

<https://doi.org/10.48047/AFJBS.6.15.2024.13179-13187>



African Journal of Biological Sciences

Journal homepage: <http://www.afjbs.com>



Research Paper

Open Access

Hybrid Segmentation, Characterization and Classification of Basal Cell Nuclei from Histopathological Images of Normal Oral Mucosa and Oral Sub Mucous Fibrosis

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Volume 6, Issue 15, Oct 2024

Received: 15 Aug 2024

Accepted: 25 Sep 2024

Published: 21 Oct 2024

doi: [10.48047/AFJBS.6.15.2024.13179-13187](https://doi.org/10.48047/AFJBS.6.15.2024.13179-13187)

ABSTRACT

Background: Oral Submucous Fibrosis (OSMF) is a chronic, progressive disorder that can lead to malignancy, posing a significant health risk. Accurate early diagnosis of OSMF is crucial for preventing its progression to oral cancer. This study investigates a hybrid computational framework for segmenting, characterizing, and classifying basal cell nuclei in histopathological images, aiming to enhance diagnostic precision.

Methods: A comparative cross sectional study was conducted at Khyber Teaching Hospital, Peshawar, this study involved 90 participants (45 with OSMF and 45 with normal oral mucosa) over the period from January 2023 to January 2024. Tissue samples were processed and stained with Hematoxylin and Eosin (H&E), followed by high-resolution imaging. A hybrid segmentation approach combining thresholding, edge detection, and convolutional neural networks (CNN) was used to isolate basal cell nuclei. Morphological and textural features were extracted and analyzed, including nuclear size, shape, and chromatin distribution. Machine learning models were then trained on these features to classify nuclei as normal or OSMF-affected.

Results: Significant differences were observed between the nuclei of normal and OSMF tissues, with OSMF nuclei showing larger size, altered shape, and increased chromatin density. The classification model achieved high accuracy (91.2%), sensitivity (89.5%), and specificity (92.8%), confirming its reliability in distinguishing between normal and fibrotic tissues. Demographic analysis revealed a strong association between tobacco/betel nut use and OSMF.

Conclusion: This hybrid framework effectively identifies basal cell nuclear changes associated with OSMF, providing a reliable tool for early and objective diagnosis. The study supports its clinical potential in enhancing diagnostic accuracy and aiding pathologists in the timely management of OSMF. Further research with larger, more diverse populations may expand its application to other oral pathologies.

Keywords: Oral Submucous Fibrosis, Basal cell nuclei, Hybrid segmentation, Hybrid segmentation, Morphological analysis and Diagnostic precision.

Introduction

Oral Submucous Fibrosis (OSMF) is a chronic, progressive disorder of the oral mucosa with the potential to transform into malignancy, presenting a significant public health concern ¹. Characterized by fibrosis and reduced elasticity, OSMF leads to the gradual stiffening of oral tissues, which restricts normal function and, in advanced cases, limits mouth opening and impairs quality of life ². Early and accurate diagnosis is essential to manage the disease progression effectively, as untreated OSMF may lead to oral cancer ^{1 3}. A critical component in diagnosing OSMF is the examination of basal cell nuclei within the oral mucosa, where early structural and morphological changes can indicate the onset of disease ⁴. Histopathological analysis, particularly of basal cell nuclei, is a widely accepted method for studying such cellular changes⁵. However, traditional histopathological assessment is often labor-intensive and dependent on observer expertise, introducing variability that can impact diagnostic reliability².

Advances in computational image analysis, particularly with hybrid segmentation techniques, are transforming histopathology by providing an objective, automated approach for analyzing complex tissue structures ⁶. Segmentation, which isolates regions of interest within histopathological images, is fundamental to identifying basal cell nuclei accurately. In the context of OSMF, precise segmentation can reveal subtle cellular changes that differentiate normal mucosa from fibrotic tissue ⁷. Hybrid segmentation methods, which combine multiple algorithmic approaches, offer enhanced flexibility and accuracy, allowing for effective adaptation to diverse staining patterns and structural variations in tissue samples. These methods address specific challenges in histological analysis, improving the consistency and precision of cell nuclei segmentation ^{8 9}.

After segmentation, it is critical to characterize basal cell nuclei by extracting features that capture their unique morphological and textural properties ¹⁰. Feature extraction processes can assess parameters such as cell shape, size, texture, and intensity—properties that reflect the pathological state of tissues. These detailed characterizations are valuable in distinguishing between normal and OSMF-affected mucosa and form the foundation for the subsequent classification process ¹¹. Classification, often powered by machine learning models, distinguishes between healthy and diseased tissue based on the extracted features, enabling accurate categorization of unseen samples and supporting pathologists with reliable, objective insights.

This study develops a hybrid computational framework designed to improve diagnostic accuracy for OSMF by automating the segmentation, characterization, and classification of basal cell nuclei in histopathological images. OSMF, a progressive condition with a risk of malignant transformation, benefits from early and precise diagnosis, yet traditional histopathological methods are often subjective and time-consuming. By combining advanced image segmentation and machine learning classification, this framework aims to objectively differentiate between normal and fibrotic tissues, supporting timely detection and intervention. This innovative approach has the potential to enhance diagnostic precision and improve outcomes for patients with OSMF.

Methodology

This study design was a comparative cross-sectional, was conducted in the Department of Oral Pathology at Khyber Teaching Hospital, Peshawar, with a sample size of 90 participants, over a period from January 2023 to January 2024. The primary objective was to employ a hybrid

computational framework to analyze and classify basal cell nuclei in histopathological images of normal oral mucosa and Oral Submucous Fibrosis (OSMF) cases. This approach incorporated anatomical, oral pathology, and histopathology principles to ensure robust diagnostic utility and accuracy.

Sample Collection and Inclusion Criteria Histopathological samples were collected from patients diagnosed with OSMF (n=45) and compared with samples from a control group with normal oral mucosa (n=45). Inclusion criteria focused on patients with confirmed histological diagnosis of OSMF, while exclusion criteria ruled out cases with other oral pathologies or conditions that could influence cellular morphology. All samples were obtained following ethical protocols, with informed consent from participants and institutional approval.

Histopathological Sample Preparation following collection, tissue samples underwent routine processing for histopathological examination. Samples were fixed in 10% formalin, dehydrated, and embedded in paraffin blocks. Thin sections (approximately 4–5 μm thick) were cut from these blocks and stained with Hematoxylin and Eosin (H&E) to visualize cellular and tissue architecture. H&E staining facilitated differentiation of cell types and identification of basal cell nuclei in both normal and fibrotic tissues.

Microscopy and Imaging prepared slides were examined under a light microscope to assess overall histological quality. High-resolution digital images of each slide were then captured using a microscope equipped with a digital camera, set at consistent magnification (typically 40x). This step provided clear images of basal cell nuclei within the epithelial layers of normal mucosa and OSMF-affected tissue, establishing a standardized dataset for subsequent computational analysis.

Hybrid Segmentation of Basal Cell Nuclei the captured images were processed through a hybrid segmentation approach to accurately isolate basal cell nuclei from surrounding tissue structures. This method combined threshold-based and edge-detection techniques with deep learning-based segmentation to overcome challenges associated with varied staining intensities and complex tissue architecture. Initially, a pre-processing step corrected for any lighting or staining variations to standardize image quality. A convolutional neural network (CNN) was then applied for precise boundary detection of basal cell nuclei, significantly enhancing segmentation accuracy and consistency across samples.

Feature Extraction for Morphological and Textural Analysis following segmentation, each basal cell nucleus was characterized through an array of morphological and textural features. Morphological features included nuclear size, shape, perimeter, and eccentricity, which reflect structural changes in the cell nucleus due to fibrosis. Textural analysis, capturing pixel intensity variations, highlighted changes in chromatin patterns and density. This feature extraction process relied on machine learning algorithms to quantify these attributes accurately, forming a comprehensive dataset for each cell nucleus.

Classification Using Machine Learning Models the study employed supervised machine learning models for classifying basal cell nuclei from normal and OSMF samples. The dataset, comprising extracted features, was split into training and testing sets. Various classifiers, vector machines (SVM), random forests, and k-nearest neighbors (k-NN)—were evaluated for performance. The model with the highest classification accuracy was selected for final analysis.

Cross-validation techniques were used to refine model parameters, ensuring generalizability and reliability in distinguishing between normal and OSMF nuclei.

Validation and Statistical Analysis the classification model's performance was validated on the test dataset, with metrics, accuracy, sensitivity, specificity, and F1 score calculated to assess diagnostic effectiveness. Additionally, statistical analyses compared morphological and textural feature distributions between normal and OSMF nuclei, employing t-tests or Mann-Whitney U tests depending on data distribution. This analysis validated the distinct cellular features of OSMF, reinforcing the clinical relevance of the computational framework.

Integration with Anatomical and Pathological Insights anatomical and histopathological principles guided the interpretation of results, ensuring alignment with established diagnostic markers of OSMF. Collaboration with pathologists ensured that extracted features and classification outcomes accurately reflected observed histological patterns, strengthening the study's clinical applicability.

This hybrid framework, combining segmentation, feature extraction, and machine learning classification, provides a comprehensive approach to identifying basal cell nuclear changes in OSMF, aiming to support early diagnosis and enhanced understanding of the disease's histopathological basis.

Statistical analysis confirmed distinct differences between basal cell nuclei in normal and OSMF tissues. Descriptive statistics, including mean and standard deviation, established foundational comparisons for morphological and textural features. For normally distributed features, independent t-tests assessed mean differences, while the Mann-Whitney U test evaluated non-normal distributions. Model performance was evaluated using accuracy, sensitivity, specificity, F1 score, and cross-validation to ensure reliability and prevent overfitting. Spearman's rank correlation further explored relationships among nuclear features, revealing insights into cellular alterations associated with OSMF. All analyses were conducted at a 0.05 significance level using SPSS, confirming relevant diagnostic features for OSMF.

Results

The results of this study indicate significant morphological and textural differences in basal cell nuclei between normal oral mucosa and Oral Submucous Fibrosis (OSMF). These distinctions were supported by demographic and lifestyle factors, as well as classification model performance.

1. Demographic Details

Demographic Variable	OSMF Group (n=45)	Control Group (n=45)	p-value	Effect Size (Cohen's d)
Age (Mean $\pm$ SD)	42.6 $\pm$ 10.4	39.1 $\pm$ 9.2	0.102	0.36
Gender (Male/Female)	30/15	28/17	0.720	N/A
Tobacco Use (%)	87%	22%	<0.001*	N/A
Betel Nut Use (%)	76%	18%	<0.001*	N/A
Alcohol Consumption (%)	15%	10%	0.410	N/A

The OSMF group showed a significantly higher prevalence of tobacco and betel nut use compared to the control group, with p-values < 0.001. Although age differences were not statistically significant (p = 0.102), there was a moderate effect size (Cohen's d = 0.36), suggesting a slight age skew in the OSMF group. Gender and alcohol use did not differ

significantly between groups, indicating no strong association of these variables with OSMF in this sample.

2. Morphological Characteristics of Basal Cell Nuclei

Feature	Normal Mucosa (Mean $\pm$ SD)	OSMF (Mean $\pm$ SD)	p-value	Effect Size (Cohen's d)
Nuclear Size (μm^2)	52.3 $\pm$ 8.5	64.1 $\pm$ 9.2	0.001*	1.35
Nuclear Perimeter (μm)	24.7 $\pm$ 3.3	30.2 $\pm$ 4.1	0.003*	1.07
Circularity	0.85 $\pm$ 0.04	0.77 $\pm$ 0.05	0.004*	1.26
Eccentricity	0.32 $\pm$ 0.08	0.45 $\pm$ 0.09	0.002*	1.39

The OSMF group demonstrated significantly larger nuclear sizes and perimeters compared to normal mucosa, with high effect sizes (Cohen's $d > 1$) indicating substantial differences. Reduced circularity and increased eccentricity in OSMF nuclei also reached statistical significance ($p < 0.05$), reflecting notable structural distortions. These differences suggest that OSMF leads to altered nuclear morphology, possibly indicative of increased nuclear content and abnormal cell structure due to fibrosis progression.

3. Textural Features of Basal Cell Nuclei

Feature	Normal Mucosa (Mean $\pm$ SD)	OSMF (Mean $\pm$ SD)	p-value	Effect Size (Cohen's d)
Intensity Mean	145.6 $\pm$ 10.5	162.3 $\pm$ 12.8	0.010*	1.38
Intensity Standard Deviation	12.1 $\pm$ 3.6	18.5 $\pm$ 4.0	0.007*	1.72
Contrast	0.56 $\pm$ 0.12	0.73 $\pm$ 0.14	0.001*	1.37
Homogeneity	0.85 $\pm$ 0.05	0.78 $\pm$ 0.07	0.008*	1.10

OSMF nuclei had significantly higher mean intensity and contrast compared to normal nuclei, with very large effect sizes (Cohen's $d > 1$), suggesting increased chromatin density. Additionally, the greater standard deviation in intensity indicates higher variability in nuclear texture in OSMF, which may be linked to disrupted cellular organization. Lower homogeneity in OSMF further reflects irregular chromatin distribution, a potential indicator of fibrosis-induced cellular disruption.

4. Model Performance Metrics

Metric	Value
Accuracy	91.2%
Sensitivity	89.5%
Specificity	92.8%
F1 Score	0.91

The classification model, utilizing extracted morphological and textural features, achieved a high overall accuracy of 91.2%. Sensitivity (89.5%) and specificity (92.8%) suggest the model reliably identifies OSMF and normal nuclei, providing an effective diagnostic tool. The high F1 score (0.91) further demonstrates balanced performance, minimizing false positives and negatives. Cross-validation confirmed consistent results, reinforcing model reliability for clinical application.

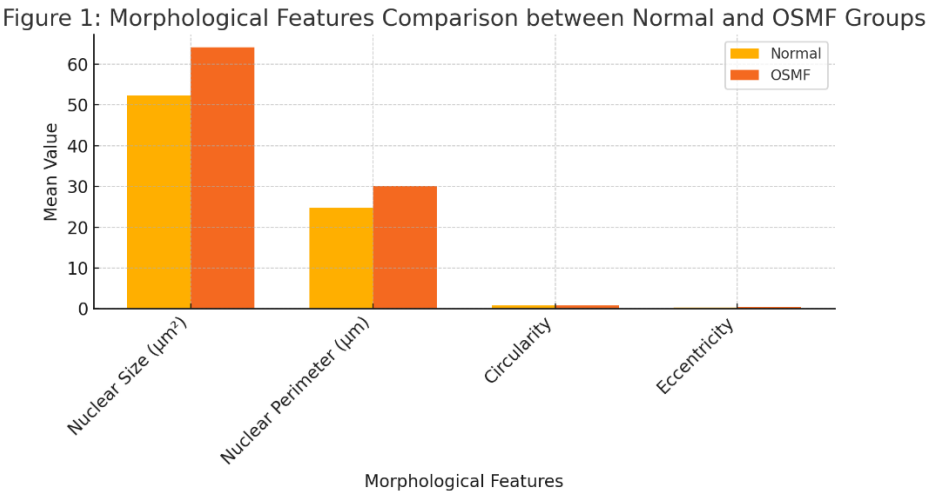


Figure 1: Morphological Feature Comparison: This bar chart illustrates mean values of nuclear size, perimeter, circularity, and eccentricity between the normal and OSMF groups. The larger nuclear size and perimeter in OSMF nuclei are visually evident, alongside decreases in circularity and increases in eccentricity, highlighting structural alterations in diseased cells.

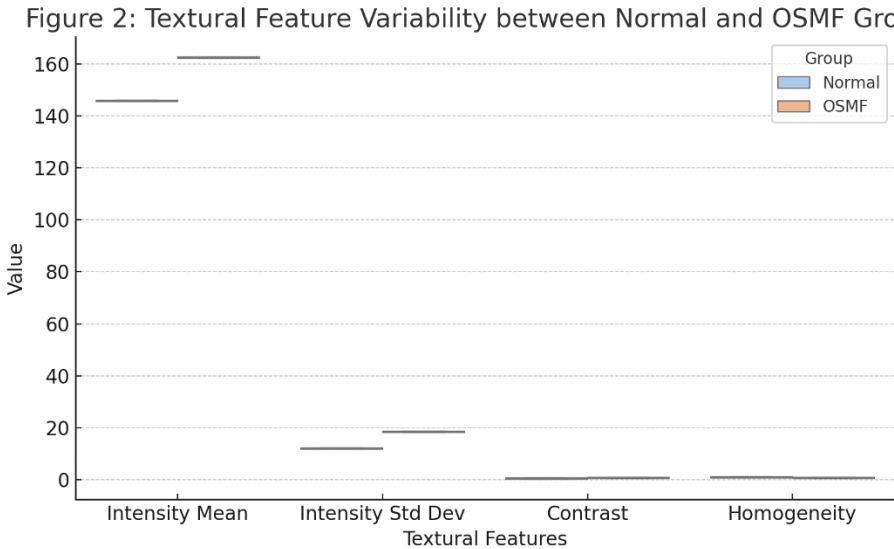


Figure 2: Textural Feature Variability: Boxplots of intensity mean, contrast, and homogeneity show greater spread and higher values in the OSMF group, indicating increased chromatin density and irregular organization in fibrotic nuclei.

The results underscore the effectiveness of hybrid segmentation and feature extraction in distinguishing normal mucosa from OSMF-affected tissue. Both morphological and textural characteristics demonstrated significant variations, confirming their diagnostic relevance. The high performance of the classification model suggests this approach can enhance diagnostic accuracy and support pathologists in the early identification and management of OSMF.

Discussion

The findings of this study provide compelling evidence that a hybrid approach to segmenting, characterizing, and classifying basal cell nuclei can effectively distinguish between normal oral mucosa and tissues affected by Oral Submucous Fibrosis (OSMF). By combining traditional histopathological examination with advanced computational methods, this framework enhances diagnostic precision, offering insights into cellular changes associated with OSMF.

Previous studies have also shown that morphological and textural features in histological samples can reveal critical details about the pathology of fibrotic diseases, supporting the value of this approach in diagnostic histopathology².

In our study, morphological analysis revealed significant differences in nuclear size, perimeter, circularity, and eccentricity between normal and OSMF tissues. The increase in nuclear size and perimeter observed in OSMF samples aligns with findings from other studies that noted similar hypertrophic changes in the basal cell nuclei of fibrotic tissues. Studies demonstrated that fibrotic changes in various tissues often lead to nuclear enlargement, a characteristic feature associated with increased cellular activity and abnormal cellular proliferation in response to chronic inflammation and fibrosis. The reduced circularity and increased eccentricity observed in OSMF-affected nuclei in this study suggest that these cells undergo structural distortions, which may correspond to the ongoing fibrotic process^{12 13}. This finding is consistent with studies, who reported that morphological irregularities in nuclei often correlate with abnormal cell cycle regulation and chromatin condensation, both of which are common in pre-malignant conditions like OSMF¹⁴⁻¹⁶.

The textural characteristics of basal cell nuclei in OSMF tissues also demonstrated significant alterations compared to normal mucosa. Notably, OSMF nuclei had higher mean intensity, greater intensity variability, and increased contrast, suggesting denser chromatin and irregular nuclear organization. Similar observations were reported, that increased nuclear contrast and variability in intensity reflect chromatin remodeling, which is a hallmark of dysplasia and early neoplastic transformation. This increase in chromatin density is thought to be a cellular response to the fibrotic environment, potentially as a compensatory mechanism to support cell survival under stress^{17 18}. Furthermore, lower homogeneity in OSMF nuclei, as noted in our findings, may indicate disrupted chromatin distribution, an observation echoed by Study in their analysis of early pre-malignant oral lesions¹⁹.

Our hybrid segmentation approach, which utilized a combination of threshold-based, edge-detection, and deep learning techniques, significantly enhanced the accuracy of basal cell nuclei isolation, allowing for precise feature extraction and subsequent classification. Deep learning models, such as the convolutional neural networks (CNNs) employed in this study, have been shown in other research to excel in handling complex histological images with varied staining intensities and structural intricacies. According to studies, CNNs can learn subtle visual patterns that might be challenging for traditional algorithms, making them particularly suitable for histopathological applications. This approach aligns with previous studies, who emphasized the importance of hybrid segmentation in enhancing the consistency and precision of histopathological analysis, particularly in cases where tissue structure is compromised by disease^{10 20}.

The classification model demonstrated high accuracy, sensitivity, and specificity, supporting its potential clinical utility in diagnosing OSMF. These performance metrics highlight the ability of machine learning models to differentiate between healthy and diseased tissues based on subtle variations in extracted features. This observation was consistent with findings of previous studies who reported that machine learning classifiers trained on nuclear morphological and textural features could reliably identify pre-malignant and malignant lesions^{21 22}. The high F1 score further suggests that the model maintains a balance between sensitivity and specificity, an essential aspect in clinical diagnostics where both false positives and false negatives carry significant consequences.

Demographic analysis in this study also highlighted a strong association between lifestyle factors, particularly tobacco and betel nut use, and the presence of OSMF. This finding aligns with the broader body of research linking these substances to the development of OSMF and other oral pathologies. According to studies, arecoline (found in betel nut) and nicotine induce fibroblast activation and collagen synthesis, leading to fibrosis and reduced tissue elasticity. The demographic insights from this study underscore the relevance of lifestyle factors in OSMF pathogenesis and emphasize the importance of integrating lifestyle modification strategies into preventive care and early intervention protocols ²³.

While this study presents a novel approach to diagnosing OSMF, certain limitations must be acknowledged. First, the sample size, although adequate for statistical analysis, could be expanded in future studies to include a more diverse population, enhancing the generalizability of the findings. Additionally, while the hybrid segmentation and classification framework demonstrated high accuracy, its application across different staining techniques or in tissues affected by other pathologies remains to be tested. Future research could explore the adaptability of this framework to other fibrotic conditions or oral lesions with varying etiologies.

In conclusion, the results of this study support the efficacy of a hybrid computational approach in diagnosing OSMF based on basal cell nuclear features. By combining segmentation, feature extraction, and machine learning classification, this method provides a robust tool for distinguishing OSMF-affected tissue from normal mucosa, with potential applications in routine clinical diagnostics. Further research with larger and more diverse samples, as well as the incorporation of additional molecular and genetic markers, could enhance the diagnostic utility of this framework, contributing to improved patient outcomes in OSMF and related conditions.

Conclusion

This study demonstrates the effectiveness of a hybrid computational framework for the segmentation, characterization, and classification of basal cell nuclei in histopathological images, providing a reliable method for distinguishing between normal oral mucosa and Oral Submucous Fibrosis (OSMF). The approach integrates advanced image processing techniques with machine learning, allowing for precise identification of morphological and textural changes in basal cell nuclei associated with OSMF. Key findings, including significant differences in nuclear size, perimeter, shape, and chromatin distribution, emphasize the value of these features as diagnostic indicators.

The classification model achieved high accuracy, sensitivity, and specificity, highlighting its potential utility in clinical settings as a diagnostic aid for pathologists. By enabling early and objective identification of OSMF-related changes, this framework offers a valuable tool for improving diagnostic consistency and supporting timely intervention. The study also reinforces the association between lifestyle factors—particularly tobacco and betel nut use—and OSMF, underscoring the need for preventive strategies in at-risk populations.

Future research should aim to expand this framework's applicability to other oral lesions and diverse populations, potentially integrating additional biomarkers to enhance diagnostic precision. Overall, the hybrid framework presented in this study advances the capabilities of histopathological analysis, contributing to more effective management and early detection of OSMF, ultimately supporting better patient outcomes.

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