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By Meenakshi Singhal , Devi Charan Shetty , Ankita Tandon & Saurabh Juneja

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Aim: To determine the perilesional tissue in oral mucosal biopsies through morphometric analysis.

Material & Methods: 50 formalin-fixed, paraffin-embedded tissue specimens were taken, which include 40 cases of oral epithelial dysplasia with perilesional tissue marked by India ink and 10 cases of the normal buccal mucosa. Histopathological examination was done to evaluate the presence or absence of dysplastic features in lesional and perilesional tissues. Morphometric analysis was done using epithelial thickness & length between the apical membrane of basal cells & the basement membrane.

Results: There was no significant differences in the epithelial thickness between lesional & perilesional tissue. However, the length between the apical membrane of basal cells & the basement membrane was found to be significantly higher on the perilesional side in comparison to lesional side.

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Meenakshi Singhal [α], Devi Charan Shetty [σ], Ankita Tandon [ρ] & Saurabh Juneja [ω]

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Results: There was no significant differences in the epithelial thickness between lesional & perilesional tissue. However, the length between the apical membrane of basal cells & the basement membrane was found to be significantly higher on the perilesional side in comparison to lesional side.

Conclusion: Numerous histological features which differentiate neoplastic tissue from healthy tissues remain subjective. Morphometric analysis can be considered as an effective guide in evaluating the progression of the normal epithelium to dysplastic epithelium in otherwise clinically normal mucosa. Objective analysis based on quantitative guidelines would be extra convenient and can be considered handy than a subjective framework.

Keywords: morphometry, epithelial dysplasia, perilesional tissue, oral potentially malignant disorders (OPMDs).

Author α: Senior Lecturer, Department of oral maxillofacial pathology & microbiology, ITSCDSR Muradnagar, Ghaziabad.
e-mail: min13021987@gmail.com
Author σ: Principal & Head of Department, Department of oral maxillofacial pathology & microbiology, ITSCDSR Muradnagar, Ghaziabad.
Author ρ: Reader, Department of oral maxillofacial pathology & microbiology, Dental Institute, RIMS, Ranchi.
Author ω: Reader, Department of oral maxillofacial pathology & microbiology, ITSCDSR Muradnagar, Ghaziabad.

I. Introduction

- Oral carcinogenesis presents a multistep model of development, as it is unlikely that there is uniformity in the way individual patients or tissues behave.[1] Although Oral squamous cell carcinoma (OSCC) is not linear in its development, it begins as simple epithelial hyperplasia progresses through epithelial dysplasia (OED), signifying more extensive genetic aberrations.[2] Such predecessor lesions are often referred to as 'Oral potentially malignant disorders (OPMDs)' since not all lesions and conditions described under this term transform to cancer.[3] The transition from normal oral epithelium to oral dysplasia and cancer results from accumulated genetic alterations.[4] Underlying the histological transition of the normal oral epithelium through a precancerous state to invasive carcinoma are multiple molecular and cellular changes.[5]
- Clinically OPMDs may present as leukoplakia, erythroplakia, oral lichen planus, and Oral Submucous Fibrosis. A variety of alterations accumulate to potentiate this transition to malignancy. Oral leukoplakia, harboring histologic features of hyperplasia and/or dysplasia is frequently encountered in the oral cavity.[6] The rate of malignant transformation in OPMDs varies from 0% to 20% in 1 to 30 years. The malignant transformation risk of leukoplakia is associated with the lesional histology. The ability to identify oral leukoplakia patients at increased risk of cancer development is critical for improving control of oral cancer.[7]
- Therefore, this study was designed to establish an objective tool to assess the perilesional tissue for histopathological changes in an otherwise clinically normal-appearing mucosa to reduce subjective bias and to objectively assess the predictive value of morphometric analysis in predicting dysplasia in otherwise normal mucosa through morphometric analysis.

II. Materials and Method

- 50 formalin-fixed, paraffin-embedded tissue specimen were taken, which include 40 cases of

oral epithelial dysplasia with perilesional tissue and 10 cases of the normal buccal mucosa. 4 micron thick sections were taken & stained with H & E. The sections were microscopically photographed using Olympus camera.

- Histopathological examination was done to evaluate the presence or absence of dysplastic features in lesional and perilesional sides of 40 cases of Oral epithelial dysplasia. The marginal areas of the tissue were excluded from the analysis because they often show hyperchromatism artifacts. All cytological and architectural alterations established by WHO in the year 2017 for OED were evaluated.[8]

Following histopathological factors were evaluated in each case- in lesional and perilesional tissue

Dysplastic features
Architectural characteristic
Irregular epithelial stratification
Loss of polarity of basal cells
Drop-shaped rete ridge
Increased number of mitotic figures
Abnormally superficial mitotic figures
Premature keratinization in single cell
Keratin pearls within rete pegs
Cellular characteristics
Abnormal variation in nuclear size
Abnormal variation in nuclear shape
Abnormal variation in cell size
Abnormal variation in cell shape
Increased N:C ratio
Atypical mitotic figures
Increased number and size of nucleoli
Hyperchromasia
Loss of epithelial cell cohesion

Morphometric analysis was done using the image analysing software Magnus Pro.

Two histopathopathological factors were quantified morphometrically in each case[9] –

i. Epithelial thickness &
ii. The length between the apical membrane of basal cells & the basement membrane. (Basal Cell Diameter)

III. Results

Table 1: Comparison of Cytological and Architectural Alterations between Lesional and Perilesional tissues of study cases

Dysplastic features	Lesional (n=40)	Perilesional (n=40)	P value
Architectural characteristic			
Irregular epithelial stratification	2	0	0.499
Loss of polarity of basal cells	10	3	0.01
Drop-shaped rete ridge	12	2	0.129
Increased number of mitotic figures	4	1	1
Abnormally superficial mitotic figures	1	0	0.317
Premature keratinization in single cell	2	0	0.499
Keratin pearls within rete pegs	0	0	0
Cellular characteristics			
Abnormal variation in nuclear size	8	2	0.317
Abnormal variation in nuclear shape	8	1	0.678
Abnormal variation in cell size	6	1	0.079
Abnormal variation in cell shape	6	1	0.079
Increased N:C ratio	16	6	0.031
Atypical mitotic figures	2	0	0.499
Increased number and size of nucleoli	21	6	0.01
Hyperchromasia	34	11	0.034
Loss of epithelial cell cohesion	3	0	0.457

The table shows cytological and architectural alterations between Lesional and Perilesional tissues of study cases. The results show that out of 40 cases of lesional and perilesional tissues of epithelial dysplasia- Loss of polarity of basal cells, Increased N:C ratio, Increased number, and size of nucleoli and Hyperchromasia showed marked difference which was found to be statistically significant ($p \leq 0.05$).

Table 2: Morphometric analysis of epithelial thickness and basal cell diameter in Lesional and Perilesional tissues of study cases

I.	Groups	N	Mean± Std Deviation (μ)	p value
Epithelial Thickness	Control	10	1310.97±58.908	.289
	Lesional	40	1734.22±520.979	
	Perilesional	40	1836.99±634.631	
Basal Cell Diameter	Control	10	50.55±3.544	.013
	Lesional	40	48.82±3.777	
	Perilesional	40	52.03±4.875	

The above table shows the morphometric analysis of epithelial thickness and basal cell diameter in lesional and perilesional tissue of study cases. The results show that out of the total 50 cases, 40 each were of lesional and perilesional tissues of epithelial dysplasia with 10 cases of the normal buccal mucosa. The epithelial thickness was 1310.97 ± 58.90 in the control group, 1734.22 ± 520.97 in the lesional group and 1836.99 ± 634.63 in the perilesional group, which was statistically not significant ($p \geq 0.05$). The basal cell diameter in the control group was 50.55 ± 3.54, in the lesional group 48.82 ±3.77 and in the perilesional group 52.03 ± 4.87, which was statistically significant ($p \leq 0.05$).

IV. Discussion

The transition from normal oral epithelium to oral dysplasia and cancer results from accumulated genetic alterations.[4] Underlying the histological transition of the normal oral epithelium through a precancerous state to invasive carcinoma are multiple molecular and cellular changes. Clinically OPMDs may present as leukoplakia, erythroplakia, oral lichen planus, and Oral Submucous Fibrosis. A variety of alterations accumulate to potentiate this transition to malignancy. Oral leukoplakia, harboring histologic features of hyperplasia and/or dysplasia, is frequently encountered in the oral cavity.[6] The rate of malignant transformation in OPMDs varies from 0% to 20% in 1 to 30 years. The malignant transformation risk- of leukoplakia is associated with the lesional histology.[7] The early identification of oral leukoplakia patient is critical for improving the control of oral cancer.

Numerous histological features which differentiates neoplastic tissue from the healthy tissues have been exhaustively discussed, but the findings remain subjective among observers. The WHO classification remains the gold standard for diagnosing OED, however, subjective bias was found, As the WHO classification includes vague histopathological factors, still there is a lack of evidence on how individual features should be weighted.

Objective analysis based on quantitative guidelines would be extra convenient and can be considered handy than a subjective framework.[10]

In the present- study, Histopathological examination was done to evaluate the presence or absence of dysplastic features in lesional and perilesional sides of 40 cases of Oral epithelial dysplasia. The marginal areas of the tissue were excluded from the analysis because they often show hyperchromatism artifacts. All cytological and architectural alterations established by WHO in the year 2017 for OED were evaluated. The results show that out of 40 cases of the lesional and the perilesional tissues of epithelial dysplasia- Loss of polarity of basal cells, Increased N:C ratio, Increased number, and size of nucleoli and Hyperchromasia showed marked difference which was statistically significant ($p \leq 0.05$) *(Table 1)*

In the present study, the morphometric analysis of epithelial thickness and basal cell diameter in the lesional and the perilesional regions of study cases was performed. The results show that the epithelial thickness was 1310.97 ± 58.90 in the control group, 1734.22 ± 520.97 in the lesional group and 1836.99 ± 634.63 in the perilesional group, which was statistically non

significant ($p \geq 0.05$). *(Figure 1)* The basal cell diameter in the control group was 50.55 ± 3.54, in the lesional group 48.82 ±3.77 and in the perilesional group 52.03 ± 4.87, which was statistically significant ($p \leq 0.05$). *(Figure 2)* The resuls of our study also reveal that there is an increase in epithelial thickness and basal cell diameter of perilesional tissue in comparison with lesional tissue. *(Table 2)* These findings are in accordance with the study done by *Okamura et al. (2016)*[9] where he demonstrated that epithelial thickness and basal cell diameter is a reliable indicator in determining and distinguishing the lesional and perilesional tissues. They further elucidated that the disordered arrangement of the basal cells is more useful than other candidate factors for determining the extent of lesional tissues. Further *Nag et al. (2018)*[11] discussed that morphometry could be administered discriminatorily on specimens having lesional and perilesional tissue, which is problematic to assess precisely through histopathology and postulated that Gutka and Pan Masala can lead to the emergence of high levels of reactive oxygen species (ROS) near the lesion leading to the damage of the surrounding normal tissue. Carcinogens decrease the capacity of the cytoplasm to mature and boost the nuclear parameters and cause a reduction in cellular parameters. Hence, they concluded that between normal and premalignant cells, cellular mean and cellular diameter was established to be lowest in premalignant cells.[9,11]

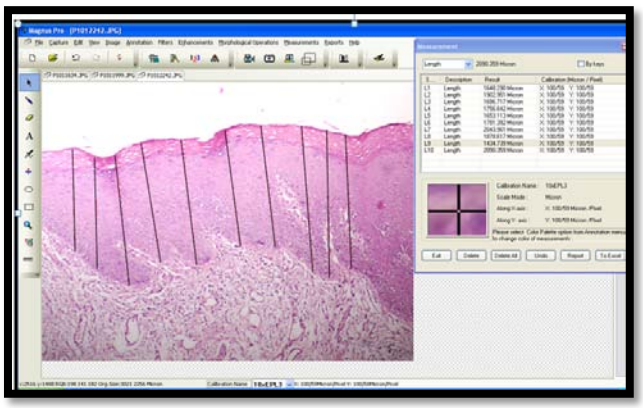

Fig. 1: Morphometric analysis of epithelial thickness (Magnus Pro Image analysis software, Olympus Inc.)

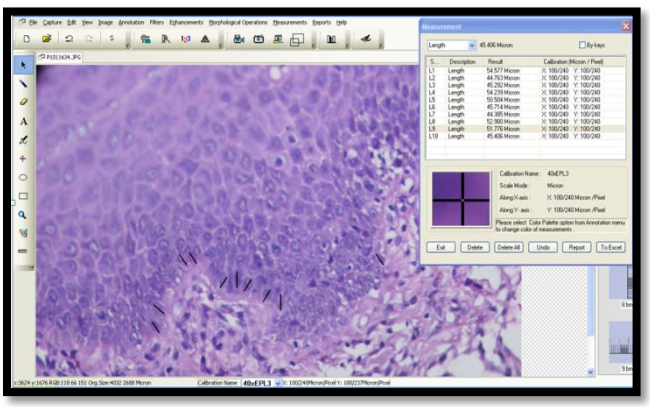

Fig. 2: Morphometric analysis of Basal cell diameter (Magnus Pro Image analysis software, Olympus Inc.)

V. Conclusion

An early and definitive diagnosis of epithelial dysplasia followed by complete excision of the lesion with safe margins is important to prevent the progression of Oral Epithelial Dysplasia into malignancy. Morphometric analysis can be considered as an effective guide to evaluate the progression of the normal epithelium to dysplastic epithelium in otherwise clinically normal mucosa. The results of the present study indicate morphometric analysis as an emerging objective tool to assist the routine histo-pathological diagnosis.

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