Morphometric Analysis of Suprabasal Cell Layer in Oral Epithelial Dysplasia: A Computer-assisted Microscopic Study

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ABSTRACT

Background: Oral leukoplakia is the most common precancerous lesion. Various grading systems based on histological findings have been proposed for dysplasia. Recently, computer-assisted morphometric analysis has been established to reduce the interobserver and intraobserver variability during the histological grading of epithelial dysplasia. This study was undertaken to establish the morphometric changes in the suprabasal cell layer of different grades of oral epithelial dysplasia.

Materials and Methods: Forty paraffin-embedded tissue sections (10 normal mucosa, 10 mild dysplasia, 10 moderate dysplasia, and 10 severe dysplasia cases) were stained using hematoxylin and eosin stain, and analyzed for cellular and nuclear morphometry using binocular microscope.

Results: Our results showed that values of nuclear area, nuclear perimeter, nuclear volume density, and nuclear/cytoplasmic (N/C) ratio were increased gradually in dysplasia compared to control groups and the values were statistically significant (P = 0.001). Nuclear diameter and cellular area were increased in dysplasia when compared to control group (P = 0.001). Mild and moderate dysplasia showed decreased value of nuclear form factor compared to control group, whereas severe dysplasia showed highest value. A fair correlation was found when comparing histological grading and grouping based on nuclear area, nuclear perimeter, N/C ratio, and nuclear volume density.

Conclusion: Nuclear features reflect cell behavior, and its morphometric analysis can be considered as a reliable tool for differentiating various grades of epithelial dysplasia.

Keywords: Epithelial dysplasia, leukoplakia, morphometric analysis, nuclear form factor, nuclear morphology

INTRODUCTION

Oral squamous cell carcinoma (SCC) is one of the most common malignancies of oral cavity and oropharynx. It occurs due to genetic damage caused by risk factors leading to uncontrolled proliferation of tumor cells, which results in dysplasia, and clinically presents as precancerous and cancerous lesions.[1] Oral leukoplakia is the most common precancerous lesion. Clinical studies showed that malignant transformation rate of leukoplakia ranges from 3% to 6%.[2] Early detection of potentially malignant oral lesion is most important, especially for high-risk groups as it shows up to 17% of cancer transformation with a mean period of 17 years after diagnosis.[3] Prediction of malignant transformation by histological method is more appropriate compared to clinical method.[4]

The histological grading of dysplasia depends on subjective evaluation of morphological changes within...
the involved tissue. However, there exists an intra- and interobserver variability during the histological assessment of grading of dysplasia. Recently, computer-assisted morphometric methods have been used to overcome this. It is a more sophisticated and quantitative method for the assessment of structural changes within the tissue, and the results have been found to be reliable and reproducible.[5-7] Only few studies have tried to evaluate the nuclear features by morphometric method for grading of dysplasia. Therefore, this study was attempted to evaluate the nuclear and cellular features in suprabasal cell layer in histologically diagnosed cases of dysplasia using a computer-assisted method and to correlate the findings with the histological grading of dysplasia.

**Materials and Methods**

The study was conducted on tissue sections retrieved from the archives of the Department of Oral Pathology and Microbiology of our institute. The control group comprised normal oral mucosal tissue specimens obtained from healthy persons with no habits (10 cases). The study group comprised of clinically diagnosed cases of leukoplakia irrespective of sex and age and histologically confirmed as epithelial dysplasia according to 1997, World Health Organization (WHO) grading of epithelial dysplasia (total 30 cases; 10 cases of mild, moderate, and severe dysplasia each).

Tissue sections (4 µm) were obtained from formalin-fixed paraffin-embedded tissue blocks using soft tissue microtome, and the sections were stained using Harris’ hematoxylin and eosin stain. The stained sections were viewed under binocular microscope using ×40 objective for morphometric analysis. Images were captured using ProgRes C3 camera and imaging software (ProgRes C3 CapturePro V2.7.7, Jenoptik Optical Systems, Jena, Germany) attached to the microscope. The final image had a magnification of ×400. For each case, five microscopic fields were randomly selected, and 20 complete, clear, nonoverlapping cells in suprabasal layer were evaluated [Table 1]. Basal cells were not included as they have indistinct cellular borders. While measuring the cells, histologically identifiable non-keratinocytes, keratinocytes undergoing mitosis, cells showing degeneration, and infiltrating lymphocytes were excluded. All the slides were blinded and histological diagnosis was not revealed during the morphometric analysis. Nuclear area, nuclear perimeter, nuclear diameter (ND), cellular area, nuclear form factor, nuclear/cytoplasmic ratio (N/C), and nuclear volume density (NVD) were analyzed in all the selected cells.

**Nuclear perimeter**

Nuclear perimeter was measured in microns. For measuring perimeter, nuclear and cellular outlines were traced and the software automatically calculated the perimeter (number of boundary pixels is converted to microns).

**Cellular and nuclear area**

From tracing of cellular and nuclear perimeter, software automatically calculated the cellular and nuclear area in square microns [Figures 1 and 2].

**Nuclear diameter**

After measurement of cellular and nuclear area, the same cells were subjected for calculation of ND. It was calculated by taking the average of minimum and maximum diameter of nucleus.

**Nuclear/cytoplasmic ratio**

It was calculated by using the following formula:

\[ \frac{N}{C} \text{ ratio} = \frac{\text{nuclear area}}{\text{cellular area} - \text{nuclear area}} \]

**Nuclear form factor**

The shape of the nucleus was assessed by form factor. It was calculated by using the following formula:

\[ \text{Form factor} = \frac{4\pi \text{ area}}{\text{perimeter}^2} \]

where \( \pi = \frac{22}{7} \).

**Nuclear volume density**

It was calculated by using the following formula:

\[ \text{NVD} = \frac{\text{nuclear area}}{\text{cellular area}} \]

All the measurements were made in microns, and the values were entered in Microsoft Excel sheet for further statistical analysis. The mean and standard deviations were calculated for different parameters. The difference in the various groups for different parameters was compared using one-way analysis of variance and Chi-square tests.

**Results**

One-way analysis of variance is used for comparing the parameters of different groups. The mean nuclear area,
nuclear perimeter, N/C ratio, and NVD were found to be increased in study groups when compared to control groups, and the values were increased in increasing grades of dysplasia and found to be statistically significant \((P < 0.001)\) as seen in Table 2 and Figures 3 and 4. ND and cellular area were increased in study group when compared to control group and were found to be statistically significant \((P < 0.001)\), but when compared within grades of dysplasia, they were not increased in a gradual manner [Figures 3 and 5]. When comparing the value of nuclear form factor between study and control group, it was found that mild and moderate dysplasia showed decreased value compared to control group, whereas severe dysplasia showed highest value and was found to be statistically significant \((P < 0.001)\) [Figure 4]. When comparing the histological grading of dysplasia and grouping based on nuclear area, nuclear perimeter, N/C ratio, and NVD, a fair correlation was found [Table 2]. It was also noted that most of the nucleus in control group was round with regular membrane outline, whereas nucleus in study group showed oval shape with irregular membrane outline. The number of nucleoli and mitotic figures also were increased in increasing grades of dysplasia.

**Discussion**

SCC of the oral cavity is one of the ten most common cancers in the world, accounting for approximately 3%–5% of all malignancies.\(^7\) It is usually preceded...
by one of the potentially malignant disorders of oral cavity. Oral leukoplakia is the most common potentially malignant disorder of the oral cavity. There exists a subjective variability during the histopathological grading of dysplasia. Therefore, interest has turned toward the sophisticated method of grading of dysplasia by using computer-assisted morphometric analysis method.\cite{5-7} Many authors have used cytomorphometric analysis for grading and for predicting prognosis in esophageal, laryngeal, uterine, and oral cancer.\cite{8-10} Various studies have also used the morphometry method for normal oral epithelium and also in various oral mucosal diseases such as lichen planus, epithelial dysplasia, oral submucous fibrosis, and traumatic keratosis.\cite{11-14}

A combination of several parameters provides a more accurate indication of tumor progression and behavior rather than a single parameter.\cite{15} Saku and Sato\cite{16} noticed an increase in the proportion of cells in the hyperdiploid to the hypertetraploid state in oral leukoplakia. In our study, we observed an increase in nuclear area, nuclear perimeter, N/C ratio, and NVD in dysplasia compared to control group and it increased gradually with increasing histological grades of oral epithelial dysplasia (mild, moderate, and severe dysplasia). This could be due to an abnormal and rapid proliferation of neoplastic cells. Increased nuclear size is related to an increased nuclear contents required for replication.\cite{17}

ND and cellular area increased from control group to dysplasia groups, but did not increase gradually
within the groups of dysplasia. This could be due to the fact that few dysplastic cells in mild dysplasia were in advanced stage, whereas cells in moderate and severe dysplasia were a stage behind in maturation or due to tissue artifacts.[18]

Variation in nuclear shape is an indicator of malignancy. Low-grade malignancy had somewhat regular nuclear outline, whereas high-grade lesions had more irregular outline.[15] This can be assessed by nuclear form factor. In our study, nuclear form factor was found to be decreased in dysplasia compared to control group, suggesting increased irregular nuclear outline in dysplasia groups. But the value was not decreased gradually within the dysplasia groups. This result was in contrary to the findings by Nandini and Subramanyam,[15] in 2011, where they had a gradual decrease in the values of nuclear form factor from mild dysplasia to severe dysplasia but the values were not statistically significant.

Eveson and MacDonald[19] noticed a progressive increase in size (represented by mean cellular area) and number of progenitor cells compared to matured cells of hamster cheek epithelium after the application of carcinogen to it. These findings correlate with our observation of increase in cellular area in the spinous cell layer compared to superficial cells.[17,19,20] Previous studies found that tissues undergoing malignant transformation typically showed a reduction in cellular area before showing reduction in nuclear area, using semiautomatic image analysis techniques.[6,17] Shabana et al.[5] studied the shape and size of the cells in basal cell layer of the oral epithelium, and they found a progressive increase in the dimensions of nuclei from normal mucosa through traumatic keratosis, lichen planus, leukoplakia, and risk group to carcinoma, with considerable variations.

Many of the studies have focused on nuclear changes in the development of malignancy, but few studies have focused on N/C ratio and NVD. In our study, N/C ratio and NVD were increased in dysplasia group compared with those in control group, and it increased gradually with increased grades of dysplasia. This was in accordance with the results of a study by White et al.[21] in leukoplakia with dysplasia; Raju Ragavendra et al.[20] and Truelson et al.[14] stated that nuclear DNA content and nuclear area were better indicators for the biologic aggressiveness of cancer in laryngeal cancers.[6] Ramaesh et al.[12,13] used cytomorphometric method for the assessment of ND and cell diameter (CD) in normal oral mucosa, dysplastic lesions, and SCCs. They found that cellular diameter was highest in normal mucosa, lower in dysplastic lesions, and lowest in SCCs, whereas ND was lowest in normal mucosa, higher in dysplastic lesions, and highest in SCCs. These studies suggested that increased ND and decreased CD can be used as early indicators of malignant transformation, and the exfoliative cytology can be used as a valuable tool for monitoring the clinically suspected lesions and for early detection of malignancy.

**Conclusion**

Nuclear morphology reflects the cell’s biological potential. Hence, the combination of several nuclear parameters indicates the behavior of tumor...
aggressiveness. Morphometric method provides an opportunity to quantify the nuclear changes and forms an objective basis for grading of dysplasia. Using various parameters with a computer-assisted method, we attempted for grading the oral epithelial dysplasia. We found that the results of computer-assisted morphometric analysis correlated well with the histological grading of dysplasia, and might help to reduce the interobserver variability, as it makes a precise objective assessment for grading of dysplasia.

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**Conflicts of interest**
There are no conflicts of interest.

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