal thickness as compared to the controls. These results are in accordance with the studies conducted by Rangaiah et al.,[3] Devathambi and Aswath,[8] and Tiwari et al.[9] which showed statistically significant increased submucosal thickness of ABM, PBM, ULM, and LLM in cases as compared to control group.

Oral submucous fibrosis is a disease characterized by increased fibrosis in the lamina propria and the submucosa. This increase in fibrosis is seen ultrasonographically as an increase in the thickness of the submucosal layer. Hence, there was increased thickness of ABM, PBM, ULM, and LLM. From this, it can be concluded that OSMF causes a significant increase in the thickness of the submucosa which can be clearly evaluated ultrasonographically.

In all the twenty study cases, it was seen that, as the severity of the disease increased clinically, the thickness of the submucosa increased ultrasonographically. This is in accordance with the study done by Devathambi and Aswath,[8] where they found a statistically significant correlation between the severity of the disease and the submucosal thickness. In the study done by Rangaiah et al.,[3] though they observed the increase in submucosal thickness with the increase in severity of the disease, no statistically significant correlation was established.

All the study cases also showed a direct correlation with the histopathological grade and the submucosal thickness ultrasonographically. In contrast, in the study by Rangaiah et al.,[3] though an increase in submucosal thickness was present from histological Grades II–III in ABM and PBM and a slight increase in submucosal thickness was present from Grades I–III; the results were statistically insignificant. This is first of a kind study which showed statistically significant correlation.

Conclusion

The present study is one of its kinds where USG is used as a diagnostic tool in OSMF. We were able to establish the normal values of submucosal thickness ultrasonographically for the small subset of Indian population. The study showed a clear association of increase in submucosal thickness in OSMF patients with good significance when compared to controls and also a statistically significant association with clinical stage and histological grade of OSMF.

However, it is said that “every coin has two sides.” i.e., along with the positive findings, there are a few limitations. A small sample size including diseased and healthy would have given more scope for better comparison and association with precision of results. An equal number of patients in each stage of OSMF would have given enhanced data regarding submucosal thickness reliability. Application of intraoral transducers for measurements of the submucosa might have yielded values which are more accurate and reliable.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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