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Ultrasonographic Evaluation of the thickness of the submucosa in Patients with Oral Submucous Fibrosis: A Descriptive Study

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Abstract

Introduction

Histopathology is the gold standard to diagnose OSF; however, it is a traumatic process with a long wait for the final diagnosis. Because OSF is a diffuse disease, a tissue sample taken from a single location may not represent the disease's true extent or severity. Ultrasonography has been used to examine soft tissues surrounding the oral cavity for the past few years, which allows for follow-up possibilities. This study aimed to use ultrasonography to assess submucosal thickness in OSF patients and compare it to normal mucosa.

Methods

The participants were split into two groups: cases and controls. The cases were 63 patients who were clinically diagnosed with OSF. In comparison, the controls were 63 patients who were randomly recruited from the outpatient clinic in the Department of Oral Medicine and Radiology. Based on the clinical findings, OSF patients were staged into four according to the criteria given by Khanna and Andrade. The submucosa was imaged using a high-resolution real-time LOGIQUE C5 ultrasound scanner with 7 to 12 MHz transducers.

Results

Results were analyzed by t-test and one-way ANOVA test.Submucosal hypertrophy was found to be associated with OSF in the study. The thickness of the submucosa measured by ultrasound in controls was statistically significant. As the duration and frequency of the habit increase and the disease progress, submucosal thickness increases in the case group.

Conclusions

USG provides non-invasive confirmation of connective tissue fibrosis with the advantage of possible repeated studies during treatment and post-treatment periods.

Keywords: *Ultrasonography (USG); Oral Submucous Fibrosis (OSF); Submucosa; Thickness; Submucosal Hypertrophy*

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I. Introduction

Oral submucous fibrosis (OSF), a chronic, insidious disease characterized by progressive submucosal fibrosis of the oral cavity and the oropharynx, is within a group of conditions that are classified under oral potentially malignant disorders.[1] It has been reported exclusively in the Indian continent and south Asians and migrants from these countries to the USA and Europe.[2, 3]

OSF has a definite potential for malignant transformation, calculated to be 4.84 % in a recent study.[4] The exact etiology of OSF is still uncertain, though many factors have been considered causative agents. However, more recent studies have confirmed areca nut as the primary (and the only) risk factor of OSF among people who probably have a genetic predisposition to the disease.[5, 6]

Previously, computed tomography (CT) or magnetic resonance imaging (MRI) was used to visualize muscle area and structural changes, including masticatory muscles (MRI).[7–9] Compared to these methods, ultrasound imaging is rapid, easily accessible, and relatively inexpensive. Ultrasonography has been used to examine soft tissues surrounding the oral cavity for the past few years, which allows for follow-up possibilities.[10, 11] It also enables the visualization of the fine detail of the superficial structures of the oral and maxillofacial tissues without the use of ionizing radiation.

However, there is a lack of studies relating to ultrasonography of masseter muscle and submucosa in OSF patients in the literature.[12–17] Hence, this study evaluated submucosal thickness in OSF patients using ultrasonography and compared it with normal mucosa. Also, to compare the cross-sectional thickness of submucosa in patients with different clinical stages & histologic grades of OSF.

II. Materials And Methods

This Descriptive study was conducted in the Department of Oral Medicine and Radiology.After describing the method and obtaining informed consent, 126 participants were included in the research. The participants were split into two groups: cases and controls. The cases were 63 patients who had been clinically diagnosed with OSF. At the same time, the controls were 63 people who were randomly chosen from the outpatient clinic in the Department of Oral Medicine and Radiology for routine dental treatment. The study participants were chosen randomly, without regard for their age, gender, or other factors. Before beginning the study, permission was obtained from the Institutional Ethical Committee. Patients who were rejected to participate in the research or who had severe systemic disorders were omitted.

To arrive at a clinical diagnosis of OSF, a comprehensive medical history and physical examination were performed. Patients exhibiting symptoms such as spicy food intolerance, burning sensation of the mouth, and progressive difficulty in opening mouth and tongue movements were taken up for a thorough examination. A proforma was used to record all of the patients' information.

According to the criteria given by Khanna and Andrade, the OSF patients were classified into four stages.[18] The four stages were,

Stage I (Very early cases) - Normal mouth opening, burning sensation, excessive salivation, acute ulceration, and recurrent stomatitis.

Stage II (Early cases) – Interincisal width during mouth opening: 26-35 mm, primarily affected areas are soft palate and faucial pillars, buccal mucosa appears mottled and marbled, with dense, pale, depigmented, and fibrosed areas alternating with normal pink mucosa, red erythematous patches, and widespread sheets of fibrosis.

Stage III (Moderately advanced cases) - Interincisal width during mouth opening: 15-25 mm, trismus, palpable vertical fibrous bands with a firm attachment to underlying tissue, fibrous bands in the soft palate appears to radiate from the anterior faucial pillar or pterygomandibular raphe in a scar-like appearance, atrophy of vermillion border of the lips, patient unable to puff out the cheeks or whistle, reduced mouth opening, and unilateral posterior cheek involvement of the soft palate and faucial pillar.

Stage IVa (Advanced cases) - Stiffness/inelasticity of the oral mucosa, mouth opening: 2-15 mm (interincisal opening), trismus, uvula seen to be involved as a shrunken, small, and fibrous bud, fauces thickened, shortened, and firm on palpation, tongue movement restricted, papillary atrophy (diffuse), the lips-circular band felt around the entire mouth, the intraoral examination is difficult.

Stage IVb (Advanced cases) - With premalignant and malignant changes, OSMF and leukoplakia, OSMF, and squamous cell carcinoma.

To confirm the diagnosis, all OSMF patients had an incision biopsy. In the control group, no biopsies were performed.

2.1 Ultrasonographic examination

With the patient in the supine posture, ultrasonographic imaging was conducted. The imaging was done using a LOGIQUE C5 high-resolution real-time ultrasound scanner with 7-12MHz transducers. To measure interobserver agreement, the investigator and sonologist independently analyzed the ultrasonographic images.

Participants were taught to indicate the mucosa by putting the fingers on the lining mucosa to define the oral cavity space before beginning USG for measuring the submucosal thickness in the buccal mucosa. To obtain precise measurements of the thickness of the submucosa, the transducer probe was brought softly into contact with the surface because excess contact pressure while imaging might affect the measurements.

An imaginary line was created between two sites for ultrasonographic imaging of the buccal mucosa. The posterior buccal mucosa was indicated by the first point, which was 1 cm anterior to the anterior border of the masseter muscle (PBM). The second point was 1 cm posterior to the oral cavity commissure, denoting the anterior buccal mucosa (ABM) (Figure 1A). Ultrasonographic imaging was performed with an extraoral approach by placing the linear transducer parallel to the lower border of the mandible (Figure 1B). The submucosal thickness was measured along the imaginary line on both the right and left sides. The USG scan fully represented the cross-section of the buccal mucosa in the muscular and submucosal planes. The submucosa was seen as a hypoechoic band supported by muscle planes and mucosal lining as a hyperechoic line. This band of hypo-echogenicity between the hyperechoic mucosa and muscle layer was measured as the submucosa thickness (Figure 2 & 3). Both anterior and posterior submucosal thickness was measured with the help of the measuring tool in USG.

2.2 Statistical analysis

The data were analyzed with IBM's Statistical Package for Social Science (SPSS®) version 23 statistical software. 'Independent sample T-test' was used to assess the difference in ultrasonographic thickness between individual study groups and males and females. 'Paired sample T-test' was used to check whether there were significant differences in values between left and right sides and between anterior and posterior buccal mucosa. Submucosal thickness in different clinical stages of OSF was compared with the control group by applying the One-way ANOVA test. A 'p' value of 0.05 or less was considered statistically significant.

Interobserver agreement on the different ultrasonographic thicknesses was analyzed using linear regression plot analysis. The Bland-Altman analysis confirmed the lack of any significant bias.

III. Results

A total of 126 people were involved in the study. The participants were split into two groups: cases and controls. The cases were 63 patients who had been clinically diagnosed with OSF. At the same time, the controls were 63 people who were randomly chosen from the outpatient clinic in the Department of Oral Medicine and Radiology for routine dental treatment.

The age of subjects in the case group in this study ranged from 27 to 73 years, with a mean age of 48.74±9.14 years. The age of subjects in the control group ranged from 26 to 71 years with a mean age of 49.05±10.05 years.

According to the criteria given by Khanna and Andrade, the OSF patients were classified into four stages.[18] The most prevalent stage was the moderate stage (stage III) with 29 patients. Three patients were in very early stage (stage I) and 11 in early-stage (stage II). The remaining 20 patients were in advanced stage (stage IV); among them, 7 had coexisting carcinoma (stage IVb).

3.1 ANALYSIS OF ULTRASONOGRAPHIC IMAGING

Ultrasonographic imaging was carried out on both right and left submucosa of all subjects included in the study, using a high-resolutionreal-time LOGIQUE C5 ultrasound scanner with 7-12MHz transducers. The thickness of both submucosae and for anterior and posterior buccal mucosa was recorded separately. The ultrasonographic findings of each group are described in the following sections.

3.1.1 The thickness of submucosa in the control group

The thickness of both anterior and posterior submucosa was more for males when compared to females. The mean thickness of the anterior submucosa was recorded as 0.0552±0.0087 for males and 0.0463±0.0092 for females. The mean thickness of the posterior submucosa was recorded as 0.0561±0.0081 for males and 0.0473±0.0091 for females (Table 1). No significant difference was noted for the thickness of both anterior and posterior submucosa on the right and left sides for both males and females

3.1.2 The thickness of submucosa in the case group

A statistically significant correlation was noted between ultrasonographic readings of anterior and posterior submucosal thickness and clinical stages of OSF. The thickness of both anterior and posterior submucosa was more for males when compared to females among each clinical stage of OSF (Table 2). No significant difference was noted for both anterior and posterior submucosa thickness on the right and left sides. A significant difference in mean thickness of both anterior and posterior submucosa was noticed between the different clinical stages of OSF (Table 3).

A statistically significant difference was noted among patients using different chewing products and the thickness of the anterior and posterior submucosa. A significant statistical correlation was detected between the

duration of chewing, the frequency of chewing, and retention of the quid in the mouth with the thickness of both the anterior and posterior submucosa (Figure 4)**.**

3.1.3 Comparison of mean thickness of anterior and posterior submucosa

A significant difference in mean thickness of anterior and posterior submucosa was noticed in different stages of OSF (Table 4).

3.1.4 Interobserver Variability

Linear regression plot analysis expressing various thicknesses measured by both observers for the whole population was highly significant, and the strength of the correlation was excellent (P<0.0001) (Figure 5). The Bland-Altman analysis confirmed the lack of any significant bias. The mean difference in thickness of anterior submucosa was -0.002 cm, with 95% of values ranged from 0.011 to -0.015 cm. The mean difference in thickness of posterior submucosa was -0.002 cm, with 95% of values ranged from 0.011 to -0.016 cm. (Figure 6). There appeared to be a substantial agreement between the two observers in evaluating the thickness of submucosa using ultrasonography.

IV. Discussion

Patients who visited Oral Medicine and Radiology department were screened. The research included a total of 126 participants. After taking a medical history and obtaining informed consent, 63 patients with symptoms suggestive of OSF were taken up for ultrasonographic investigation. As controls, an equal number of people were chosen from the Oral Medicine and Radiology outpatient clinic. They were matched for age and sex.

The mean thickness of anterior submucosa was recorded as 0.0552±0.0087 cm for males and 0.0463±0.0092 cm for females, and mean thickness of posterior submucosa was recorded as 0.0561±0.0081 cm for males and 0.0473±0.0091 cm for females, in normal subjects. These are in accordance with the values obtained by Devathambi&Aswath (0.045 - 0.056 cm) and Rangaiah *et al.* (0.056±0.011 cm).[12, 14] There was no significant difference in mean thickness of anterior and posterior submucosa among controls (P>0.05). Devathambi&Aswath and Rangaiah *et al.* also noticed no difference in mean thickness of anterior and posterior submucosa among controls.[12, 14]

It was observed that a highly significant difference in both anterior and posterior submucosal thickness between different clinical stages of OSF and controls. As the clinical stages of OSF advance, the thickness of both anterior and posterior submucosa increased. These results were in agreement with the observations made by Rangaiah *et al.* &Devathambi&Aswath.[12, 14]Also, the thickness of anterior and posterior submucosa was more for males when compared to females among each clinical stage of OSF. A statistically significant difference was noted among patients using different chewing products and the thickness of anterior and posterior submucosa. Those patients who had a habit of chewing commercial preparation had a higher submucosal thickness than those using conventional preparation. This could be due to the submucosal changes brought about by more areca nut concentrations in commercial preparation.[19]

A significant correlation was observed between the duration of chewing, the frequency of chewing, and retention of the quid in the mouth with the thickness of both anterior and posterior submucosa. It implies that as the duration of chewing, the frequency of chewing, and retention of the quid in the mouth increased, disease severity also increased. This was in accordance with the study by Shah & Sharma and Jacob *et al*.[20, 21], ButAccording to Maher *et al.* and Rangaiah *et al.*, daily usage appeared to be most important than the duration of the habit.[14, 22]

Also noticed a significant difference between the mean thickness of anterior and posterior submucosa in different clinical stages of OSF. This was consistent with the explanations made by Devathambi and Aswath.[12] This increase in thickness of the posterior submucosa compared to the anterior submucosa could be due to the increased tendency of people to keep the tobacco or betel nut in the region of the buccal pouch.

The study showed a clearassociation of submucosal hypertrophy with OSF.The normal values of ultrasonographically measured submucosal thickness for a small subset of Kerala's population were established in this study.

V. Conclusion

The prognosis of OSF during treatment can be assessed using USG non-invasively. Histopathological assessment of OSF patients provides confirmatory evidence of fibrosis in the connective tissue. In contrast, USG provides the same information non-invasively with the advantage of possible repeated studies during treatment and post-treatment periods. Also, USG takes less time studying a larger area, whereas the former takes longer to study a limited area. So, USG can be considered a valuable, radiation-free, and non-invasive better diagnostic tool than clinical and histopathological examinations for OSF evaluation.Though, further studies with a larger sample size are needed to develop an accurate algorithm for submucosal thickness to differentiate between different stages of OSF and for the early detection of malignant changes in OSF.

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TABLES

Table 1: Mean values of thickness of submucosa (cm) in controls

	Male (N=33)		Female (N=30)		T-test	
USG measurements	Mean	SD	Mean	SD	t value	Significance
ABM (Right)	0.0558	0.0094	0.0453	0.0086	4.586	<0.001
ABM (Left)	0.0545	0.0087	0.0473	0.0104	3.023	0.004
ABM (Mean)	0.0552	0.0087	0.0463	0.0092	3.912	<0.001
PBM (Right)	0.0567	0.0096	0.0463	0.0089	4.424	<0.001
PBM (Left)	0.0555	0.0079	0.0483	0.0101	3.15	0.003
PBM (Mean)	0.0561	0.0081	0.0473	0.0091	3.961	<0.001

USG: Ultrasonography, SD: Standard Deviation, ABM: Anterior Buccal Mucosa, PBM: Posterior Buccal Mucosa

Table 1

Table 2: Thickness of submucosa (cm) among cases

USG Measurements	Control Mean ± SD	Stage I Mean ± SD	Stage II Mean ± SD	Stage III Mean ± SD	Stage IVa Mean ± SD	Stage IVb Mean ± SD	ANOVA F	Significance
ABM (Male)	0.0552±0.0087	0.0800±0.0141	0.1417±0.0153	0.1729±0.0211	0.2550±0.0000	0.2430±0.0045	359.1262	<0.001
PBM (Male)	0.0680±0.0095	0.1000±0.0212	0.1733±0.0161	0.2152±0.0230	0.2863±0.0063	0.2800±0.0050	402.7821	<0.001
ABM (Female)	0.0463±0.0092	0.0700	0.1369±0.0131	0.1475±0.0071	0.2172±0.0118	0.2250±0.0141	528.2487	<0.001
PBM (Female)	0.0582±0.0097	0.0800	0.1669±0.0100	0.1844±0.0118	0.2494	0.2650±0.0095	724.6122	<0.001

USG: Ultrasonography, SD: Standard Deviation, ANOVA: Analysis of Variance, ABM: Anterior Buccal Mucosa, PBM: Posterior Buccal Mucosa

Table 2

Table 3: Comparison of mean thickness of submucosa between the different stages of OSF

Mean Difference between	Mean Difference	Standard Error	T-test t value	T-test Significance
Stage I & II (ABM)	-0.0615	0.0084	-7.352	<0.001
Stage II & III (ABM)	-0.0277	0.0070	-3.972	<0.001
Stage III &IVa (ABM)	-0.0630	0.0071	-8.880	<0.001
Stage IVa&IVb (ABM)	-0.0090	0.0084	-1.071	0.298
Stage I & II (PBM)	-0.0753	0.0085	-8.908	<0.001
Stage II & III (PBM)	-0.0381	0.0078	-4.892	<0.001
Stage III &IVa (PBM)	-0.0540	0.0078	-6.959	<0.001
Stage IVa&IVb (PBM)	-0.0149	0.0078	-1.910	0.072

USG: Ultrasonography, OSF: Oral Submucous Fibrosis, ABM: Anterior Buccal Mucosa, PBM: Posterior Buccal Mucosa

Table 3

Table 4: Comparison of mean thickness of anterior and posterior submucosa

Group	N	Paired Differences Mean	Paired Differences SEM	T-test t value	T-test Significance
Control	63	-0.0005	0.0002	-1.938	0.057
Stage I	3	-0.0167	0.0044	-3.780	0.063
Stage II	11	-0.0305	0.0022	-13.970	0.000
Stage III	29	-0.0409	0.0017	-23.751	0.000
Stage Iva	13	-0.0319	0.0014	-22.052	0.000
Stage IVb	7	-0.0379	0.0026	-14.337	0.000

SEM: Standard Error Mean

Table 4

FIGURES

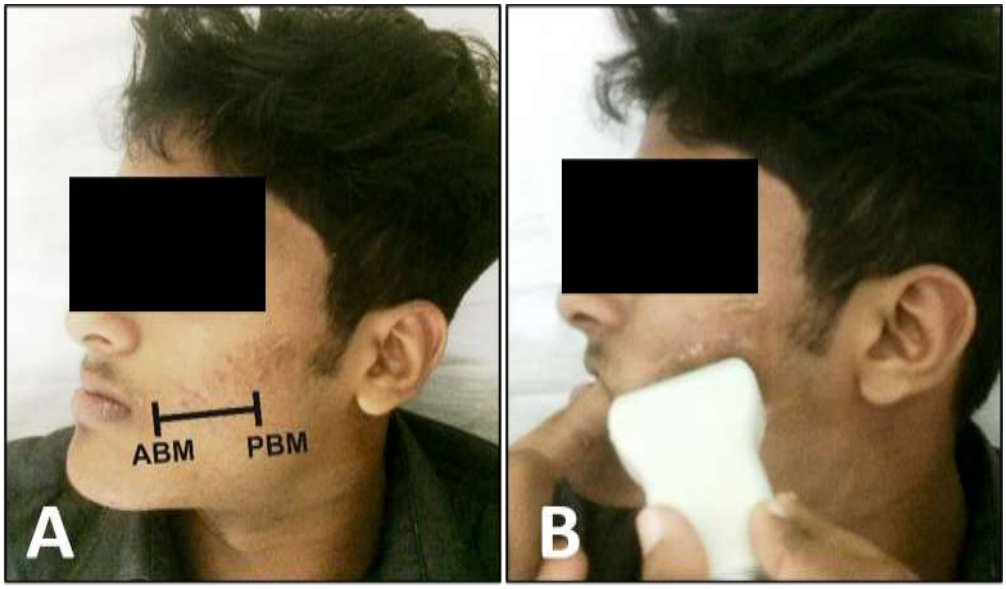

Figure 1: A) Patient's face showing anterior and posterior points for measuring the submucosal thickness in the buccal mucosa region by ultrasonogram. Figure 1B) Photograph showing the placement position of the linear transducer for measuring the submucosal thickness by ultrasonogram.

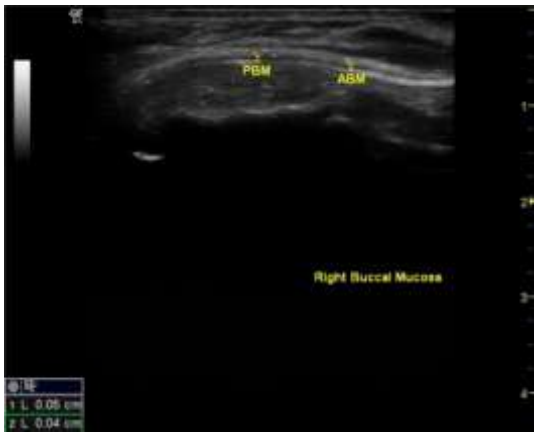

Figure 2: Ultrasound image of the buccal mucosa in a control patient showing the submucosal thickness.

Figure 3: Ultrasound image of the buccal mucosa in a patient with OSF showing the submucosal thickness.

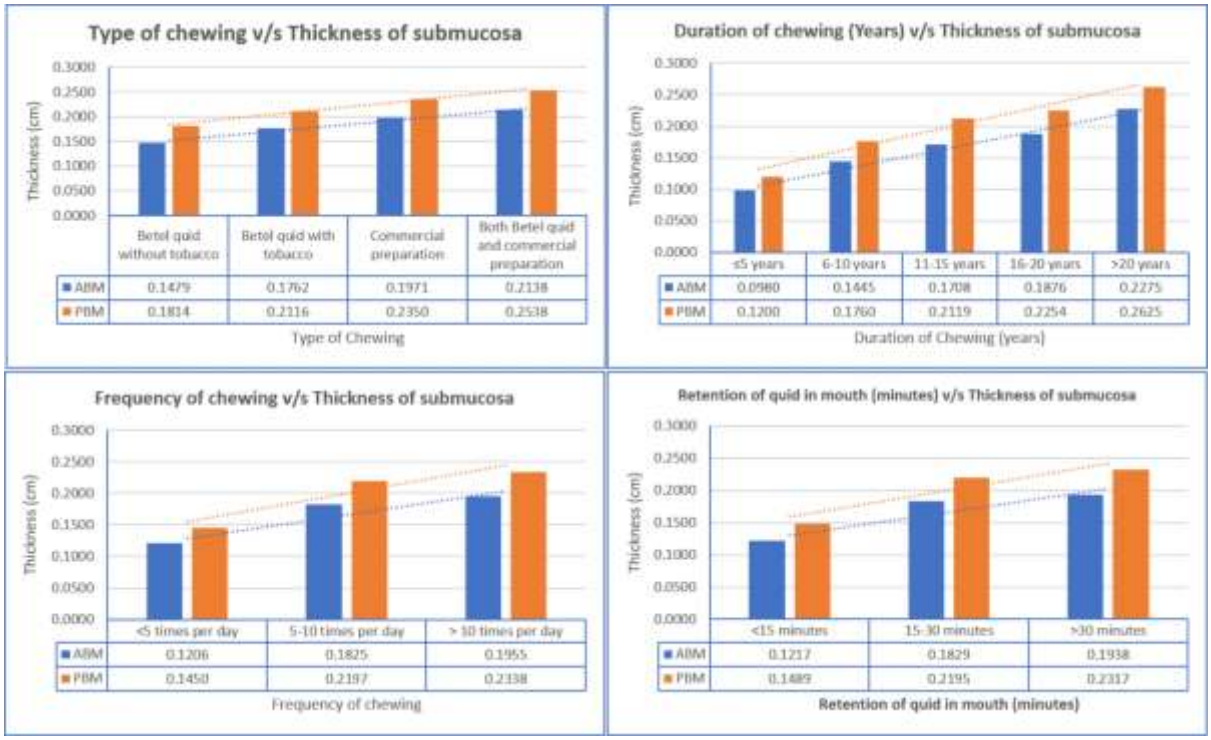

Figure 4: the relationship between submucosal thickness and type of chewing, duration of chewing, frequency of chewing, and retention of quid in the mouth.

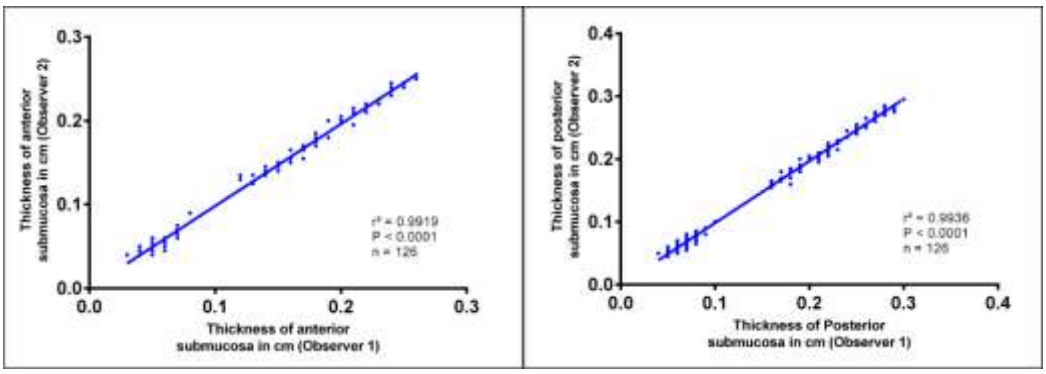

Figure 5: Linear regression plotting expressing various thicknesses obtained by both observers. *Solid lines* represent linear regression, and *dots* represent obtained values.

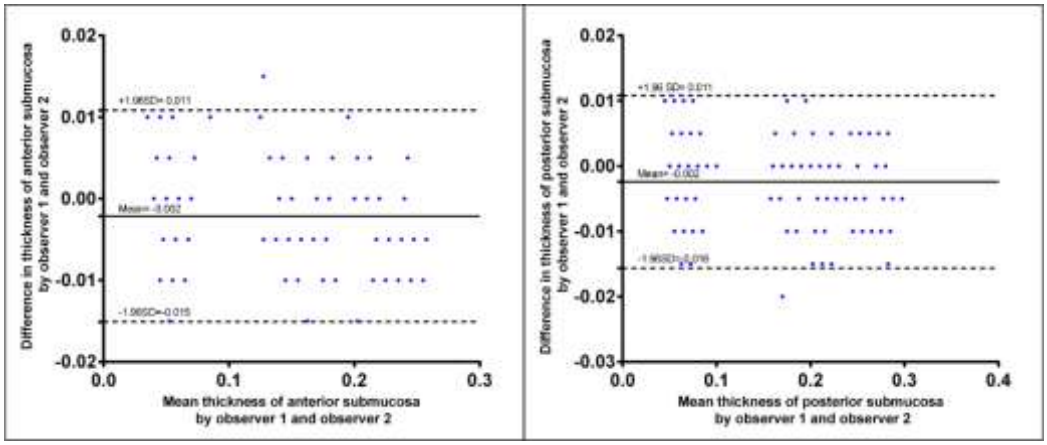

Figure.6: Bland-Altman graphs comparing the difference in thicknesses obtained by both observers. *Solid lines* represent average differences, and *dashed lines* represent 95% limits of agreement.