International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Histomorphometric Characterization of Oral Potentially Malignant Disorders and OSCC: New Age Perspective

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Abstract: ***Introduction: Early detection of potentially malignant lesions is the most crucial determinant for successful treatment, better prognosis, and survival. 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, to investigate the cellular and the nuclear changes in correlation with the histological behavior of the lesions. The quantitative and qualitative analysis of several parameters may reveal incipient cellular changes and thus offer high reliability over routine histopathological examination. Aim and Objectives: Our present study was undertaken to assess, analyze and compare the morphometric parameters with the changes occurring in epithelial cells of OPMDs and OSCC. Materials and Methods: 20 cases were obtained and stained with hematoxylin and eosin. The sections were subjected to morphometric analysis to analyze all the morphometric parameters in OPMDs and OSCC using various image analyzer softwares (FIJI). Result: Quantification of 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.***

Keywords: Morphometry. Dysplasia, Oral squamous cell carcinoma, OSMF

1. Introduction

Oral squamous cell carcinoma accounts for around 94% of all malignant lesions in the oral cavity, a region where primary malignant tumors most frequently originate in the head and neck.1

The aetiology of head and neck epithelial carcinoma is considered to be a complex multifactorial and multistep process. It is a complex malignancy where environmental factors, viral infections, and genetic alterations most likely interact, and thus give rise to the malignant condition.2

Most oral cancers are usually preceded by clinical lesions, collectively referred to as oral potentially malignant disorders (OPMDs). They are precancerous lesions that can progress to oral cancer. It is underlined that all OPMDs should be approached with caution because even small, inconspicuous lesions can reveal severe dysplasia or unexpected malignancy. Therefore, it is important to detect a lesion in the precancerous stage.3, 4

The World Health Organization has approved the most extensively used system for diagnosing and grading oral epithelial dysplaisa (OED), which serves as the foundation for patient stratification. The histological changes ensue most evidently in the basal and parabasal layer of dysplastic epithelium.4, 5

Because of its potential to provide safety assurance and boost workflow efficiency, the COVID - 19 epidemic has propelled digital technology to become one of the most critical and irreplaceable technologies in dentistry. Information of patients gathered from a digital software or smart device helps in early detection or prevention of disease.6, 7

The term "digital pathology" refers to the use of computer workstations to view whole slide images (WSIs) that were created by scanning glass microscope slides at a high resolution; applications include teaching, research, and initial diagnostic reporting. The accuracy, consistency, and uniformity of research inclusion criteria and results could be improved with the help of digital pathology and image analysis. Image analysis, in general, can give more reproducible quantification of the morphology of individual cells or key tissue components.8

In the histological grading of OPMDs and oral squamous cell carcinoma (OSCC), the evaluation of cellular and nuclear characteristics is crucial. To investigate its relationship with the prognosis of various malignancies, the morphometry of epithelial tumor cells has been researched.9 Quantitative analysis of several parameters, such as nuclear cytoplasmic ratio, cellular and nuclear area, may reveal incipient cellular changes and thus offer high reliability over routine histopathological examination in impending and frank oral cavity malignancies, allowing for earlier diagnosis and better treatment.1^0

The purpose of this study was to evaluate the histomorphometric parameters (cellular area, nuclear area, N/C ratio, nuclear diameter, nuclear perimeter and nuclear volume density) in OPMD and OSCC cases.

2. Materials and Method

The study was conducted in the Department of Oral Pathology and Microbiology, Shree Bankey Bihari Dental College, Ghaziabad. Tissue specimens were retrieved from the archives. The control group normal buccal mucosa.15 biopsy specimens of histologically confirmed Oral Epithelial Dysplasia, Oral Submucous Fibrosis with dysplasia and Oral comprised of 5 paraffin embedded blocks of Squamous Cell Carcinoma were analyzed morphometrically.

All biopsy specimens were subjected to routine tissue processing, embedded in paraffin wax and tissue blocks were prepared. Tissue sections of 4 µm thickness were cut using a soft tissue microtome and stained with routine hematoxylin and eosin stain. The stained sections were observed under microscope using WHO criteria to establish the histopathological grade of Epithelial Dysplasia, OSMF and OSCC.

EXCLUSION AND INCLUSION CRITERIA: Healthy individuals without any systemic illness and without any deleterious habit, were included in the control group. Histopathological diagnosis of OPMDs (Leukoplakia and OSMF) with histological evidence of Oral epithelial dysplasia, OSMF and OSCC were included in the study. The most representative areas were selected from sections for morphometric analysis and improperly fixed tissues were excluded.

Morphometric Analysis

Images were acquired for morphometric analysis using a digital camera mounted to a Binocular research microscope (Olympus 40X magnification). Image analysis program Image - J was used to perform the measurements. Microscopic fields were chosen at random from each section for image analysis software. The selected field includes representative cells with discrete cellular and nuclear outlines, avoiding the overlapping of cells. Each selected field included 25 cells from both parabasal and spinous cell compartments.6 parameters - Nuclear area (NA), nuclear perimeter (NP), nuclear diameter (ND), cellular area (CA), nuclear/cytoplasmic ratio (N/C), and nuclear volume density (NVD) were analyzed in all the selected fields. Following criteria was utilised as adapted from **Harikrishnan et al**:

Cell area (CA), nuclear area (NA), Nuclear Perimeter: Cellular area, nuclear area and nuclear perimeter were calculated after tracing the nuclear and cellular outlines through the software.

Nuclear Diameter: It was estimated by taking the average of the minimum and maximum nucleus diameters.

Nuclear/cytoplasmic ratio: It was calculated by using the formula:

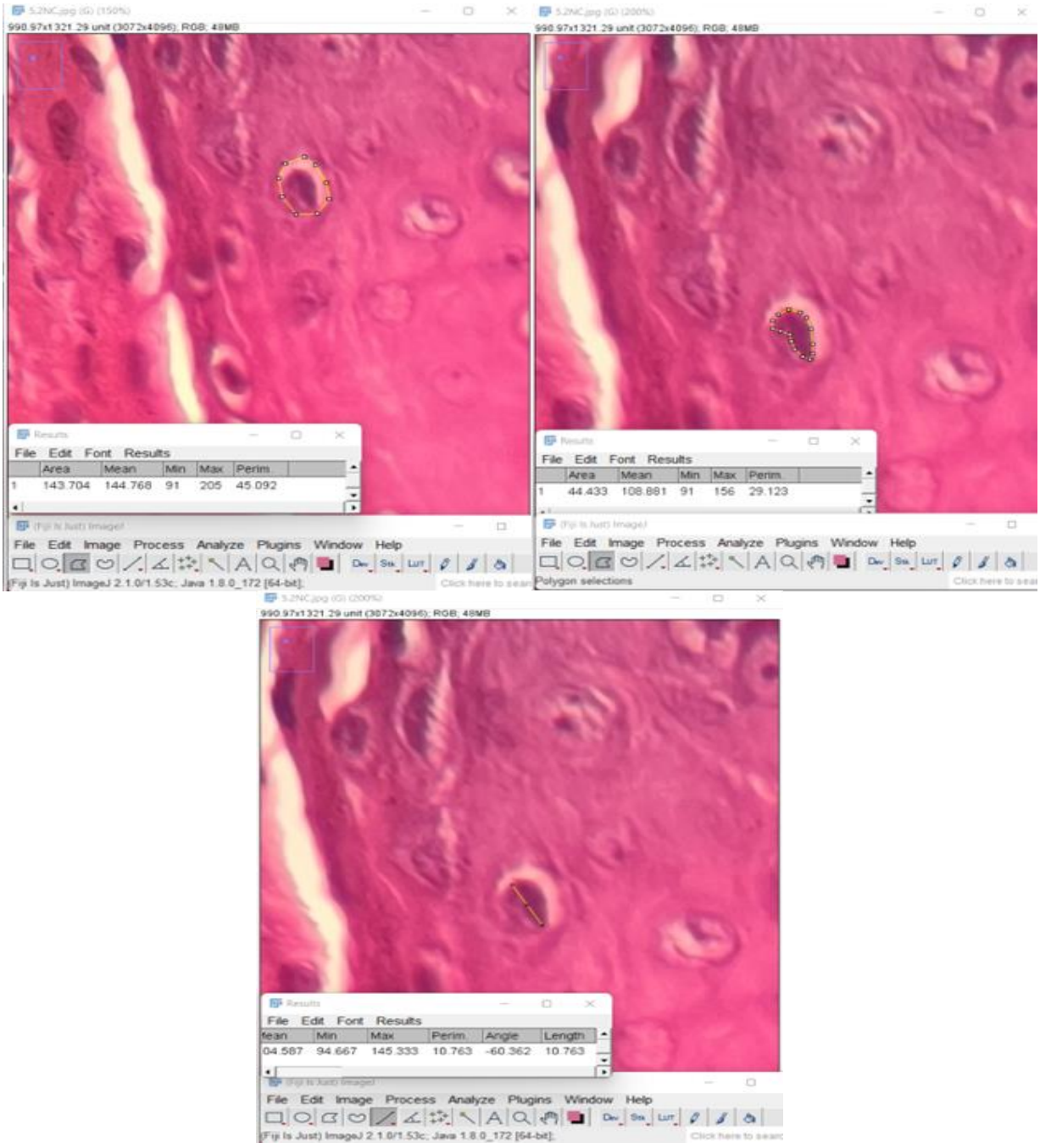
Nuclear area / [Cellular area − Nuclear area]

Nuclear volume density: It was calculated by using the formula:

NVD = Nuclear area / Cellular area

One - way ANOVA test was used to assess the differences between the groups. The analysis of variance revealed a significant difference in the cell area of suprabasal cells across the groups. p - value ≤ 0.05 with 15% confidence interval was considered statistically significant.

Figure



3. Result

OSCC exhibited the smallest cellular area (130.16) followed by controls (131.92), OSMF (134.36) and dysplasia (145.52) (statistically significant $p \leq 0.05$). However, the nuclear area was found to be lower in controls (35.8) than OSMF (36.84), dysplasia (41.68) and maximum in OSCC (46.76) (statistically significant ($p \leq 0.05$). The nuclear perimeter was found to be highest in OSCC followed by dysplasia, control and OSMF. Nuclear diameter was highest in OSCC followed by dysplasia, OSMF and control. (**Table - 1)**

The ratio of nuclear/cytoplasmic area was observed to be lowest in control (0.373) followed by OSMF (0.377), Oral Epithelial Dysplasia (0.402) and highest in OSCC (0.564). Nuclear Volume Density in suprabasal cells were lower in control (0.271), OSMF (0.274), Dysplasia (0.286) and OSCC (0.359). (**Table - 2)**

Table 1

Groups	Cell Area	Nuclear Area	Nuclear Perimeter	Nuclear Diameter
Controls	131.92±1.85	35.8±1.41	12.08±0.72	8.72±0.67
OSMF	134.36±0.67	36.84±0.61	12.04±0.35	9.36±0.59
Dysplasia	145.52±4.76	41.68±2.08	18.24±0.35	11.28±0.75
OSCC	130.16±0.84	46.76±4.34	19.68±0.64	13.24±0.32
p - value	0.00001	0.0002	0.00001	0.00001

Table 2

GROUPS	NC RATIO	NVD
Controls	0.373±0.02	0.271±0.01
OSMF	0.377±0.01	0.274±0.005
Dysplasia	0.402±0.02	0.286±0.01
OSCC	0.564±0.07	0.359±0.03
p - value	0.00002	0.00001

4. Discussion

OSCC is one of the most common cancers in the world, accounting for around 3%–5% of all malignancies. It is generally preceded by the potentially malignant disorders of oral cavity. The term "potentially malignant" implies that a particular lesion will eventually become malignant. Clinically, it is critical to determine the likelihood of malignant transformation of OPMD into invasive carcinoma, because predicting fate for an individual patient remains challenging once transformation has happened. As a result, early and careful identification is critical so that measures can be done to halt the progression of OPMD to OSCC.[11]

There exists a subjective variability during the histopathological examination. Therefore, interest has turned toward the sophisticated method of computer - assisted morphometric analysis method. A combination of several parameters provides a more accurate indication of tumor progression and behaviour rather than a single parameter.[3]

In this study, cell area in OSCC (130.16±0.84 μm²) was less than in normal mucosa, OSMF and dysplasia (131.92±1.85 μm², 134.36±0.67 μm² and 145.52±4.76 μm²). Our findings are consistent with Ramesh et al[12] who documented a decrease in cell area from normal to dysplastic and tumor cells in OSCC. According to Gao et al[13], as carcinogenesis continues, shrinkage of cell junctions leads to a rise in intercellular spaces and, ultimately, a decrease in cell area.

The mean values of NA and NP, which are 46.76 μm and 19.68 μm respectively, were found to be higher in OSCC. These findings were discovered to be similar to those by Gupta et al.[14], Smith et al.[15], and Shabana et al[16]. According to their findings, NA and NP gradually increased from OSMF, leukoplakia, to OSCC. The rapid and abnormal proliferation of neoplastic cells as well as an increase in DNA synthesis are the main causes of this increase in NA and NP in OPMDs and OSCC.

Abdel - Salam et al[18] analyzed oral hyperplasia and dysplasia using image cytometry. They discovered that nuclear area is a useful measure for differentiating various groups. There was overlap between the moderate and severe dysplasia groups. In comparison to normal, there was an increase in cell area, nuclear area, and N/C ratio in suprabasal cell layers of dysplastic epithelium. The current study's findings are comparable to those of the previous study.

An increase in the N/C ratio is listed as a standard hallmark of cellular atypia and is regarded as an indication of premalignant transformation in suspicious lesions. One of the persistent results during the transformation from benign to malignant, according to Callimeri and Smith[19], was an increased nuclear to cytoplasmic ratio. The present results showed the N/C ratio of OPMDs, i. e., OSMF (0.377μm), Dysplasia (0.402 μm) was significantly lower than that of OSCC (0.564μm), which is similar to the findings of Jin et al[20] Ramaesh et al[12] and Gupta et al[14] where they observed a decrease in the N/C ratio from OSCC to OPMD to normal mucosa. According to our study, there was an increase in NVD in OPMDs and OSCC compared to the control group, which may be the result of neoplastic cells proliferating abnormally and quickly.

5. Conclusion

Early intervention is the key factor in managing potentially malignant lesions and conditions. Histopathological considerations may help in recognizing early changes and thereby promoting early diagnosis. Oral pathologists may find morphometry to be a useful tool for estimating the number of cells and cell structures and thereby aid in the more precise assessment of lesions that are highly proliferative and dysplastic, as well as their malignant potential. Nuclear morphometric measurements made with image analysis tools are thought to be an objective and reliable approach for predicting patient survival. Hence, computer - assisted histomorphometric approaches can be utilized to objectively determine the degree of malignancy of oral tissues.

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