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A Histomorphometric Analysis of Basal cells in Oral Epithelial Dysplasia

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KEYWORDS:

Histomorphological Analysis, Epithelial Dysplasia, Oral Precancer, Basal Cells

ABSTRACT:

Aim: This study aims to evaluate objectively, the atypically appearing basal cell layer of oral lesions showing epithelial dysplastic features and compare it with normal buccal mucosa.

Materials and Methods: 30 cases from department archives were included and were divided into three groups: ten healthy controls, ten cases with low risk oral epithelial dysplasia and ten cases of high risk epithelial dysplasia. Ten fields were selected in each case and ten basal cells were selected in each field. Nine nuclear parameters were selected and assessed objectively and then compared statistically within the three groups using ANOVA test.

Results: The results indicated an increase in nuclear area with the grade of dysplasia, with the highest value found in the high-risk group. The mean aspect ratio also varied, with normal buccal mucosa showing significantly higher values compared to both low-risk and high-risk groups. A statistically significant difference was observed in the angle between the groups, with low-risk groups showing the highest angle and high-risk groups showing the lowest.

Conclusion: In conclusion, the increase in nuclear size, length from the superior border of the nucleus to basal side of cell membrane and length from the apex of the basal epithelial cells to the basement membrane and a marked reduction in aspect ratio in dysplasia strongly correlated with high-risk dysplasia.

Introduction

Oral squamous cell carcinoma (OSCC) is the most common type of epithelial neoplasm in the oral cavity, often resulting from genetic mutations that lead to uncontrolled cell growth of abnormal cells.[1] Dysplasia, derived from the Greek term "abnormal formation," refers to structural abnormalities in tissue development, specifically the transition from normal epithelium to epithelial dysplasia (OED).[10,11] As the disease progresses, both tissue-level changes (epithelial dysplasia) and cellular-level abnormalities (atypical cells) become evident.[2] These morphological and physical alterations are significant both diagnostically and prognostically, often referred to as precancerous changes. A potentially malignant disorder, such as leukoplakia, is characterized by a white oral mucosal lesion that cannot be attributed to any other specific condition.[3] The presence of dysplasia forms the cellular basis for this potentially malignant condition. Histopathological assessments of dysplasia may vary

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between pathologists, with some focusing on different features, leading to discrepancies in how dysplasia is evaluated. There is growing recognition of these subjective variations, prompting the search for more objective evaluation methods.[4-8] Recently, computer-assisted morphometry has been employed to study nuclear and cellular changes in relation to the histological behaviour of lesions.[9] This technique allows for the analysis of various features observed in microscopic images, yielding more consistent, objective, and reproducible results. From a morphological standpoint, some studies have suggested that abnormal representation of the basal cell layer in oral epithelial dysplasia could predict progression to carcinoma.[10,11,12,13] However, objective evidence on the prognostic significance of the basal layer's appearance remains limited (14). This study aims to provide data on the relevance of an atypical basal layer in OED for predicting significant progression to invasive cancer.

Materials and Methods

The study used cases from the Departmental archives of Oral and Maxillofacial Pathology at Karnavati School of Dentistry, Uvarsad, Gandhinagar. A total of 30 histopathologically confirmed cases were included, comprising 10 cases each of oral leukoplakia with high-risk oral epithelial dysplasia, low-risk oral epithelial dysplasia, and 10 healthy controls without lesions. Cases with clear basal cell layers without overlap in histopathological sections were selected for inclusion, while lesions not diagnosed as leukoplakia, those without epithelial dysplasia, insufficient epithelium, improper orientation, or lacking depth were excluded.

Methods

Tissue sections from biopsy specimens obtained at the Department of Oral Pathology and Microbiology, Karnavati University, Uvarsad, Gujarat, were used in this study. The H&E sections were confirmed and graded according to the Kujan et al. (2006) binary classification system. Images were captured for further morphometric analysis.

Measurements

Stage micrometers were used for precise measurement of tissue sections. The micrometer and images were adjusted to the same size and resolution (40X magnification). The distance between two lines in the micrometer was measured and divided by 10, as each line represented 10 micrometers. The factors outlined by Okamura et al. (16) were followed in the study and are illustrated in Figure 1.

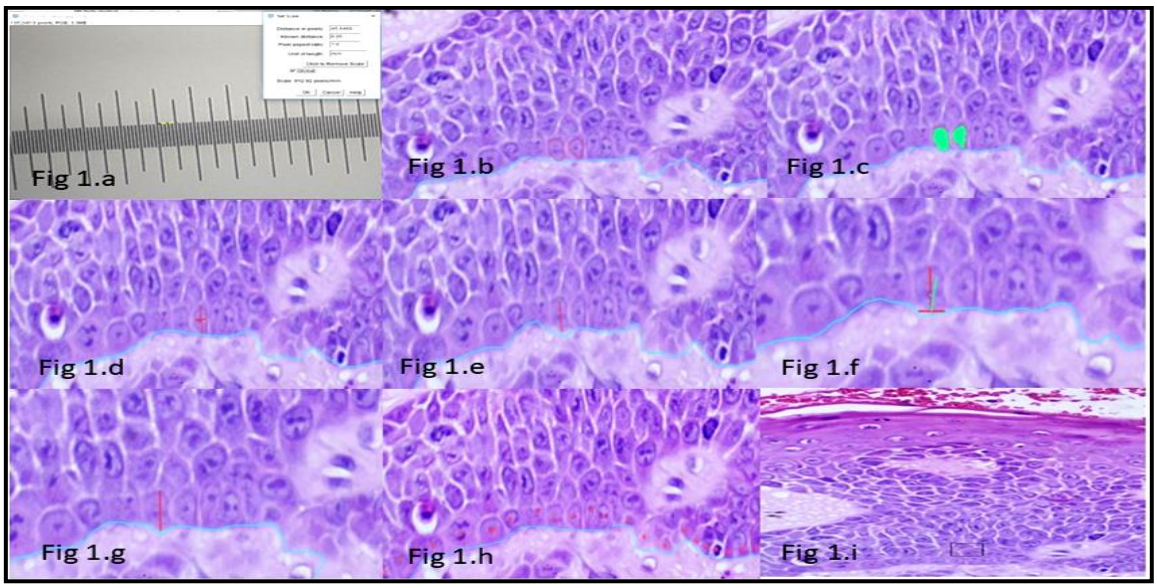

Figure 1: (***a***) Calibration of scale micrometer. (***b***) Factor 1, Nuclear-Area. (***c***) Factor 2, Circularity of nucleus of basal cells. (***d***) Factor 3, Aspect ratio of nucleus of basal cells. (***e***) Factor 4, Length between upper border of nucleus and basal cell membrane. (***f***) Factor 5, Long axial angle of nuclei of the basal layer and the lines perpendicular to the tangent line of interaction point. (***g***) Factor 6, length between the apex of the basal cells and basement membrane. (***h***) Factor 7, Basal cell density. (***i***) Factor 8, Density of cells in quadrant

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Statistics

A total of 24,000 calculations were performed and compiled in Microsoft Excel 2020 for statistical analysis.

Results

The nuclear area of normal buccal mucosa was found to be 23.53±13.00, compared to 37.33±17.92 for low-risk epithelial dysplasia and 57.48±33.21 for high-risk epithelial dysplasia. These results indicate an increase in nuclear area as the grade of dysplasia rises, with the highest value found in the high-risk group. Significant differences were observed between all groups. The mean aspect ratio also varied, with normal buccal mucosa showing significantly higher values compared to both low-risk and high-risk groups. There were no significant differences between low-risk and high-risk groups ($p > 0.05$). A statistically significant difference was observed in the angle between the groups, with low-risk groups showing the highest angle and high-risk groups showing the lowest. Post hoc testing revealed non-significant differences in factors 2 and 3 between low-risk and high-risk epithelium (p-values: 0.923 and 0.941, respectively), and in factors 7 and 8 between high-risk and normal groups (p-values: 0.771 and 1, respectively).

Table 1: Morphometric Analysis

Total sections:10 normal mucosa + 10 low riskdysplasia + 10 high risk dysplasia	30 sections
Number of microscopic field per sections	10 fields
Cells per field (basal layer)	10 cells
Total cells per section	100 cells (10 fields x 10 cells)
Total nuclei measured	3000
Total number of measurements	27000

Table 2: Mean and Standard deviation of different variables (ANOVA)

STUDY GROUPS	Nuclear Area (Mean± SD)	CIRCULARITY (Mean± SD)	ASPECT RATIO (Mean± SD)	Length nucleus (Mean± SD)	Angles (Mean± SD)	Length cell (Mean± SD)	Density of Basal cells (Mean± SD)	Density in Quadrant (Mean± SD)
GROUP1	23.53±13.00	.159±.15	2.78±1.38	10.14±4.41	4.86±4.85	12.20±4.91	.11±0.02	.01±.001
GROUP2	37.33±17.92	.145±.14	2.31±.98	11.66±4.95	5.78±4.07	14.12±5.62	.22±1.13	.12±1.14
GROUP3	57.48±33.21	.154±.15	2.30±1.03	13.87±5.93	3.83±3.46	16.83±6.95	.09±0.02	.01±0.002
'F' value	548.8	50.01	56.41	133.307	54.50	155.232	9.67	10.40
'P' value	.00	.00	.00	.00	.00	.00	.00	.00

Group 1- Normal Buccal Mucosa, Group 2- Low Risk Dysplasia, Group3- High Risk Dysplasia

SD= Standard Deviation. P<0.005

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Table 3: Intergroup comparison of various parameters representing Nuclear Shape and Nuclear Size among study groups. (Post hoc)

	SIZE			SHAPE					
	Nuclear Area			Circularity			Aspect Ratio		
	Mean diff	S.E	Sig	Mean diff	S.E	Sig	Mean diff	S.E	Sig
Group 1-2	13.793	1.03	.000	-.060	.006	.000	.464	.051	.000
Group 2-3	20.15	1.03	.000	.006	.006	.923	.017	.051	.941
Group 3-1	33.94	1.03	.000	-.058	.006	.000	.481	.051	.000

Group1- Normal Buccal Mucosa, Group2- Low Risk Dysplasia, Group3- High Risk Dysplasia. S.E= Standard Error, Sig= Significance, diff= Difference. P<0.005

Table 4: Intergroup comparison of various parameters representing Loss of Polarity and Nuclear Hyperplasia among study groups. (Post hoc)

	Loss of Polarity									Basilar Hyperplasia					
	Mean Length			SD of Length			SD of Angles			Density of Basal Cells			Density of Quadrant		
	Mean diff	S.E	Sig	Mean diff	S.E	Sig	Mean diff	S.E	Sig	Mean diff	S.E	Sig	Mean diff	S.E	Sig
Group 1-2	1.51	.229	.00	1.91	.263	.00	.920	.186	.000	.100	.029	.002	.117	.029	.000
Group 2-3	2.21	.229	.00	2.70	.263	.00	1.94	.186	.000	.120	.029	.000	.116	.029	.000
Group 3-1	3.73	.229	.00	4.62	.263	.00	1.02	.186	.000	.020	.029	.771	.000	.029	1.000

Group 1- Normal Buccal Mucosa, Group2- Low Risk Dysplasia, Group3- High Risk Dysplasia

S.E= Standard Error , Sig= Significance, diff= Difference<0.005

Discussion

Grading squamous cell carcinoma is crucial as it correlates directly with prognosis. While OSCC is less prevalent in Western countries, notable exceptions exist, and many cases present with visible mucosal alterations, such as red or white patches.[11] Precancerous lesions are

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often diagnosed based on clinical appearance and graded through histopathology. Recently, attention has turned to using morphometric techniques to analyze histomorphological changes in lesions.[17] Morphometric analysis offers a reliable method to examine nuclear characteristics, enabling early identification of cellular alterations. The results from this approach are more consistent, objective, and reproducible.[18,19] Smitha et al. concluded that an increase in basal cellular and nuclear size in leukoplakia makes it more prone to malignant transformation, suggesting that these features may help predict malignant potential.[15] Okamura et al. proposed that the loss of polarity plays a significant role in predicting malignant potential.[16] Ananjan et al. found that both cellular and nuclear variables could indicate tumor aggressiveness.[19] In contrast, this study focused solely on nuclear parameters of basal cells to assess whether these could be useful for predicting dysplasia from normal buccal mucosa. Nigudkar et al. observed that the ratio of nuclear to cellular perimeter increases with OSCC grade.[20]

Conclusion

A gradual increase in nuclear size, disruption of nuclear polarity, and a decrease in aspect ratio during dysplasia may provide an objective means for assessing epithelial dysplasia. The findings of this study suggest that histomorphometric analysis may uncover features that routine histological examination might overlook, offering a more reliable approach to predicting the progression of dysplastic lesions.

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