

ORIGINAL RESEARCH

Apoptosis: A Prognostic Marker for Premalignant and Malignant Squamous Cell Lesions of the Oral Cavity: A Study of 60 Cases

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ABSTRACT

Introduction- Apoptosis is the process of programmed cell death, is highly regulated biochemical process used by organism for the deletion of cell in normal and malignant tissue. Oral cavity cancers are the most common cancers in men and the third among the women's in India. Different prognostic factors for premalignant and malignant squamous cell lesion of oral cavity but they do not give any information regarding the biological characteristic of tumour. Therefore we need new prognostic factor that give information regarding the aggressiveness of tumour. The present study was design to assess the significance of apoptotic index as a prognostic marker in the premalignant and malignant squamous cell lesion of oral cavity.

Materials and Methods: The study included 60 patients presenting with oral lesions. Evaluation of apoptotic index (AI) (using light microscopy) was performed on haematoxylin and eosin-stained sections. Student's t test was performed.

Results: The maximum mean AI was observed in WD SCC 0.7600 ± 0.0966 and with progression towards higher grades. A fall was noted, the values being 0.5417 ± 0.0669 in MD SCC and 0.4500 ± 0.0535 in PD SCC. The difference between WD SCC and PD SCC was found to be significant ($p < 0.001$; highly significant). Similarly, despite an increase in the mean AI with an increasing degree of dysplasia (mild dysplasia = 0.2333 ± 0.0888 , moderate dysplasia 0.4600 ± 0.0966 , severe dysplasia = 0.6000 ± 0.0756). The mean AI increased progressively from dysplasia (0.4067 ± 0.1760) to SCC (0.5900 ± 0.1470).

Conclusion: This study result showed that tumour with high AI has good prognosis in contrast to tumour that exhibit less apoptosis and tend to show aggressive behaviour and greater potential for metastasis.

Keywords: Apoptosis, premalignant, malignant, squamous cell carcinoma

How to cite this article: Anjana Arya, Nitesh Mohan, Som Dutt Singh. Apoptosis: A prognostic marker for premalignant and malignant squamous cell lesions of the oral cavity: a study of 60 cases. International Journal of Contemporary Medical Research 2015;2(4):852-855

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Source of Support: Nil

Conflict of Interest: None

INTRODUCTION

Apoptosis is the process of programmed cell death, is highly regulated biochemical process used by organism for the deletion of cell in normal and malignant tissue. Oral cavity cancers are the most common cancer faced by mankind today. High proportion cases of oral cavity cancer seen in males and the third most common in cancer in females in India. Different prognostic factors for squamous cell lesion of oral cavity but they do not give any information regarding the biological characteristic of tumour. Therefore it necessary to search for new prognostic factor that give information regarding the aggressiveness of tumour. Most common oral cancer was squamous cell carcinoma and several factors like tobacco and tobacco related product, premalignant lesion which present as persistent lesion like leukoplakia or oral submucosal fibrosis are predisposing factor for oral cancer.¹ It was originally believed that cancer occur due to cellular proliferation but now a day it reveals that due to decrease in cell death. Cell proliferation and cell death are two histological parameters for identification of individuals who are at a high risk of developing carcinoma and has prognostic significance. Hence the aim and objective of our study to assess the significance of apoptotic index as a prognostic marker in the premalignant and malignant squamous cell lesion of oral cavity.

MATERIALS AND METHODS

The study included 60 patients presenting with oral

lesions. A clinical workup and relevant investigations were carried out in each case. The excisional/incisional oral biopsy specimens were then processed. All sections were routinely stained with hematoxylin and eosin (H and E) stain. The H and E sections were examined using high magnification (oil immersion ×100x lens). From each section, 10 fields devoid of any preservation or fixation artifact, inflammation and necrosis were selected. Apoptotic cells/bodies in the stroma around the tumors were not counted. In each section, 1000 tumor cells were evaluated for the presence of apoptotic cells and apoptotic bodies. AI was calculated as the number of apoptotic cells and apoptotic bodies, expressed as a percentage of the total number of non-apoptotic tumor cells counted in each case.

STATISTICAL ANALYSIS

Statistical evaluation was carried out using the Student t test, with P < 0.05 being significant.

RESULTS

Apoptotic index was evaluated in 60 cases of pre-malignant and malignant squamous cell lesions of the oral cavity, out of which 30 cases of oral dysplasia (mild=12, moderate=10, severe=8), and 30 cases of squamous cell carcinoma (well differentiated [WD] = 10, moderately differentiated [MD]=12, poorly differentiated [PD]=8) were included. The apoptotic cells showing deep eosinophilic cytoplasm and pyknotic round nucleus (Figure 1). Apoptotic bodies are randomly distributed in squamous cell carcinoma which appeared as tiny, round and pyknotic nucleus (Figure 2).

The maximum mean AI was observed in WD SCC 0.7600 ± 0.0966 and with progression towards higher grades. A fall was noted, the values being 0.5417 ±

0.0669 in MD SCC and 0.4500 ± 0.0535 in PD SCC. The difference between WD SCC and PD SCC was found to be significant (p < 0.001; highly significant). Similarly, increase in the mean AI with an increasing degree of dysplasia (mild dysplasia = 0.2333 ± 0.0888, moderate dysplasia 0.4600 ± 0.0966, severe dysplasia = 0.6000 ± 0.0756) was statistically significant (Table-1).

The mean AI was increased progressively from dysplasia (0.4067 ± 0.1760) to SCC (0.5900 ± 0.1470). It was also statistically significant (Table-2).

Statistical significance was observed on correlation between mild dysplasia and moderate dysplasia (p < 0.001; Highly significant) (Table-3) and between mild dysplasia and severe dysplasia dysplasia (p < 0.001; Highly significant) (Table-4).

Comparison between moderate dysplasia and severe dysplasia was not statistically significant (p < 0.01; significant at 1%) (Table-5).

Group	N	Range	Apoptotic Index Mean ± SD
Oral Dysplasia	30	0.0 – 0.7	0.4067 ± 0.1760
Mild Dysplasia	12	0.0 – 0.3	0.2333 ± 0.0888
Moderate Dysplasia	10	0.3 – 0.6	0.4600 ± 0.0966
Severe Dysplasia	8	0.5 – 0.7	0.6000 ± 0.0756
Oral Squamous cell carcinoma	30	0.4 – 0.9	0.5900 ± 0.1470
Well Differentiated	10	0.6 – 0.9	0.7600 ± 0.0966
Moderately Diff.	12	0.4 – 0.6	0.5417 ± 0.0669
Poorly Diff.	8	0.4 – 0.5	0.4500 ± 0.0535

Table-1: Apoptotic index in squamous cell lesions of the oral cavity

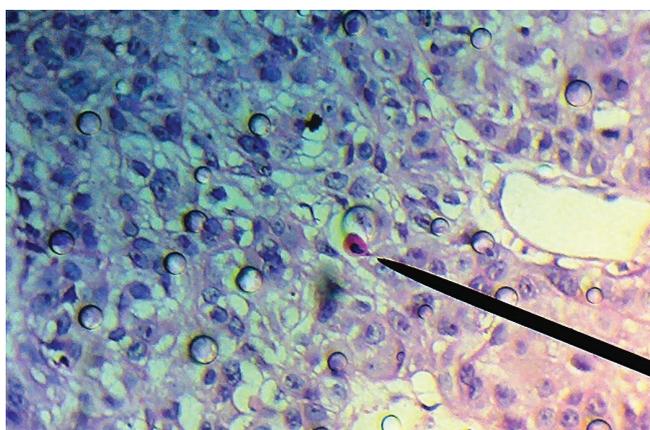


Figure-1: Apoptotic cell showing pyknotic nucleus (H&E; X 100)

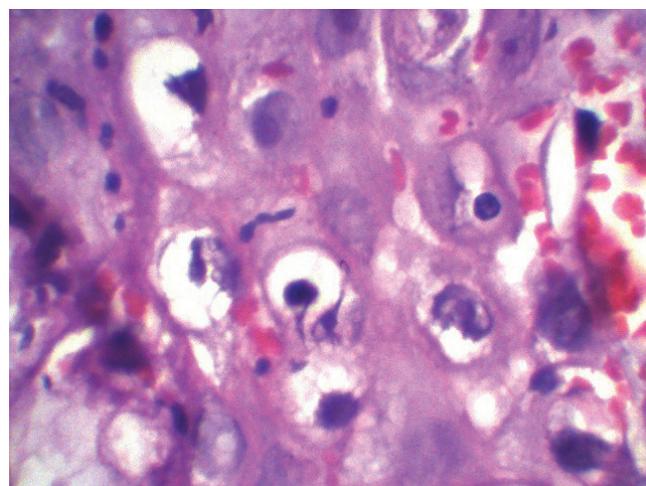


Figure-2: Apoptotic bodies randomly distributed in SCC (H&E; X 100)

Group	Apoptotic Index Mean \pm SD	Mean Difference	't' value	P value
Dysplasia	0.4067 \pm 0.1760	0.1833	4.378	<0.001*
SCC	0.5900 \pm 0.1470			
* p < 0.001; Highly significant				
Table-2: Comparison of Apoptotic index between Dysplasia and SCC				

Group	Apoptotic Index Mean \pm SD	Mean Difference	't' value	P value
Mild Dysplasia	0.2333 \pm 0.0888	0.2267	5.731	<0.001*
Moderate Dysplasia	0.4600 \pm 0.0966			
* p < 0.001; Highly significant				
Table-3: Comparison between Mild Dysplasia and Moderate Dysplasia				

Group	Apoptotic Index Mean \pm SD	Mean Difference	't' value	P value
Mild Dysplasia	0.2333 \pm 0.0888	0.3667	9.576	<0.001*
Severe Dysplasia	0.6000 \pm 0.0756			
* p < 0.001; Highly significant				
Table4:-Comparison between Mild Dysplasia and Severe Dysplasia				

Group	Apoptotic Index Mean \pm SD	Mean Difference	't' value	P value
Moderate Dysplasia	0.4600 \pm 0.0966	0.1400	3.353	0.004*
Severe Dysplasia	0.6000 \pm 0.0756			
* p < 0.01; Significant at 1%				
Table-5: Comparison between Moderate Dysplasia and Severe Dysplasia				

DISCUSSION

The present study is based on the evaluation of AI in 60 oral premalignant and malignant squamous cells lesions on light microscopy. Apoptotic bodies is identified by its morphological feature have smaller in size, smooth and round margin separating it from neighboring cell, pyknotic nucleus and eosinophilic cytoplasm. Apoptotic bodies were counted using 100x magnification (under oil immersion).² It was observed that a fairly accurate assessment of apoptosis is possible by light microscopy. Mitotic figures were readily identifiable and were morphologically distinct from apoptotic cell. Most cellular area is selected for counting of Apoptotic bodies. Areas showing marked inflammation, necrosis and hemorrhage are not included because they cause also condensation of the chromatin. Other leukocytes are not included in AI counting.³ In our study WD SCC show high AI, possibly suggests that tumors that ex-

hibit more apoptosis may be slower growing and have less aggressive behavior. Where as other author study suggested that diminished apoptotic response have an increased propensity for metastatic survival.³ In the present study it was observed that as the AI increases, the nature of the lesion changed from oral dysplasia to SCC. The results are in accordance with other author studies.^{4,5} Viswanathan⁶ studied that AI was found to increase gradually from normal to dysplasia to carcinoma and highest AI index was seen in well-differentiated squamous cell carcinomas (SCCs). Similarly in our study we found that WD SCC have high AI. Birchall⁷ found that AI increased from 0.12% \pm 0.07 S.E.M. (normal) to 0.58 \pm 0.13 (CIS) but fell to 0.14 \pm 0.14 in SCC. Xie et al⁸ studied that proliferative and apoptotic markers, especially their combinations, have prognostic value in tongue SCC. Whereas other author reported that apoptosis prevent the development of aneuploidy and other genetic abnormalities com-

monly associated with cancer cells and progression of neoplasia.³

CONCLUSION

In India incidence of oral cancer rises because of tobacco chewing habits. Mortality and morbidity is very high due to oral cancer. For prevention of this oral cancer we need new prognostic marker that give information regarding aggressiveness of tumour and also help in management of oral cancer. In present study we found that oral cancer with low AI and higher grade show aggressive behavior and more potential for metastasis. Also low grade lesion that high AI may be more sensitive to chemotherapy and hence likely to have a better prognosis.

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