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ORIGINAL ARTICLE

Apoptosis: A Prognostic Marker in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma

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ABSTRACT

Introduction: Oral cancer is a major health problem especially in India. In cancer, the equilibrium between proliferation and apoptosis is disturbed. The defect in apoptotic pathway allows cells to proliferate with genetic abnormalities. Thus, the aim of this study is to assess the significance of apoptotic index (AI) as a putative prognostic marker in oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC). **Materials and Methods:** Study constituted of 60 previously histopathologically confirmed cases, 30 cases of OED and 30 cases of OSCC of different histopathological grades. A uniform section of 3-4 μm thickness was cut from all the blocks and was then stained using hematoxylin and eosin stains. AI was assessed using a binocular research light microscope. The results were sent for statistical analysis using Student's *t*-test. **Results:** The mean AI increased progressively with increasing grades of OED and decreased with increasing grades of OSCC. A maximum mean AI was reported in well-differentiated squamous cell carcinoma (WDSCC) 0.7600. The results observed were significant ($P < 0.001$) on comparing WDSCC with moderately differentiated SCC (MDSCC) and with poorly differentiated SCC (PDSCC) but were insignificant on comparing MDSCC with PDSCC. **Conclusion:** This suggest tumors that exhibit less apoptosis tend to show aggressive behavior, hence AI can offer an idea about the nature and course of the lesion. Thereby helping in prognosis and predicting its outcome.

Key words: Apoptosis, apoptotic index, dysplasia, oral squamous cell carcinoma

INTRODUCTION

In India, oral cancer is the most common cancer in males and the third most common cancer in females.[1] An early diagnosis of these lesions improves the prognosis with minimum impairment and deformity.

Apoptosis, a genetically programed cell death is clearly distinct from necrosis and it is a physiologic phenomenon which happens spontaneously in the process of normal tissue growth. Apoptosis is an indispensable phenomenon for normal growth and the development of all organisms. It plays a significant role in the maintenance of the normal physiologic state but its alteration may lead to disease state.[2] A deregulated apoptotic pathway may lead to either excessive removal or prolonged survival of cells.[3] Many researchers have reported deregulation in apoptosis may lead to malignant transformation thus leading to tumor proliferation.[4,5]

A technique of counting of apoptotic cells and apoptotic bodies has been discussed by various authors.[6-8] It is an easy and cheap method that can be performed on hematoxylin and eosin (H and E) stained sections using light microscope.

This study attempts to summaries the significance of apoptotic index (AI) as a biological marker in the premalignant lesion and malignant lesion of the oral cavity.

MATERIALS AND METHODS

The present *in vitro* study constituted of histopathologically diagnosed, formalin-fixed, paraffin embedded tissue samples of 30 cases of oral epithelial dysplasia (OED) and 30 cases of oral squamous cell carcinoma (OSCC) which were collected from the archives of the Department of Oral Pathology and Microbiology, Institute of Dental Sciences, Bareilly. Among 30 cases of OED, 12 were mild, 10 were moderate and 8 cases were of severe dysplasia. 30 cases of OSCC were divided into different histopathological grades based on Border's criteria, among which 10 cases were well-differentiated squamous cell carcinoma (WDSCC), 12 cases were moderately differentiated SCC (MDSCC) and 8 were poorly differentiated SCC (PDSCC).

A uniform section of 3-4 μm thickness was cut from all the blocks and was routinely stained with H and E stain. All counts

were performed on a research microscope using oil immersion lens (×100). For estimation of AI, 10 representative fields were selected randomly devoid of any artifacts. A total of 1000 tumor cells were screened for apoptotic cells and apoptotic bodies. AI was assessed as the percentage of apoptotic cells and bodies, among the total number of non-apoptotic cells, that were counted in each case.[5,6,9]

Statistical evaluation was carried out using Student *t*-test, with $P < 0.001$ being significant.

Criteria to Identify the Apoptotic Cells

The apoptotic cells stained by H and E stain show few well-define features, which includes cell shrinkage, condensed dark eosinophilic cytoplasm and dense pyknotic, round to oval, irregular-shaped nucleus [Figure 1].[10-12] The shrunken cell fragments as apoptotic bodies which appear either scattered or forming cluster among tumor cells [Figure 2].

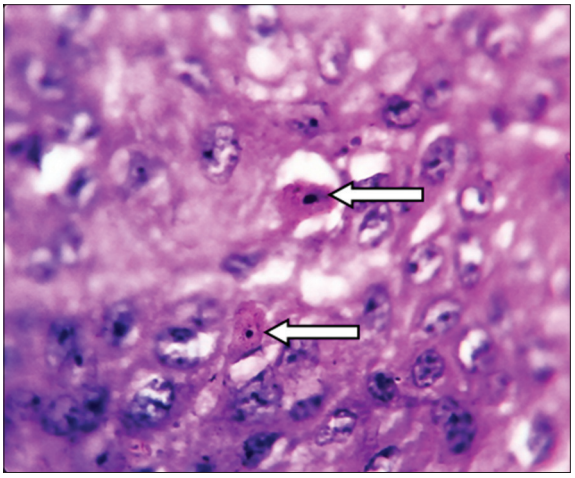

Figure 1: Apoptotic cell showing deep eosinophilic cytoplasm and pyknotic nucleus in moderately differentiated variant of squamous cell carcinoma

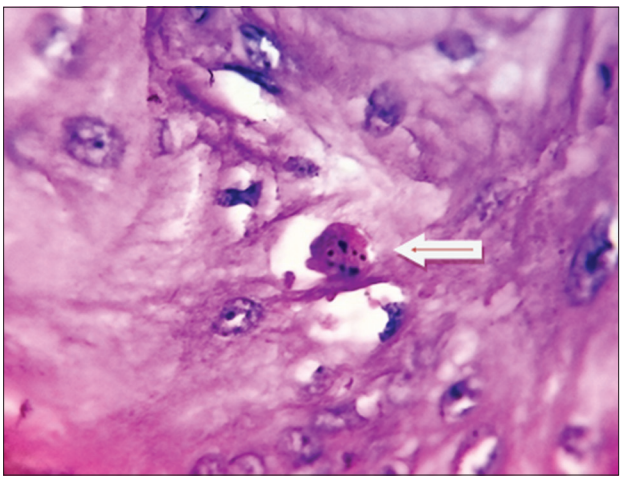

Figure 2: Apoptotic bodies seen in clusters in well-differentiated squamous cell carcinoma

RESULTS

In mild to moderate dysplasia, apoptotic bodies were most commonly seen in the basal and suprabasal layers while in severe dysplasia and OSCC; they were randomly distributed. The mean AI increased progressively with increasing grades of OED and decreased with increasing grades of OSCC. However, the maximum mean AI was reported in WDSCC (0.7600 ± 0.0966) [Table 1].

On comparing the mean AI of OED with OSCC, the results were highly significant. Similarly, on comparing mean AI among groups of OED, i.e., mild dysplasia with moderate dysplasia and mild dysplasia with severe dysplasia the results were significant, ($P < 0.001$), but no statistical significance was found on comparing moderate dysplasia with severe dysplasia [Table 2].

AI was significantly higher on comparing WDSCC with MDSCC and WDSCC with PDSCC. However, on comparing MDSCC with PDSCC, no significant correlation was observed [Table 3].

DISCUSSION

OED and OSCC are most commonly diagnosed oral lesions in India.[13] A dysfunction in the apoptotic system can either lead to

Table 1: Mean AI and SD among all groups

Histological grades	Total number of cases	Apoptotic index Mean±SD
OED	30	0.4067±0.1760
Mild OED	12	0.2333±0.0888
Moderate OED	10	0.4600±0.0966
Severe dysplasia	8	0.6000±0.0756
OSCC	30	0.5900±0.1470
WDSCC	10	0.7600±0.0966
MDSCC	12	0.5417±0.0669
PDSCC	8	0.4500±0.0535

AI: Apoptotic index, SD: Standard deviation, OED: Oral epithelial dysplasia, OSCC: Oral squamous cell carcinoma, WDSCC: Well-differentiated squamous cell carcinoma, MDSCC: Moderately differentiated squamous cell carcinoma, PDSCC: Poorly differentiated squamous cell carcinoma

Table 2: Comparisons between different histological grades of OED

Histological grades	Mean difference	*t* value	*P* value
OED	0.1833	4.378	<0.001*
OSCC			
Mild OED	0.2267	5.731	<0.001*
Moderate OED			
Mild OED	0.3667	9.576	<0.001*
Severe OED			
Moderate OED	0.1400	3.353	0.004
Severe OED			

*P<0.001; being significant. OED: Oral epithelial dysplasia, OSCC: Oral squamous cell carcinoma

Table 3: Comparisons between different histological grades of OSCC

Histological grades	Mean difference	*t* value	*P* value
WDSCC MDSCC	0.2183	6.249	<0.001*
WDSCC PDSCC	0.3100	8.106	<0.001*
MDSCC PDSCC	0.0917	3.240	0.005*

*P<0.001; being significant. OSCC: Oral squamous cell carcinoma, WDSCC: Well-differentiated squamous cell carcinoma, MDSCC: Moderately differentiated squamous cell carcinoma, PDSCC: Poorly-differentiated squamous cell carcinoma

excessive removal or prolonged survival of cells or a combination of both processes which leads to a wide variety of diseases including oral cancers.[14]

As light microscopy has its own drawbacks, hence to avoid chances of human error in counting apoptotic bodies there are various advanced methods that have been developed for detection of apoptotic cells; e.g., electron microscopy, flow cytometry, gel electrophoresis, immunohistochemistry, *in situ* end labeling of fragmented DNA, and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate biotin nick end labeling technique. Although it is accepted, these methods are better for evaluation of apoptotic cells. Moreover, these are expensive and specialized techniques, so their setup and standardization are difficult in many institutes.

In this study, the biologic and clinical significance of AI were evaluated in 60 cases of OED and OSCC. A fair an accurate assessment of apoptosis is possible by light microscopy. Apoptotic cells were most commonly seen in the suprabasal and basal regions of early dysplastic lesions, but as the severity of the lesion increased the apoptosis becomes more generalized.[15,16] In the case of carcinomas, the apoptotic bodies were counted in the substance of the tumor. The apoptotic cells that were present in the stroma surrounding the tumor, and those that were observed in the areas of necrosis and inflammation were excluded.

It was observed that there was an increase in AI with increasing grades of OED. An increase in AI was reported as the nature of the lesion changed from OED to SCC.[4,17]

A decrease in AI with the increasing severity of OSCC was observed with a maximum value in WDSCC. Various authors have suggested that increase in apoptosis occurs with disease progression, gradually up to carcinoma *in situ* but falls again in SCC.[3-5,9] In this study, AI increased progressively from dysplasia to carcinoma but decreased with decreasing differentiation of the tumor.

The above results and interpretation are in accordance with the previously reported study which suggested that apoptosis was induced more frequently in keratinizing region of SCC of the esophagus, than in the non-keratinizing region,[18] possibly because of well-balanced cell death and cell proliferation seen in well-differentiated and keratinizing regions. On the other side, few studies have contradictory observation where the AI is significantly lower in differentiated gastric carcinomas than in undifferentiated tumors, possibly because cellular proliferation was quiet higher in less-differentiated tumor tissue than in well-differentiated tissue. Thus, the enhanced cell proliferation also led to an increase in the number of apoptotic cells.[19]

In this study, the clinical implication of apoptosis is emphasized. Although it has been proved experimentally, apoptosis is also induced by anticancer agents or irradiation. Hence, calculation of AI can be applied before and after chemotherapy to evaluate its outcome.[20] Tumors, which display increased apoptosis after one cycle of chemotherapy, are more likely to achieve pathological regression. High AI after chemotherapy predicts that patient may have a good pathological response.[21]

CONCLUSION

This study demonstrates the clinical significance of apoptosis in assessing disease progression. In near future, it will be better if the histopathology reports of all premalignant and malignant lesions of the oral cavity are submitted with their AI. This would help in providing timely surgical intervention and less deformity, thus helping in prognosis and its outcome.

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