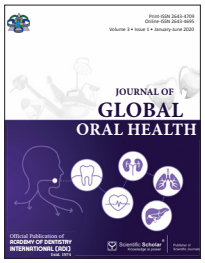
www.jglobaloralhealth.org

Journal of Global Oral Health



Research Article

Morphometric analysis of epithelium in oral submucous fibrosis using open-source software

Arthi Prakasam[1], Preetha Selvan[1], Kannan Ranganathan[1]

[1]Department of Oral Pathology, Ragas Dental College and Hospital, Tamil Nadu Dr. MGR Medical University, Chennai, Tamil Nadu, India.

***Corresponding author:**
Arthi Prakasam,
Department of Oral Pathology,
Ragas Dental College, Chennai,
Tamil Nadu, India.

arthiprakasam67@gmail.com

Received: 14 August 2024
Accepted: 14 October 2024
EPub Ahead of Print: 17 December 2024
Published: 26 December 2024

DOI
10.25259/JGOH_27_2024

Quick Response Code:

ABSTRACT

Background: Oral submucous fibrosis (OSMF) is a chronic oral mucosal disease characterized by progressive deposition of collagen in subepithelial connective tissue and epithelial atrophy. ImageJ is open-source software that aids researchers to visualize, inspect, quantify, and validate scientific image data. **Objectives:** To measure and compare the changes in epithelial thickness and cell morphology in OSMF in comparison with normal mucosa using computer-aided image analysis software. **Materials and Methods:** 15 OSMF and 10 cases of normal buccal mucosa were analyzed. Epithelial thickness and cell morphology of spinous cells of OSMF cases were quantified by ImageJ software and compared with that of normal tissue. **Results:** An increase in the cellular area of the spinous cells in OSMF compared to that of the normal tissue was observed. **Conclusion:** ImageJ represents a powerful tool that enhances the precision and efficiency of epithelial analysis in OSMF research compared to historical methods.

Keywords: Oral submucous fibrosis, ImageJ, Morphometric analysis, Cell morphology, Epithelial thickness

INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic oral mucosal disease characterized by progressive deposition of collagen in subepithelial connective tissue and epithelial atrophy. More and Rao defined OSMF as a debilitating, progressive, irreversible collagen metabolic disorder induced by chronic chewing of areca nut and its commercial preparations; affecting the oral mucosa and occasionally the pharynx and esophagus; leading to mucosal stiffness and functional morbidity with a potential risk of malignant transformation.[1] Nayanar *et al*., reported a malignant transformation rate between 1.9-12%. In India, the prevalence of OSMF is highest and in South East Asia, it is 0.62–6.42%.[2] OSMF is linked to the use of areca nut products. Arecoline, an alkaloid present in areca nut, is believed to stimulate excessive collagen production, contributing to the development of OSMF. Betel quid with tobacco usage was higher in females at about 23.5%. OSMF associated with the use of pan masala has the highest odds ratio (OR) of 81.5, followed by areca nut with alcohol use of 69.9.[3]

Pindborg and Sirsat classified OSMF from a histopathological perspective, dividing the condition into four stages based on the observed tissue features. The stages included are very early stage, early stage, moderately advanced stage, and advanced stage, with each stage reflecting different levels of progression and severity of the disease.[4] The dual classification systems provide a comprehensive framework for understanding and categorizing OSMF, incorporating both clinical manifestations related to mouth opening and histopathological changes for a more nuanced

characterization of the condition. In the study conducted by Ranganathan *et al.*,[3] OSMF was categorized into four distinct groups according to the degree of mouth opening. Group I encompassed cases where individuals exhibited only symptoms without any restriction in mouth opening. Group II included those with limited mouth opening of 20 mm and above. Group III comprised individuals with mouth opening measuring <20 mm. Finally, Group IV represented advanced stages of OSMF, characterized by both limited mouth opening and the presence of precancerous changes throughout the mucosa.

In biological studies, parameters such as cell area and perimeter, as well as epithelial thickness, play crucial roles in characterizing and understanding various physiological and pathological processes. The cell area and perimeter are used to quantify the size and shape of individual cells. These measurements reflect cellular behaviors, such as migration, proliferation, and differentiation. Changes in cell area and perimeter can indicate alterations in cellular functions or responses to external stimuli.[5] Epithelial thickness is a vital parameter when examining tissues composed of surface epithelium. The thickness of the epithelial layer is a key determinant of its structural integrity and barrier function. Variations in epithelial thickness can be indicative of physiological changes and severity of pathological conditions.[6] Cell behavior, including malignant transformation, is dependent on changes in epithelial cell size and shape in OSMF. A major challenge to oral oncologists is to determine and identify the degree of tissue morphological alteration and to predict the exact transition of a normal tissue/oculopharyngeal muscular dystrophy to cancer.[7,8]

Various systems are currently accessible to an anatomical pathologist for nuclear morphometry image analysis. However, a significant limitation is that many of these systems necessitate expensive software and hardware attachments for image acquisition, analysis, and storage. Consequently, a cost-effective alternative for image analysis would be useful for pathologists and researchers alike. ImageJ is an open-source, Java-based image processing and analysis program software that aids researchers to visualize, inspect, quantify, and validate scientific image data. Image analysis allows users to extract information from images in a reproducible manner [Figure 1].[9] ImageJ, being publicly available, presents a practical option for nuclear morphometric assessments on stained sections, offering the flexibility to download specific plug-ins directly from the ImageJ website which is a definite advantage for better and easy diagnosis. The aim of the study was to measure and compare the changes in cell area and perimeter and epithelial thickness in OSMF in comparison with normal mucosa using ImageJ.

MATERIALS AND METHODS

A retrospective study was conducted in the Department of Oral and Maxillofacial Pathology and Microbiology, Ragas Dental College, Chennai, between September 2023 and November 2023. The study samples comprised 25 hematoxylin and eosin-stained soft tissue sections (n = 25) of normal oral mucosa and OSMF. The samples were categorized into two groups, namely Group I - 15 cases of OSMF (n = 15) and Group II - 10 cases of normal oral mucosa (n = 10). Hematoxylin and eosin-stained OSMF and normal mucosa slides were retrieved from archives of our department. Photographs of five random fields of interest were obtained from the hematoxylin/eosin-stained slides. Accurate calibration for (×40) magnification was done using a stage micrometer before measurements using ImageJ software [Figure 2]. Image analysis was done using ImageJ by measuring the region of interest. Images of normal mucosa and OSMF were morphometrically measured (in µm) at 3 points from epithelial surface to epithelial connective tissue interface using ImageJ software in 3 different fields [Figure 3]. The mean of the 3 measurements in 3 fields was taken as the thickness of the epithelium. Five random cells with clear outline from each of the five fields were traced in spinous cell layer of normal mucosa and OSMF, and thereby, cell area and cell perimeter were calculated by ImageJ software [Figure 4].

The following inclusion and exclusion criteria were considered for the selection of tissues for the study. Inclusion criteria were histopathologically confirmed cases of OSMF, availability of clear hematoxylin and eosin-stained slides, photographic representations of five random fields at ×40 magnification, and slides examined under a calibrated microscope with accurate calibration. Exclusion criteria were incomplete or poorly stained slides, no histopathological diagnoses of OSMF, presence of other oral pathologies or lesions, history of previous treatment for OSMF, insufficient information, or clinical data. The parameters used were cell area and perimeter and epithelial thickness. ImageJ analyzer (version 1.53) software was used.

Statistics

Paired t-test was used to analyze the correlation of epithelial thickness, cell area, cell perimeter, and normal mucosa using the Statistical Package for the Social Sciences (version 2.0).

RESULTS

The mean cell area of normal mucosa was 620.86 (standard deviation [SD] = ±117.74) and mean cell area of OSMF was 943.433 (SD = ±114.43). An increase in the cell area in OSMF is observed in comparison with normal mucosa.

The mean of cell perimeter of normal mucosa was 105.57 (SD = ±9.707) and mean cell perimeter of OSMF was 134.72 (SD

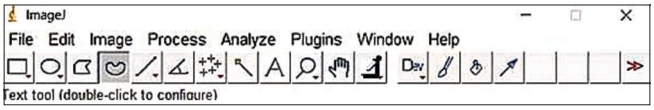


Figure 1: ImageJ software.

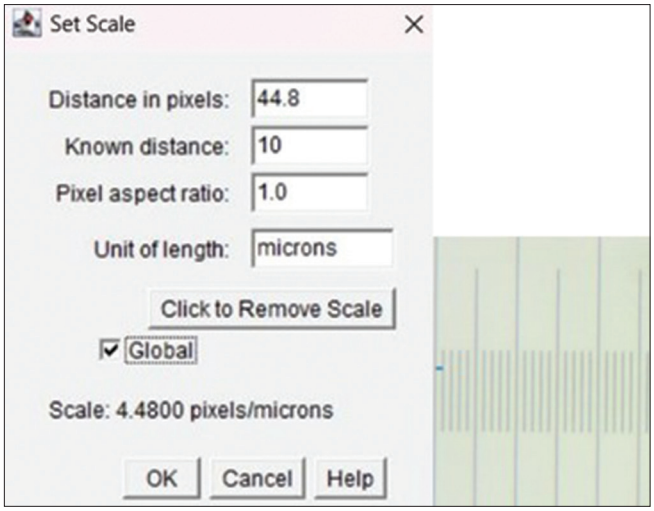


Figure 2: Set scale for (×40) magnification and calibration using set scale straight line selection tool.

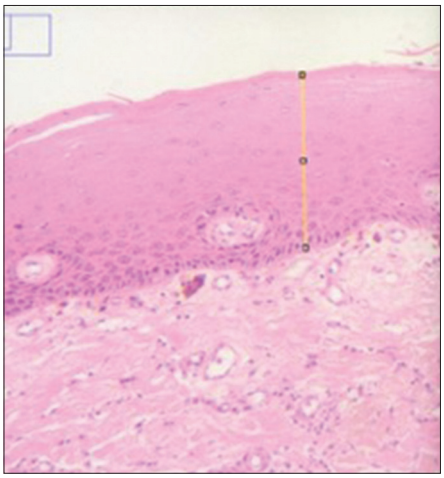


Figure 3: Measurement of epithelial thickness. 3 points from the epithelial surface to epithelial connective tissue interface are measured in 3 different fields.

= ±12.40). An increase in the cell perimeter in OSMF was observed in comparison with normal mucosa. The analysis shows that the correlation between cell area and perimeter was highly significant.

The mean thickness of epithelium of normal mucosa was 405.02 (SD = ±214.96) and mean thickness of epithelium OSMF was 134.72 (SD = ±12.40) [Table 1]. No significant increase in thickness of epithelium in OSMF was observed in comparison with normal mucosa [Graph 1].

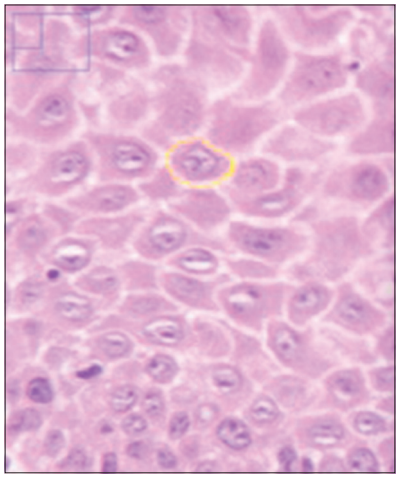


Figure 4: Measurement of cell area. Five random cells with clear outline from each of the five fields were traced in spinous cells and measured.

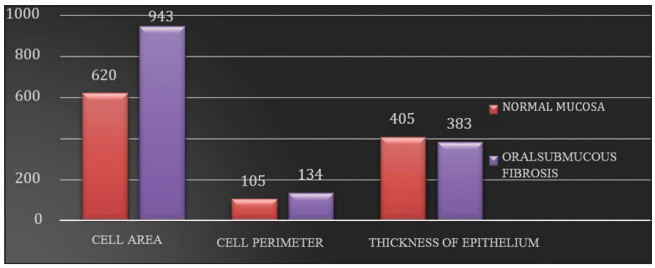
Table 1: Morphometric values of OSMF and normal mucosa

Parameter	*N*=25	Mean	Standard deviation (±)	*P*-value*
Cell area - normal mucosa	10	620.86	±117.74	*P*=0.00
Cell area -OSMF	15	943.43	±114.43	
Cell perimeter - normal mucosa	10	105.57	±9.77	*P*=0.00
Cell perimeter - OSMF	15	134.722	±12.40	
Thickness of epithelium - normal mucosa	10	405.02	±214.96	*P*=0.75
Thickness of epithelium - OSMF	15	383.23	±94.55	

*$P>0.05$, OSMF: Oral submucous fibrosis

DISCUSSION

Pathological alterations usually begin with fibrosis of lamina propria and epithelium responds to it in the form of atrophy (thinning of epithelium). In this study, morphometric analysis of epithelial thickness, cell perimeter, and cell area was done in OSMF compared to normal epithelium. Pindborg and Sirsat showed 91% of atrophic epithelium with cell layers as low as 3 – 4 in a study of 23 cases of OSMF. Pindborg and Sirsat studied 53 biopsies of OSMF and noted that 71.7% had atrophic epithelium, 26.4% had normal, and 1.9% had hyperplastic epithelium when compared to normal epithelium.[4] Gao *et al.*, studied the morphometry of spinous cell in 16 specimens of OSMF using Interactive Image Analysis System IBAS-II a progressive decrease of cell area reflected a malignant progress.[10]



Graph 1: Graphical representation of morphometric values of oral submucous fibrosis (OSMF) and normal mucosa.

In our study, the mean value of cell area and perimeter increased in OSMF when compared with normal. There is a significant increase in both cell area and perimeter within OSMF. Increase in cell dimensions (area, perimeter) in OSMF indicates OSMF in its early stage as compared to normal mucosa. This aligns with observations by Pindborg and Sirsat who also noted enlarged epithelial cells in the fibrotic condition.[4] Such cellular hypertrophy could be attributed to several mechanisms. Chronic cellular stress associated with the fibrotic process might trigger compensatory hypertrophic responses within epithelial cells, leading to increased size without corresponding proliferation. This stress can trigger cellular adaptations, including changes in cell morphology and size. In addition, the ongoing remodeling of the extracellular matrix due to fibrosis may directly influence cell shape and area.[11,12]

There was a significant increase in cell area and perimeter with cells being more oblong and flattened in contrast to the polygonal shape. Epithelium was considered atrophic based on a reduction in the number of cell layers and loss of rete ridges. The thickness of the epithelium was similar for both OSMF and normal mucosa ($P = 0.75$). Rathore *et al.*, stated a decrease in epithelial thickness with the increase in severity/stage of OSMF.[13] However, Kapoor *et al.*, did the morphometric analysis of epithelium thickness in OSMF patients and found that thickness did not consistently decrease with increasing grades of the disease.[14] This could be explained by the fact that the increased cell size in OSMF results in overall unchanged epithelial thickness, though cell numbers are reduced. This is due to alterations in cell proliferation and apoptosis. Studies have shown decreased proliferation and increased apoptosis in OSMF epithelium compared to normal mucosa.[15,16]

Since alteration in cellular morphology and altered tissue architecture presently contribute majorly to the determination and confirmation of pathological states, morphometric analysis using ImageJ, an open-source software, aids to validate scientific image data in a reproducible manner.

These results have shown that with the use of ImageJ, it is possible to evaluate change in cell morphology of the individual cells of specific or all epithelial thickness. Studying the epithelial thickness in different stages of OSMF can throw light on stage-specific changes in this parameter. Analyzing the stratification pattern of the epithelium in OSMF can reveal potential changes in the number of cell layers and increase in cell area. Investigating the mechanisms behind epithelial flattening due to submucosal fibrosis can also help in shedding light on the pressure-induced changes in cell morphology.

Deviations in cell area, perimeter, and thickness of epithelium in OSMF compared to normal epithelium are promising a morphometric feature for histopathological assessment of disease process. The use of quantitative analysis using ImageJ offers a valuable and objective criterion, thus improving the differentiation of this condition from lesions carrying a significant risk of malignant change.

Morphometric analysis of the epithelium in OSMF using ImageJ offers significant advantages over traditional histological techniques. This approach provides precise, quantitative data on epithelial thickness, cell size, and nuclear dimensions, enhancing the objectivity and reproducibility of measurements compared to subjective manual assessments. ImageJ's automated features streamline data processing, allowing for the analysis of large sample sizes with reduced human error and time investment. In addition, the software's capability to handle various image formats and its extensive plugins facilitate comprehensive morphological evaluations. This study demonstrates the potential of ImageJ software for quantitative morphometric analysis in pathology, which could make advanced image analysis more accessible to researchers and clinicians worldwide. However, the small sample size and retrospective design of this study suggest further research to confirm and expand upon these findings. The other limitations include the need for high-quality images and the potential for variability due to differences in image acquisition methods. Moreover, the interpretation of morphometric data still requires a certain level of expertise, and the software's accuracy depends on proper calibration and standardization of protocols. Despite these challenges, ImageJ represents a powerful tool that enhances the precision and efficiency of epithelial analysis in OSMF research compared to historical methods.

CONCLUSION

ImageJ can significantly contribute in understanding structural changes in epithelium, aiding in diagnostic, therapeutic and research application, particularly in fields like pathology, histology and tissue engineering.

Ethical approval

The research/study was approved by the Institutional Review Board/Institutional Ethics Committee at Ragas Dental College, Chennai number - RIEC/20240616/OP, dated 06/08/2024.

Declaration of patient consent

Patient's consent is not required as there are no patients in this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

REFERENCES

1. More CB, Rao NR. Proposed clinical definition for oral submucous fibrosis. J Oral Biol Craniofac Res 2019;9:311-4.
2. Nayanar G, Reddy MG, Kakodkar P, Dony EC. Malignant transformation rate of oral submucous fibrosis: A systematic review and meta-analysis. Oral Surg 2023;16:220-7.
3. Ranganathan K, Devi MU, Joshua E, Kirankumar K, Saraswathi TR. Oral submucous fibrosis: A case-control study in Chennai, South India. J Oral Pathol Med 2004;33:274-7.
4. Pindborg JJ, Sirsat SM. Oral submucous fibrosis. Oral Surg Oral Med Oral Pathol 1966;22:764-79.
5. Agley CC, Velloso CP, Lazarus NR, Harridge SD. An image analysis method for the precise selection and quantitation of fluorescently labeled cellular constituents: Application to the measurement of human muscle cells in culture. J Histochem Cytochem 2012;60:428-38.
6. Khaled ML, Helwa I, Drewry M, Seremwe M, Estes A, Liu Y. Molecular and histopathological changes associated with keratoconus. Biomed Res Int 2017;2017:7803029.
7. Shabana AH, El-Labban NG, Lee KW. Morphometric analysis of basal cell layer in oral premalignant white lesions and squamous cell carcinoma. J Clin Pathol 1987;40:454-8.
8. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 2012;9:671-5.
9. Schroeder AB, Dobson ET, Rueden CT, Tomancak P, Jug F, Eliceiri KW. The ImageJ ecosystem: Open-source software for image visualization, processing, and analysis. Protein Sci 2021;30:234-49.
10. Gao S, Liu S, Shen Z, Peng L. Morphometric analysis of spinous cell in oral submucous fibrosis. Comparison with normal mucosa, leukoplakia and squamous cell carcinoma. Chin Med J 1995;108:351-4.
11. Wynn TA, Ramalingam TR. Mechanisms of fibrosis: Therapeutic translation for fibrotic disease. Nat Med 2012;18:1028-40.
12. Kropski JA, Blackwell TS. Endoplasmic reticulum stress in the pathogenesis of fibrotic disease. J Clin Invest 2018;128:64-73.
13. Rathore AS, Gupta A, Shetty DC, Kumar K, Dhanapal R. Redefining epithelial characterization in oral submucous fibrosis using morphometric analysis. J Oral Maxillofac Pathol 2017;21:36-40.
14. Kapoor R, Sansare K, Tamgadge S, Karjodkar F, Mehra A, Mishra I, *et al*. Epithelial atrophy, fibrosis and vascularity correlation with epithelial dysplasia in oral submucous fibrosis, a prospective study. J Microsc Ultrastruct 2022;10:1-6.
15. Ranganathan K, Kavitha R. Proliferation and apoptosis markers in oral submucous fibrosis. J Oral Maxillofac Pathol 2011;15:148-53.
16. Gayathri K, Malathi N, Gayathri V, Adtani PN, Ranganathan K. Molecular pathways of oral submucous fibrosis and its progression to malignancy. Arch Oral Biol 2023;148:105644.

How to cite this article: Prakasam A, Selvan P, Ranganathan K. Morphometric analysis of epithelium in oral submucous fibrosis using open-source software. J Global Oral Health. 2024;7:93-7. doi: 10.25259/JGOH_27_2024