Comparison of Morphometric Parameters in Suprabasal Cells in Epithelial Hyperplasia, Leukoplakia and Squamous Cell Carcinoma: an Image Analysis

Shilpi[1], Shaleen Chandra[2], Fahad M Samadi[3], Gadiputi Sreedhar[4], Jiji George[5], SH Thippeswamy[6]

ABSTRACT

Introduction: Computerized image analysis (CIA) based assessment of routine H and E staining pattern enables objective interpretation and quantification for differential diagnosis of the state of pathology elucidated by the tissue being investigated. Aim and objectives: The aim of this study was to establish the morphometric parameters of normal oral epithelial cells in the supra-basal cell layers.

Material and methods: This was a comparative study conducted on H and E stained tissue sections from epithelial hyperplasia, dysplasia, squamous cell carcinoma and normal oral mucosa. The Morphometric analysis of Images captured using digital camera under 10x magnifications was done using image analyzer software, IMAGE PROPLUS 4.1. The software automatically calculated cell and nuclear area with manually traced cells. The N/C ratio was calculated manually.

Results: Quantification of suprabasal cells gave accreditation for identification of cell layers. Morphometric parameters showed significant variations to differentiate grades of dysplasia and sites of oral epithelium.

Conclusion: The simple, inexpensive and easy morphometric analysis method can make the histomorphological study of tissues with premalignant lesions a more objective and practically applicable one for the early detection of cancer. Thus, morphometric analysis could serve as a discriminatory model and help in more accurate assessment of lesions which are highly proliferating and dysplastic and their malignant potential.

Keywords: Morphometry, OSCC, suprabasal cells

INTRODUCTION

Oral cancer is the sixth most common cancer worldwide and has been marked by high morbidity and poor survival rates that have changed little over the past few decades. Beyond prevention, early detection is the most crucial determinant for successful treatment, better prognosis, and survival of cancer. Yet current methodologies for cancer diagnosis based upon pathological examination alone are insufficient for detecting early tumor progression and molecular transformation.[1] In India, approximately 94% of oral malignancies are those of oral squamous cell carcinomas (OSCC) whose etiology is multifactorial with various intrinsic and extrinsic factors.[2]

Computerized image analysis (CIA) based assessment of immunostaining pattern enables objective interpretation and quantification for differential diagnosis of the state of pathology elucidated by the tissue being investigated. Yaziji and Barry[3] in their investigations on Diagnostic Immuhistochemistry reported the main biases in conventional methods of semi-quantitative diagnostic reporting viz. reaction bias (in specimen fixation, tissue processing, antigen retrieval and detection system) and interpretation bias (in the selection of antibody panels, sensitivity of the chosen panel, choice of antibody types and clones, results and literature interpretation).

The quantitative and qualitative analysis of several parameters such as nuclear cytoplasmic ratio and cellular/nuclear area, may reveal incipient cellular changes and thus offer high reliability over routine histopathological examination, in impending and frank malignancies of the oral cavity, in terms of early diagnosis and better treatment.

The aim of this study was to establish the morphometric parameters of normal oral epithelial cells in the supra-basal cell layers.

MATERIAL AND METHODS

This was a comparative study conducted in the Department of Oral Pathology and Microbiology, Babu Banarasi Das College of Dental Sciences, Lucknow. The study was approved by the Ethical Committee of the Institute and consent from each individual was taken before enrolling in the study. The study was conducted on tissue specimens retrieved from the archives and from freshly biopsied formalin fixed tissues. The control group (n=20) comprised normal tissue from healthy adult individuals irrespective of age with no habits. The tissue was retrieved from adjacent to surgical area during routine surgical procedure. The study group consisted of 60 paraffin blocks with tissues obtained during biopsy which were confirmed histopathologically for Epithelial hyperplasia, Dysplasia and Squamous Cell Carcinoma, 20 each.

Inclusion and exclusion criteria

Healthy individuals without any systemic and mental ailments and without any habit, irrespective of age and gender were included in the study. Clinically diagnosed white lesions with histological evidence of epithelial hyperplasia and without histological evidence of epithelial dysplasia were included in the study. Clinically diagnosed cases of leukoplakia, with histological evidence of epithelial dysplasia and histopathologically

[1]Senior Resident, [2]Professor and Head, [3]Assistant Professor, Department of Oral Pathology and Microbiology, King George Medical University, [4]Professor and Head, [5]Professor, [6]Ex Reader, Department of Oral Pathology and Microbiology, Babu Banarasi Das College Of Dental sciences, India

Corresponding author: Dr. Shilpi, B.D.S, M.D.S., Senior Resident, Department Of Oral Pathology and Microbiology, King George Medical University, Lucknow – 226003, India

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confirmed cases of oral squamous cell carcinoma, irrespective of its aetiology were included in the study. For every case, the most representative areas were selected from sections which could be subjected to morphometric analysis. Those patients with hyperkeratinized tissue were excluded. Improperly fixed tissues were excluded.

All biopsy specimens were fixed in 10% formalin for 24 hours, dehydrated in increasing concentrations of ethanol, cleared in Xylene, impregnated in paraffin wax and embedded in paraffin wax and tissue block were prepared. Tissue sections of 5 µm thickness were cut using a soft tissue microtome. The sections obtained were stained with Harris Hematoxylin and Eosin.

The stained sections were observed under microscope using WHO 2005 criteria to establish the grade of Epithelial Dysplasia.

Morphometric technique

Morphometric analysis of tissue sections

For morphometric analysis, images were captured using digital camera attached to a Binocular research microscope with a 4x objective. The actual measurements were done using the Image Proplus 4.1 after accurate calibration. Images were captured, stored and arranged according to the study groups.

Microscopic fields were selected randomly, commencing with first representative field on the left hand side of the section, then moving the stage to the next field and then continuing the selection to include a minimum of 7 fields from each section. For each section, the selected field included representative largest cells where distinct cellular and nuclear outlines were seen avoiding areas of overlapping cells. Histologically identifiable non-keratinocytes were not measured. The images were classified, transferred and stored in the computer. The measurements were done using Image Proplus 4.1 on the same fields (figure-1).

Measurements of morphometric parameters

Cell area (CA): It was measured in microns Square. For measurement, the cell perimeter was traced and software automatically calculated the cell area. For each field 5 largest cells with clear outlines were selected.

Nuclear Area (NA): Similar to cell area, nuclear outlines of the same cells which were used for cell area were traced.

The 5 largest cells and nuclei in each compartment were selected on the assumption that the plane of section would have passed through the centre of the cell or nuclei being measured and would more closely represent the actual size. This method also assumed that the cells in one compartment were more or less of similar size.

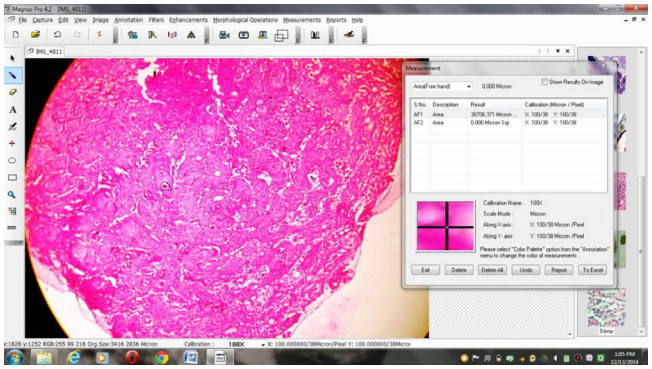

Figure-1: Shows software for image analysis and measurements

Nuclear- Cytoplasmic ratio (N/C):

The Nuclear cytoplasmic ratio was calculated as below:

N/C =Nuclear area/ Cell area- Nuclear area

The outline of cells and nuclei where a complete outline could be clearly seen was traced on the screen. The cellular and nuclear measurements were carried out using measurement toolbars of the software.

STATISTICAL ANALYSIS

The results are presented as mean area and the data collected in this study were analyzed statistically by computing descriptive statistics, viz., mean and standard deviation. The differences in the control group and study groups for various diagnostic variables were compared by means of analysis of variance (ANOVA) followed by Turkey's test for pairwise comparisons. The comparison of morphometric parameters between buccal and gingival mucosa were compared by using Unpaired t-test. The results were considered statistically significant whenever $p<0.05$. All the analysis was carried out by using SPSS 16.0 version (Chicago, Inc., USA).

RESULTS

The analysis of variance showed that there was significant ($p=0.0001$) difference in the cell area in suprabasal cells among the groups. The post-hoc intergroup comparison test revealed that there was significant ($p=0.0001$) difference in cell area between pair of groups. The cell area was found to be lower in SCC (144.71±1.17) than dysplasia (155.27±1.37), hyperplasia (159.72±2.03) and controls (149.50±1.37). However, the nuclear area was observed to be lower in controls than controls (50.68±1.06) than hyperplasia (51.91±1.64), dysplasia (61.36±1.21) and OSCC (68.74±1.01) which statistical significant ($p=0.0001$). Similar observation was found in the ratio of nuclear/cytoplasmic area in suprabasal cells among the groups (Table-1).

There was significant ($p<0.01$) difference in the cell and nuclear area among different grades of dysplasia in suprabasal cells. The cell area was significantly ($p=0.001$) lower in normal (149.50±1.37) than mild dysplasia (155.98±1.88), moderate dysplasia (156.17±1.95) and severe dysplasia (157.19±1.26). The similar pattern was observed for nuclear area and Nuclear - Cytoplasmic ratio (Table-2).

The cell and nuclear area were significantly lower ($p=0.0001$) in buccal mucosa than gingival mucosa in suprabasal cells. However, reverse relationship was found for Nuclear - Cytoplasmic ratio (Table-3).

DISCUSSION

In this study, the suprabasal cell area in well differentiated OSCC (144.71±1.17 µm²) was reduced in comparison to normal mucosa and dysplasia (149.50±1.37 µm², 155.27±1.37µm²). The findings of our study are in accordance with Ramesh et al[4] who has observed the decreased cell area from normal >dysplastic > SCC. The reason for the same was explained by Gao et al[5] that as the carcinogenesis progresses shrinkage of cell junctions lead to increase in intercellular spaces and ultimately there is decrease in cell area.

Suneet et al[6] found increased NA in smears from keratinocytes

Groups	Cell area (in micron square)	Nuclear area (in micron square)	Nuclear-Cytoplasmic area ratio
Control	149.50±1.37 [a]	50.68±1.06 [a]	0.51±0.01 [a]
Epithelial Hyperplasia	159.72±2.03 [a]	51.91±1.64 [a]	0.48±0.04 [a]
Dysplasia	155.27±1.37 [a]	61.36±1.21 [a]	0.65±0.01 [a]
OSCC	144.71±1.17 [a]	68.74±1.01	0.90±0.02
ANOVA p-value	0.0001*	0.0001*	0.0001*
*Significant, [a]p=0.0001			

Table-1: Comparison of morphometric parameters in suprabasal cells

	Cell area	Nuclear area	Nuclear - Cytoplasmic ratio
Normal (control)	149.50±1.37[a]	50.68±1.06 [a]	0.51±0.03 [a]
Mild dysplasia	155.98±1.88 [a]	60.98±2.01 [a]	0.64±0.01 [a]
Moderate dysplasia	156.17±1.95 [a]	61.76±1.27 [a]	0.65±0.02 [a]
Severe dysplasia	157.19±1.26 [a]	61.98±0.57 [a]	0.65±0.03 [a]
ANOVA p-value	0.0001*	0.001*	0.001*
*Significant, [a]p=0.001			

Table-2: Comparison of cell, nuclear area and nuclear - cytoplasmic ratio in suprabasal cells of different grades of dysplasia

	Cell area	Nuclear area	Nuclear - Cyto-plasmic ratio
Buccal	149.50±1.37	50.68±1.06	0.51±0.02
Gingival	187.27±1.56	58.02±0.99	0.44±0.01
p-value[1]	0.0001*	0.0001*	0.0001*
[1]Unpaired t-test			

Table-3: Comparison of Cell and Nuclear area between Buccal and Gingival mucosa in suprabasal cells

of OSCC and concluded that increased NA is due to increased DNA synthesis. Callimeri and Smith[7] found that an increased nuclear to cytoplasmic ratio was one of the consistent findings during progression from benign to a state of malignancy.

In a morphometric study on gastric precancerous lesions, morphometry was effective in distinguishing mild, moderate, severe epithelial dysplasia and carcinoma. The carcinogen induced cellular changes in epithelia. There was progressive increase in size and number of progenitor cells with slight increase in the more matured cells.[8]

Abdel – Salam et al[9] conducted an image cytometry study in oral hyperplasia and dysplasia. They found that nuclear area is a useful parameter for discriminating among the various groups. Overlap between the moderate and severe dysplasia group were seen. There was increase in cell area, nuclear area and N/C ratio in suprabasal cell layers of dysplastic epithelium as compared to normal. Morphometric parameters showed intergrade difference to differentiate among grades of dysplasia with significantly highest nuclear area in severe dysplasia and lowest cumulative values in mild dysplasia. The findings of the present study is similar to the above mentioned study.

The site wise variation in the N: C ratio was explained by Jin. et al[10] that the mean N: C ratio in palatal mucosa was 0.618±0.98. It was observed that N:C ratio in palatal mucosa are higher compared to the values of normal buccal mucosa in our study.

There are many qualitative studies on the cellular morphology of normal and abnormal epithelia, but few have recorded actual size or area of individual cell types, with very few studies involving oral epithelium. Hence, no comparisons could be made with their data.

CONCLUSION

The simple, inexpensive and easy morphometric analysis method can make the histomorphological study of tissues with premalignant lesions a more objective and practically applicable one for the early detection of cancer. Thus, morphometric analysis could serve as a discriminatory model and help in more accurate assessment of lesions which are highly proliferating and dysplastic and their malignant potential.

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