Original Article

Operational Role of Apoptotic Index in Premalignant and Malignant Squamous Lesions: An Apprise

Abstract

Background: The aim of the study was to evaluate the role of apoptotic index (AI) in premalignant and malignant squamous lesions of different sites on light microscopy. **Materials and Methods:** Retrospective study of 75 cases of premalignant and malignant squamous epithelial lesions of different sites was done. All slides were H and E stained, screened for apoptosis under ×40. AI was calculated as the number of apoptotic cells and apoptotic bodies, expressed as percentage of total number of tumor cells counted in each case. **Results:** On statistical analysis, it was found that the difference in the apoptotic indices in all the subgroups of dysplasia was not statistically significant. However, the significant statistical difference was found within the malignant group, *P* value between well-differentiated squamous cell carcinoma (SCC) and moderately differentiated SCC (MDSCC) was <0.0001. *P* value obtained between MDSCC and poorly differentiated SCC was 0.0006. **Conclusion:** We conclude that apoptotic indices are useful in distinguishing between benign and malignant squamous lesions. Several indices such as proliferating index (Ki-67) and AgNOR count are not routinely available in various hospitals, especially in developing countries. The advantage of this technique is that it can be calculated in routine H and E stained sections, and so it saves time. Although it is labor-intensive, it is cost-effective method which can benefit the patient as it correlates well with tumor aggressiveness and thereby increasing the prognosis of the patients.

Keywords: *Apoptotic index, dysplasia, squamous cell carcinoma*

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Introduction

Cell proliferation and cell death maintain equilibrium between various cellular reactions such as regeneration, hyperplasia, dysplasia, hypertrophy, atrophy or metaplasia in multicellular organisms. Cell death appears to be a basic biological phenomenon which is maintained by the human body. It physiologically removes these cells, which is required for the regulation of tissue homeostasis.[1]

Apoptosis is defined as physiological and pathological processes of programmed cell death. It is characterized by cell shrinkage, blebbing of the plasma membrane, and nuclear condensation and fragmentation. Dysregulation and dysfunction of apoptosis may contribute to a variety of conditions such as cancer, viral infections, and immunological diseases which involve the oral cavity also.

Our study emphasizes on the role of apoptosis in premalignant and malignant lesions in different sites. Apoptosis is seen to increase with increasing grades of dysplasia and cancer. Hence, quantifying apoptosis can help us to identify the tumor aggressiveness. The percentage of apoptotic bodies in the tumor cells and dysplastic cells is designated as an apoptotic index (AI). Assessment of cell death is performed by counting the apoptotic cells and apoptotic bodies using a light microscope. Since this is relatively an easy method and is feasible under routine circumstances, this technique has been used widely.[2-5] The rate at which a tumor proliferates has long been considered to have a relationship to its clinical course, thus providing an easy means of accurately assessing the growth fraction of normal, dysplastic, and neoplastic tissue.[6-9] Early diagnosis of these lesions greatly increases the chance of cure and significantly reduces deformity in the patients. In the recent past, histological techniques that identify the parameters such as cell proliferation and cell death have become quite significant. Measuring these parameters may not only help in identifying the individuals who are

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at a greater risk of developing carcinomas but they also carry a significant prognostic value and also represent a good model of tumor development.[2,6]

It offers another advantage that tumors showing apoptosis are more sensitive to chemotherapy and likely to have a better prognosis, thereby, indicating the fact that the beneficial anti-cancer effects of chemotherapy are predominantly mediated through induction of apoptosis in tumor cells.[10]

Hence, the present study was undertaken with the purpose of evaluating the role of AI in premalignant and malignant squamous cell lesions affecting different sites.

Materials and Methods

The study population comprised of 75 cases with the diagnosis of squamous epithelial dysplasia and squamous cell carcinomas (SCC) of all grades, (H and E stained) in the pathology department of a tertiary medical college. We excluded all cases of patients undergoing chemotherapy and radiotherapy. In addition, improper sections, where tumor cells are not properly identifiable or infiltrated by necrosis/inflammatory cells were excluded from the study.

Counting and scoring technique

In the study sample, in each section, 1000 dysplastic cells/tumor cells were evaluated for the presence of apoptotic bodies/cells in ×40 magnification. A particular area was selected in the slide; a total number of epithelial cells were first counted and noted. Then, the number of apoptotic cells in that particular area was also counted. Then, the field was changed, and apoptotic bodies were counted in that particular area. Thus, in a given slide, apoptotic bodies present within the total of 1000 cells were counted. AI was calculated as the number of apoptotic bodies/cells expressed as a percentage of the total number of nonapoptotic tumor/dysplastic cells counted in each case. From each section, 10 fields devoid of artifacts were selected. There were two observers in this study, and the common results were formulated to avoid interobserver variability.

Morphologically, apoptosis is characterized by a series of morphological changes, which can be appreciated by light microscopy. On histologic examination with H and E stain, apoptosis involves single cells or small clusters of cells. The apoptotic cell appears as a round or oval mass with dark eosinophilic cytoplasm and dense purple nuclear chromatin fragments [Figure 1]. Nuclei show various stages of chromatin condensation and aggregation and ultimately, karyorrhexis.

Student's *t*-test was used to calculate *P* value. $P < 0.05$ was considered as statistically significant. Statistical analysis was performed using SPSS 12.0 for Windows (Microsoft, Seattle, WA, USA).

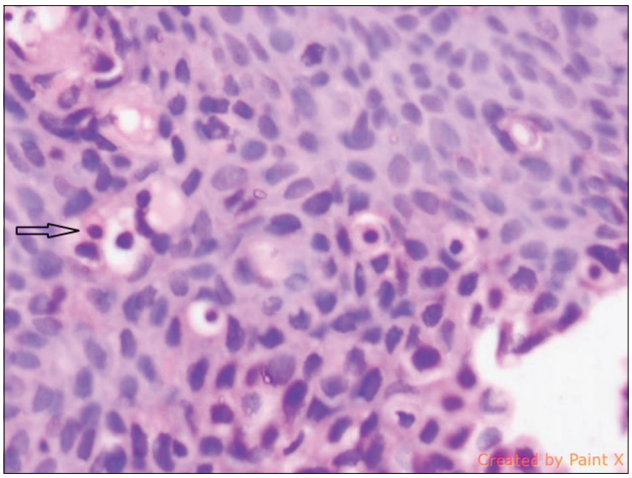

Figure 1: Tissue section showing squamous cell carcinoma showing apoptotic bodies in island of tumor cells (H and E, ×200)

Results

The *P* value obtained between mild dysplasia and moderate dysplasia is 0.13. *P* value between moderate and severe dysplasia is 0.7. *P* value between mild and severe dysplasia is 0.11.

The *P* value obtained between well-differentiated SCC (WDSCC) and moderately differentiated SCC (MDSCC) is <0.0001. *P* value obtained between WDSCC and poorly differentiated SCC (PDSCC) is <0.0001. *P* value obtained between MDSCC and PDSCC is 0.0006.

Discussion

Cellular proliferation and apoptosis are an important feature of dysplasia and carcinoma. They have been known to play an important role in tumor progression and development. The interrelationship and the role of each of these entities in the progression of the tumor are yet to be defined. Several indices can be used to measure cell proliferation such as AI, mitotic index, proliferating cell nuclear antigen, Ki-67, and AgNOR count; these have been recognized as useful prognostic indicators of tumor.[11] Likewise, there are various methods to detect apoptosis-like electron microscopy, flow cytometry, electrophoresis, *in situ* nick translation and terminal deoxynucleotidyl transferase-mediated dUTP biotin nick labeling method.[12] Although apoptosis has been evaluated and shown to correlate with tumor grade and subtype in various malignant lesions by various complicated and sophisticated methods, yet there are very few studies available in literature from India, which have evaluated apoptosis using simple light microscopy method in premalignant and malignant lesions of various sites. The present study is based purely on morphology as it is fairly reliable, easily practiced, and inexpensive method for detection of apoptosis.

Apoptotic bodies were seen in the suprabasal and basal regions of the normal mucosa and early dysplastic lesions,

but as the severity of the premalignant or malignant lesion increases, the apoptosis becomes generalized.[13,14] They are well recognized morphologically on light microscopy in formalin fixed H and E stained slides.

The present study was conducted on 75 cases of various lesions at different sites to evaluate AI and to correlate these indices with different grades of squamous premalignant and malignant lesions.

In the present study, majority of the lesions were malignant 58 cases (77.33%) and 17 cases (22.66%) were premalignant. In our study, we found a maximum number of premalignant and malignant lesions in the age group of 50–60 years (42.66%) which were followed by age group of 60–70 years (24%) [Table 1]. Vijaya *et al*. 2008 reported maximum number of premalignant lesions between 35–54 years (61.1%) and malignant lesions were in age group 35–64 years (80.2%).

Various sites were evaluated, with maximum propensity in base of tongue (20%). In fact, most of the premalignant and malignant lesions were reported in head-and-neck region (81.33%). Rest were observed in cervix, leg, foot, and vaginal vault [Table 2].

Histologically, we divided the premalignant lesions as mild, moderate, and severe. The most common type of premalignant lesion identified was mild and moderate dysplasia (16%). We observed that there was a mild reduction in AI in severe dysplasia when we compared the value to moderate dysplasia [Table 3].

In the present study, we further subdivided the malignant lesions into WDSCC, MDSCC, and PDSCC. There was statistically significant difference between the AI of all three groups and the majority of malignant lesions were in moderately differentiated group (26 cases i.e., 34.66%).

In the carcinomas, the apoptotic bodies were counted in the substance of the tumor avoiding the apoptotic cells that are present in the surrounding stroma and also those that are seen in the areas of necrosis and inflammation. AI increases gradually up to carcinoma *in situ* but decreases again in SCC.[15,16] In the present study also, AI increased progressively from normal to carcinoma but decreased with decreased differentiation of the tumor.

This study suggests that apoptotic function is not altered during progressive stages of dysplastic change in the epithelium, while proliferation is triggered only in late stages of dysplasia. To conclude, the apoptotic cells can be easily demonstrated in routine H and E stained sections, though a high degree of variability still exists in the AI reported by various authors. To avoid this interobserver variability, the established criteria for recognition and counting of apoptotic cells should be strictly adhered so that the lesions can be characterized properly according to their potential for invasiveness.

Table 1: Distribution of cases according to age

Age (years)	Number of cases (%)
20-30	3 (4)
30-40	4 (5.33)
40-50	3 (4)
50-60	32 (42.66)
60-70	18 (24)
>70	15 (20)

Table 2: Distribution of number of cases in percentage according to site

Site of lesion	Number of cases (%)
Tongue	5 (6.66)
Supraglottic	8 (10.66)
Border of tongue	15 (20)
Retromolar trigone	12 (16)
Left pyriform fossa	5 (6.66)
Cervix	10 (13.33)
Right pyriform fossa	6 (8)
Base of tongue	2 (2.66)
Uvula	8 (10.66)
Vaginal vault	1 (1.33)
Leg	2 (2.66)
Foot	1 (1.33)

Table 3: Mean apoptotic index in dysplastic and malignant squamous cell lesions in different sites

Type of lesion (number of cases)	Mean (AI%)
Mild dysplasia (6)	0.477±0.047
Moderate dysplasia (6)	0.533±0.047
Severe dysplasia (5)	0.525±0.043
WDSCC (24)	0.757±0.064
MDSCC (26)	0.607±0.082
PDSCC (8)	0.275±0.433

WDSCC: Well-differentiated squamous cell carcinoma, MDSCC: Moderately differentiated squamous cell carcinoma, PDSCC: Moderately differentiated squamous cell carcinoma, AI: Apoptotic index

Conclusion

We conclude that apoptotic cells can be readily and accurately demonstrated on routine H and E stained sections. AI is the simplest, easily available and time-saving technique that can be employed in any laboratory especially in a developing country like ours. Proliferative and apoptotic indices are useful in distinguishing between benign and malignant lesions. Few more studies are required for utilizing apoptosis as target in the anti-cancer therapy development and thus improving the prognosis.

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Conflicts of interest

There are no conflicts of interest.

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