Original Article

Morphometric analysis of basal cells of oral epithelium in predicting malignant transformation of oral potentially malignant disorders in patients with tobacco chewing habit

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Abstract

Background: Oral potentially malignant disorders (OPMDs) are a heterogenous group of disorders which precede the development of oral cancer. These are characterized by increased risk of malignant transformation to Oral cancer.

Aims & Objectives: In this study, an attempt has been made to assess the morphological alterations of the nuclei of the basal cells in OPMDs and oral squamous cell carcinoma (OSCC). Our objective was to compare the alterations and to assess the predictive factor of such alterations of basal cells in malignant transformation of OPMDs to OSCC.

Materials and Methods: This was a retrospective study conducted on tissue sections of 150 formalin fixed, paraffin embedded blocks obtained from the archives. The specimens were grouped into OSCC group (n= 50) and OPMDs (n= 100). Nuclear features were evaluated using computer- assisted microscopic image analysis. One- way ANOVA analysis was done to verify the difference between the groups for all variables.

Results: Our results showed statistically significant difference for all parameters between the groups. Among OPMDs, leukoplakia showed significant increase in nuclear area, nuclear perimeter, Nuclear/Cytoplasm (N/C) ratio, density and loss of polarity, while OSF showed significance with only perimeter, density and loss of polarity.

Conclusion: Based on findings of present study, it is concluded that measurements using computer- aided morphometric analysis may provide an objective means for predicting the malignant transformation OPMDs to OSCC. Among OPMDs, Leukoplakia has a higher chance of malignant transformation than OSF

Keywords: Leukoplakia, malignant transformation, morphometric analysis, nuclear parameters, oral squamous cell carcinoma, oral submucous fibrosis

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Submitted: 07-Feb-2020, **Accepted:** 07-Oct-2020 , **Published:** 09-Jan-2021

INTRODUCTION

Despite the advancement of medical technologies and prognostic aids, cancer of oral cavity (COC) still remains as one of the highly prevalent cancers and

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.JOMFP_55_20

How to cite this article: Santoshi CK, Kumar JV, Bhagirath PV, Vinay BH, Prakash YJ. Morphometric analysis of basal cells of oral epithelium in predicting malignant transformation of oral potentially malignant disorders in patients with tobacco chewing habit. J Oral Maxillofac Pathol 2020;24:579-80.

also leading cause of mortality in developing countries, contributing nearly one-third of all cancers.[1] In 2018, COC accounted for an estimated 350,000 new cases and 177,000 deaths. In India alone, an estimated 120,000 new patients with OCC were diagnosed in 2018, of which about 72,000 patients died.[2] It was found to be that cancer of mouth and tongue, overshadow cancer of lung, making cancer of mouth (excluding tongue) as second most common cancer among males in major cities of India. The projected burden of cancer by the year of 2020 will be lung (102,300), mouth (99,495), prostate (61,222), tongue (60,669) and larynx (36,079) that makes COC, leading cancer site for men in most of India.[3] Among all oral malignancies, squamous cell carcinoma (SCC) is considered the lesion with the highest prevalence, accounting up to 90% of malignant tumors of the head-and-neck region.[4,5]

Many researchers believed that early diagnoses of oral lesions are important in both prevention and therapeutic procedures. Most of the oral cancers are usually preceded by asymptomatic clinical lesions, collectively referred to as oral potentially malignant disorders (OPMDs).[6] Moreover, the most commonly encountered OPMDs are leukoplakia, erythroplakia, oral submucous fibrosis (OSMF), oral lichen planus (OLP), etc. The prevalence rate of OPMDs ranges from 1% to 5% worldwide.[7] The term OPMD stands for clinical diagnosis, for which the histological diagnosis will be hyperplasia, hyperkeratosis, oral epithelial dysplasia (OED) or oral SCC (OSCC).

Current research has shown that malignant transformation of OPMD is mainly due to increased proliferation of basal layer cells under the influence of mediators from inflammatory infiltrate that in turn activates different pathways, leading to the development of tumor.[8] It was found to be that the overall malignant transformation rate (MTR) of leukoplakia was 3.5%, with rate varied between 0.13% and 34%,[9] MTR of OLP is 1.1% with variation between 0% and 10%[10,11] and MTR of OED ranges between 1.4% and 36%.[12,13] It is believed that variation in transformation is mainly attributed to differences in follow-up times, selection of patients and tobacco habits.

It is emphasized that all OPMDs should be viewed with suspicion because even small, subtle lesions can manifest significant dysplasia or unsuspected carcinoma.[14] Recognition of dysplastic changes in earlier stage is vital in preventing carcinoma changes.[15] Dysplasia refers to abnormal growth and grading of OED into mild, moderate and severe is used to assess the probability of malignant transformation. It has been reported that 7%–50% of severe, 3%–30% of moderate and <5% of mild dysplastic lesions are capable of transforming into carcinomas.[4,16,17] The more are the dysplastic changes occur, the more unusual is the basal and parabasal epithelium across the whole oral epithelium. Dysplasia comprises a loss in the uniformity of individual cell as well as loss in architectural orientation.[18] Commonly seen dysplastic changes are nuclear hyperplasia, hyperchromatism, prominent nucleoli, increased nuclear-to-cytoplasmic ratio, increased cellular density, abnormal mitotic figures, epithelial pearls, basal cell hyperplasia, loss of polarization, etc.

Owing to unreliability associated with the subjective evaluation of epithelial dysplasia and histopathological diagnosis, many researchers sought for more objective approach regarding the predictive value of the atypical appearance of the basal layer. Computer-aided morphometric analysis reduces the errors of interobserver variation, would be of value to improve reproducibility and histopathological diagnosis. In this study, an attempt has been made to assess the morphological alterations of the nuclei of the basal cells in OPMDs and OSCC. Our objective was to compare the alterations and to assess the predictive factor of the such alterations of basal cells in the malignant transformation of OPMDs to OSCC.

MATERIALS AND METHODS

Sample

Our study comprised tissue sections of 150 formalin-fixed, paraffin-embedded blocks obtained from the archived blocks of biopsy specimens, department of oral pathology and microbiology. These specimens were then divided into two groups as Group I and Group II. Group I included fifty samples of OSCC which is a control group and Group II is a study group with a total of 100 samples. Group II is again divided into two subgroups, i.e., IIA comprises fifty samples of leukoplakia and IIB comprises fifty samples of OSMF. The study was approved by the ethics committee of the institute (PMVIDSandRC/IEC/OMFP/DN/0096-16). Informed consent regarding the use of tissue blocks for the purpose of the study was obtained from all patients in accordance with institutional guidelines.

Tissue sectioning

Tissue sections of approximately 5 μm thickness were cut using a soft-tissue microtome. The sections were stained using Harris's hematoxylin and eosin. All slides were coded to make sure that the observer must not be aware regarding clinical diagnosis and histological grading during

the measurements. The stained sections were observed under research microscope and were then microscopically photographed using camera.

Computer-aided morphometric analysis

For each case, five microscopic fields were randomly selected. Photographs of selected fields were taken, captured on to the hard drive of the computer where they were analyzed using T- Capture image analysis software (Version 3.9.0.605) Tucsen Scientific Camera, Photonics Co., Ltd., 2011 (Basic version), Fuzhou, Fujian Province of China. In each case, nuclei of tumor cells with clear, complete and nonoverlapping outlines were randomly selected to analyze variables such as nuclear area (NA), nuclear perimeter (NP), nuclear-cytoplasmic ratio (N/C), density of basal cells and loss of polarity (LOP) of the basal layer.

- NA and NP: NA was measured in μm^2 and NP was measured in μm (microns) by tracing around the nucleus. The software then automatically calculates and presented the output of NA and NP. Schematic illustrations of measurement of NA and NP were presented in Figure 1. For each field, five largest nuclei with clear outlines were selected
- N/C ratio: The N/C ratio was calculated using the formula N/C ratio = NA/Cell area − NA
- The density of the basal cells: Basal cell density was measured to represent the degree of abnormal proliferation of basal cells. It was calculated to evaluate the density per unit length [Figure 2]. The calculation formula was as follows:
  The density of basal cells = number of the basal cells/length of the basement membrane
- LOP: LOP of basal layer is considered as one of the representative findings for predicting the progression of OED into invasive carcinoma. In the present study, we quantified LOP using morphometric methods, i.e., (1) length between the upper pole of the nucleus and the basal cell membrane (LOP1) and (2) length between the apical membrane of the cells and the basement membrane (LOP2) [Figure 3].

The measurement units for the morphometric analysis include μm^2 for NA and cytoplasmic area, whereas it is μm for the rest of the other parameters. All the measurements are saved in Microsoft Excel for further statistical analysis.

Statistical analysis

Statistical analysis was performed using SPSS package version 20.0 (IBM, Chicago, IL, USA). $P < 0.05$ was considered statistically significant. One-way ANOVA was performed to compare the differences between the groups (control and study), followed by Tukey's honestly significant difference multiple comparison test. Pearson's correlation was performed to analyze correlation between all parameters in OSCC, leukoplakia and OSF.

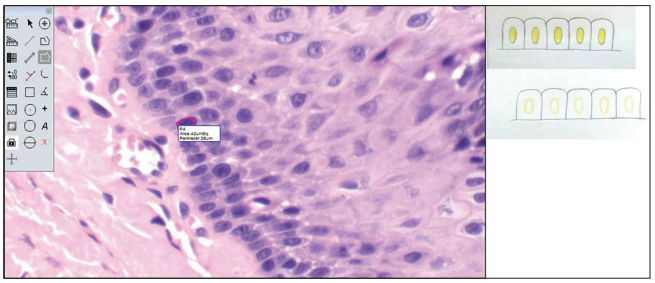

Figure 1: Measurement of nuclear and nuclear perimeter

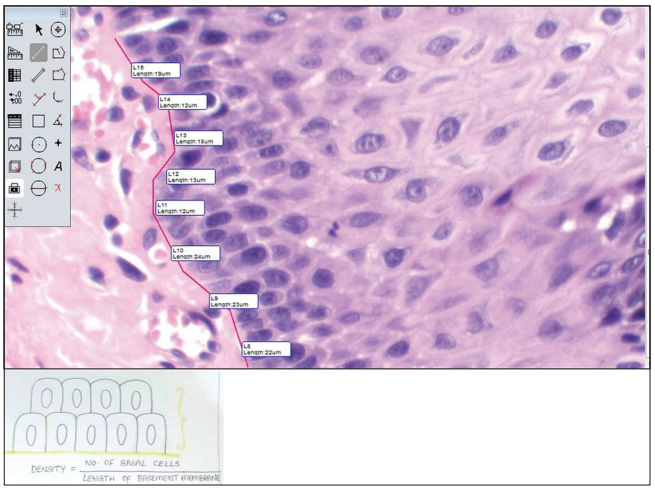

Figure 2: Measurement of density of basal cells

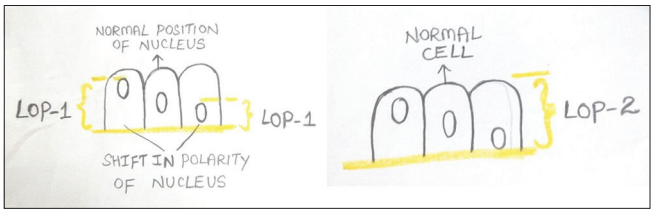

Figure 3: Measurement of loss of polarity (1 and 2)

RESULTS

The mean NA for control group (OSCC) was 44.33 μm^2, whereas for leukoplakia and OSF groups, it was 40.65 and 36.48 μm^2, respectively. The mean NA was progressively decreased from OSCC to OPMDs. One-way ANOVA showed that there was a statistically significant difference in the NA among the groups ($P = 0.0001$; i.e., <0.05) [Table 1].

The mean NP for control group (OSCC) was 25.12 μm, 24.06 μm for leukoplakia and 23.1 μm for OSF. It was

Table 1: Analysis of variance of nuclear area between study and control groups

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	*F*	*P*
Between groups	2	1543.43	771.7155	11.1432	0.0001*
Within groups	147	10180.35	69.2541		
Total	149	11723.78			

*Statistically significant ($P<0.05$)

Table 2: Analysis of variance of nuclear perimeter between study and control groups

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	*F*	*P*
Between groups	2	102.09	51.046	8.396	0.0004*
Within groups	147	893.70	6.079		
Total	149	995.8			

*Statistically significant ($P<0.05$)

Table 3: Analysis of variance of nuclear-cytoplasmic ratio between study and control groups

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	*F*	*P*
Between groups	2	0.5533	0.2766	6.9353	0.0013*
Within groups	147	5.8638	0.0399		
Total	149	6.4171			

*Statistically significant ($P<0.05$)

Table 4: Analysis of variance of density of basal cells between study and control groups

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	*F*	*P*
Between groups	2	0.0350	0.0175	11.1506	0.0001*
Within groups	147	0.2309	0.0016		
Total	149	0.2659			

*Statistically significant ($P<0.05$)

Table 5: Analysis of variance of loss of polarity 1 between study and control groups

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	*F*	*P*
Between groups	2	83.28	41.64	10.843	0.0001*
Within groups	147	564.55	3.84		
Total	149	647.83			

*Statistically significant ($P<0.05$)

Table 6: Analysis of variance of loss of polarity 2 between study and control groups

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	*F*	*P*
Between groups	2	140.52	70.26	14.868	0.0001*
Within groups	147	694.64	4.72		
Total	149	835.16			

*Statistically significant ($P<0.05$)

also observed that there was a gradual decrease in mean NP from OSCC to OPMDs. One-way ANOVA showed that there was a statistically significant difference in the NP among the groups ($P = 0.0004$; i.e., <0.05) [Table 2].

The mean N/C ratio of OSCC group was 0.836 μm, leukoplakia was 0.982 μm and OSF was 0.889 μm. It was also observed that the mean of OPMDs was significantly higher when compared to the mean of OSCC individuals ($P = 0.0013$; i.e., <0.05) [Table 3].

The mean density of basal cells for OSCC group was 0.154 μm, leukoplakia was 0.189 μm and OSF was 0.182 μm. It was also observed that the mean of OPMDs was significantly higher when compared to the mean of OSCC individuals ($P = 0.0013$; i.e., <0.05) [Table 4].

Tables 5 and 6 show a statistically significant difference (One-way ANOVA) between the groups in terms of loss of polarity 1 and 2. Mean values of LOP 1 for OSCC, leukoplakia and OSF were 14.5, 13.4 and 12.6, and the mean values for LOP 2 were 17.5, 16.1 and 15.2, respectively. It was also observed that loss of polarity (both 1 and 2) was found to be more in OSCC group and then gradually decreased for both leukoplakia and OSF.

DISCUSSION

The term "premalignant" is commonly used and widely understood, it entails considerable risks that an individual lesion may inevitably become malignant.[19] The term "potentially premalignant" is referred when the risk regarding the progression to malignancy is statistically increased.[20] From the clinical point of view, it is absolutely vital to assess the risk of malignant transformation of OPMD into invasive carcinoma, owing to the fact that the prediction of outcome for an individual patient remains difficult once transformation occurred. Therefore, the early and careful diagnosis is of extreme significance so that efforts can be made to arrest the progression of OPMD to OSCC. Researchers have believed that so far, the prediction of malignant transformation from epithelial dysplasia is subjective, suggested the need for sophisticated techniques to improve the histologic assessment of OED.[18] This led to the introduction of computer-assisted morphometric techniques, by which one can investigate the cellular and nuclear changes with respect to histological behavior of the lesions.

The present study carried out morphometric analysis of the cellular and nuclear measurements of basal and suprabasal cells among patients diagnosed with OSCC, leukoplakia and OSF. Numerous studies were done in the past applying morphometry for grading and for predicting prognosis of various cancers. And also, studies with respect to oral cavity were done applying morphometric techniques to normal mucosa as well as OPMDs such as leukoplakia, lichen planus, OSF and OEDs.[21-25]

The present results have shown that there is steady in NA and NP dimensions from OPMDs i.e., leukoplakia and OSF to OSCC. The mean values of NA and NP were found to be larger in OSCC, which are 44.3 μm^2 and 25.1 μm. These results were found to be compatible with those reported by Gupta *et al*.,[26] Smitha *et al*.[18] and Shabana *et al*.[23] Their results showed a progressive increase in NA and NP from OSF, leukoplakia to OSCC. This increase in NA and NP in OPMDs and OSCC is mainly attributed to rapid and abnormal growth of neoplastic cells and also due to increase in DNA synthesis.[24,27]

An increase in N/C ratio is cited as standard feature of cellular atypia and considered as a suggestive feature of premalignant change in suspicious lesions. White *et al*. expected the lowest N/C ratio to be seen in normal controls, nonneoplastic lesions without malignant potential and benign neoplasms, followed by potentially premalignant group and the malignant group would have substantially higher values or perhaps similar to that of potentially premalignant group.[28] The present results showed the N/C ratio of basal cells of OPMDs, i.e., leukoplakia (0.982 μm) and OSF (0.889 μm) was significantly higher than that of OSCC (0.836 μm), which is similar to the findings of Shabana *et al*.[23] Moreover, these findings were found contrary to that Jin *et al*.,[29] Ramaesh *et al*.[30] and Gupta *et al*.,[26] where they showed a decrease in the N/C ratio from OSCC to leukoplakia to normal mucosa.

The results of the present study showed that a significant relation is observed between OSCC and OPMDs in terms of density of basal cells. Within OPMDs, it showed a decrease in the density of basal cells from OSCC to leukoplakia and from OSCC to OSF, with a significant value, indicating high proliferative activity in the OPMDs. This proliferation may be due to release of local acetylcholine transmitters by the action of carcinogens, resulting in increased signal transduction pathways.

Cell community in human body comprises billions of cells; it reflects a dynamic system with a well-regulated balance between the cell proliferation and death. When this balance is skewed in favor of cell accumulation, the result is tumor development which in turn destructs the entire cell community.[31] Cell polarity is one such regulatory factor that governs cellular proliferation, death and their replenishment. The fundamental role of apical-basal polarity with respect to tumor suppression is to upkeep tight apical junctional complex and conversation of asymmetric cell division. In addition, evidence suggests that the proteins involved in cell polarity are usually targeted by several oncogenes.[32] The findings from morphometric study by Okamura *et al*. suggested that the disordered arrangement of the basal cells as loss of polarity may be helpful to predict the malignant transformation of OED.[33] Results of the present study showed a significant difference between OSCC and OPMDs, with a decrease in the loss of polarity from OSCC to leukoplakia and from OSCC to OSF. This might be due to increased expression of matrix metalloproteinases, resulting in a shift in the position of basal cell nuclei, leading to epithelial–mesenchymal transition and finally to invasion. Due to this, there observed a shift in the polarity of the nucleus and position of the basal cells in OPMDs.

CONCLUSION

A significant relation of morphometric parameters such as NA, nuclear perimeter, N/C ratio, density and loss of polarity of the basal cells is observed between OSCC and OPMDs. Within OPMDs, leukoplakia showed significance with almost all the factors included, than OSF with OSCC, indicating high chances of malignant transformation of leukoplakia to OSCC, compared to OSF. Morphological changes in the nucleus of the basal cells act as a diagnostic tool in predicting the malignant transformation of OPMDs to OSCC. Overall, from the results of the present study, it can be inferred that measurements using computer-aided morphometric analysis may provide an objective means for the assessment of epithelial dysplasia.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009;45:309-16.
2. Mummudi N, Agarwal JP, Chatterjee S, Mallick I, Ghosh-Laskar S. Oral Cavity cancer in the Indian subcontinent - Challenges and opportunities. Clin Oncol (R Coll Radiol) 2019;31:520-8.
3. Gupta B, Johnson NW. Oral cancer: Indian pandemic. Br Dent J 2017;222:497.
4. Shirani S, Kargahi N, Razavi SM, Homayoni S. Epithelial dysplasia in oral cavity. Iran J Med Sci 2014;39:406-17.
5. Tampa M, Caruntu C, Mitran M, Mitran C, Sarbu I, Rusu LC, *et al*. Markers of oral lichen planus malignant transformation. Dis Markers 2018;2018:1-13.
6. Reibel J. Prognosis of oral pre-malignant lesions: Significance of clinical, histopathological, and molecular biological characteristics. Crit Rev Oral Biol Med 2003;14:47-62.
7. Ranganathan K, Kavitha L. Oral epithelial dysplasia: Classifications and clinical relevance in risk assessment of oral potentially malignant disorders. J Oral Maxillofac Pathol 2019;23:19-27.
8. Liu Y, Messadi DV, Wu H, Hu S. Oral lichen planus is a unique

disease model for studying chronic inflammation and oral cancer. Med Hypotheses 2010;75:492-4.
9. Warnakulasuriya S, Ariyawardana A. Malignant transformation of oral leukoplakia: A systematic review of observational studies. J Oral Pathol Med 2016;45:155-66.
10. Aghbari SMH, Abushouk AI, Attia A, Elmaraezy A, Menshawy A, Ahmed MS, *et al*. Malignant transformation of oral lichen planus and oral lichenoid lesions: A meta-analysis of 20095 patient data. Oral Oncol 2017;68:92-102.
11. Landini G, Mylonas P, Shah IZ, Hamburger J. The reported rates of transformation of oral lichen planus. J Oral Maxillof Sur Med Pathol 2014;26:213-20.
12. Ho PS, Chen PL, Warnakulasuriya S, Shieh TY, Chen YK, Huang IY. Malignant transformation of oral potentially malignant disorders in males: A retrospective cohort study. BMC Cancer 2009;9:260.
13. Lumerman H, Freedman P, Kerpel S. Oral epithelial dysplasia and the development of invasive squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995;79:321-9.
14. Waldron CA, Shafer WG. Leukoplakia revisited. A clinicopathologic study 3256 oral leukoplakias. Cancer 1975;36:1386-92.
15. Neville BW, Day TA. Oral cancer and precancerous lesions. CA Cancer J Clin 2002;52:195-215.
16. Warnakulasuriya S, Reibel J, Bouquot J, Dabelsteen E. Oral epithelial dysplasia classification systems: Predictive value, utility, weaknesses and scope for improvement. J Oral Pathol Med 2008;37:127-33.
17. van der Waal I. Oral potentially malignant disorders: Is malignant transformation predictable and preventable? Med Oral Patol Oral Cir Bucal 2014;19:e386-90.
18. Smitha T, Sharada P, Girish H. Morphometry of the basal cell layer of oral leukoplakia and oral squamous cell carcinoma using computer-aided image analysis. J Oral Maxillofac Pathol 2011;15:26-33.
19. Speight PM, Khurram SA, Kujan O. Oral potentially malignant disorders: Risk of progression to malignancy. Oral Surg Oral Med Oral Pathol Oral Radiol 2018;125:612-27.
20. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med 2007;36:575-80.
21. Raju Ragavendra T, Rammanohar M, Sowmya K. Morphometric computer-assisted image analysis of oral epithelial cells in normal epithelium and leukoplakia. J Oral Pathol Med 2010;39:149-54.
22. Hegde V. Cytomorphometric analysis of squames from oral premalignant and malignant lesions. J Clin Exp Dent 2011;3:441-44.
23. Shabana AH, el-Labban NG, Lee KW. Morphometric analysis of basal cell layer in oral premalignant white lesions and squamous cell carcinoma. J Clin Pathol 1987;40:454-8.
24. Nandini DB, Subramanyam RV. Nuclear features in oral squamous cell carcinoma: A computer-assisted microscopic study. J Oral Maxillofac Pathol 2011;15:177-81.
25. Sunitha B, Kiran Kumar K, Hallikeri K, Rekha K. Morphometric analysis of nuclear changes in invasive tumor front of squamous cell carcinoma. J Orofac Sci 2010;2:9-12.
26. Gupta K, Gupta J, Miglani R. Computer aided morphometric analysis of oral leukoplakia and oral squamous cell carcinoma. Biotech Histochem 2016;91:251-4.
27. Böhm N, Sandritter W. DNA in human tumors: A cytophotometric study. Curr Top Pathol 1975;60:151-219.
28. White FH, Jin Y, Yang L. An evaluation of the role of nuclear cytoplasmic ratios and nuclear volume densities as diagnostic indicators in metaplastic, dysplastic and neoplastic lesions of the human cheek. Histol Histopathol 1997;12:69-77.
29. Jin Y, Yang LJ, White FH. Preliminary assessment of the epithelial nuclear-cytoplasmic ratio and nuclear volume density in human palatal lesions. J Oral Pathol Med 1995;24:261-5.
30. Ramaesh T, Mendis BR, Ratnatunga N, Thattil RO. Cytomorphometric analysis of squames obtained from normal oral mucosa and lesions of oral leukoplakia and squamous cell carcinoma. J Oral Pathol Med 1998;27:83-6.
31. Lee M, Vasioukhin V. Cell polarity and cancer-cell and tissue polarity as a non-canonical tumor suppressor. J Cell Sci 2008;121:1141-50.
32. Royer C, Lu X. Epithelial cell polarity: A major gatekeeper against cancer? Cell Death Differ 2011;18:1470-7.
33. Okamura T, Izumo T, Yagishita H, Mori T, Sakamoto K, Harada K. Disordered arrangements of basal cells as a prognostic factor for oral epithelial dysplasia: A morphometric study of 96 cases. Oral Surg Oral Med Oral Pathol Oral Radiol 2016;122:355-61.