Evaluation of Apoptotic Index in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma

Sonia Gupta[1], Suheel Hamid Latoo[2], Mohammad Shafi Dar[3]

ABSTRACT

Introduction: Oral cancers are a major health problem in India. An early diagnosis of these lesions improves the prognosis with minimum impairment and deformity. The defect in apoptotic pathway allows cells to proliferate with genetic abnormalities. Recently, parameters of cell death have emerged as important diagnostic and prognostic tools. Study aimed to determine and compare the apoptotic index (AI) in oral epithelial dysplasia and oral squamous cell carcinoma using light microscopy in order to evaluate whether apoptosis can be used as a prognostic marker.

Material and methods: A retrospective study was carried out on 30 formalin fixed, paraffin embedded tissue blocks in the Department of Oral pathology, GDC Srinagar and the study comprised of 15 cases of oral epithelial dysplasia (05 cases for each grade) and 15 cases of oral squamous cell carcinoma (05 cases for each grade). All the cases were histopathologically diagnosed using H & E so as to evaluate and compare the apoptotic index (AI).

Results: In the present study, the mean apoptotic index increased progressively with increasing grades of OED and decreased with increasing grades of OSCC. The p-value was found to be statistically significant.

Conclusion: Apoptosis can be used as a prognostic marker in oral epithelial dysplasia and oral squamous cell carcinoma.

Keywords: Apoptotic Index, Dysplasia, Squamous Cell Carcinoma, Hematoxylin and Eosin Stain

INTRODUCTION

OSCC is one of the major causes of morbidity and mortality and is the sixth most common malignancy known. It is the most common form of cancer affecting males and account for 50-70% of all cancers diagnosed in India. Early diagnosis of oral cancer improves the prognosis as well as reduces the mortality and morbidity rate. Five year mean survival rate remains very low, despite improvements in diagnostic and treatment modalities.[1,2] Two-thirds of oral cancer patients are diagnosed at advanced tumour stages, where survival drops to a little more than 30% and its prognosis is unpredictable. Individuals with dysplastic lesions in the oral cavity are at high risk for developing OSCC.[3] Majority of these are unequivocally associated with tobacco chewing and usually preceded by potentially malignant disorders of oral cavity like leukoplakia, erythroplakia, sideropenic dysphagia, oral lichen planus (OLP), and oral submucous fibrosis (OSMF).[4,5] Oral leukoplakia is the most common and prevalent form. To treat oral cancer effectively, an early diagnosis of these potentially malignant disorders is essential. Tissue alterations that occur in these disorders must be recognized clinically and histologically, and at the molecular level as early as possible. A significant transformation at cellular level is the appearance of apoptosis. [6]

Apoptosis is a form of programmed cell death. Apoptosis can be quantified in an attempt to understand the balance of cell proliferation or death in a particular physiologic or pathologic process.[7, 8] Apoptosis is involved in removing or eliminating the tumour cells. Hence, regulation of apoptosis plays a key role in carcinogenesis, tumour progression and metastasis. Accumulation of genetic or epigenetic alterations, eventually lead to deregulation of apoptosis resulting in uncontrolled cell proliferation leading to cancer, autoimmune diseases and neurodegenerative diseases. Apoptotic cell identification is one such early diagnostic marker to identify cancer.[9] The present study was done to determine and compare the apoptotic index (AI) in oral epithelial dysplasia and oral squamous cell carcinoma using light microscopy in order to evaluate whether apoptosis can be used as a prognostic marker.

MATERIAL AND METHODS

A retrospective study was carried out on 30 formalin fixed, paraffin embedded tissue blocks in the Department of Oral pathology, GDC Srinagar in the year 2021 and the study comprised of 15 cases of oral epithelial dysplasia (05 cases for each grade) and 15 cases of oral squamous cell carcinoma (05 cases for each grade). All the cases were histopathologically diagnosed using H & E so as to evaluate and compare the apoptotic index (AI). Specimen including any other associated pathology and treated cases of OED as well as OSCC were excluded from the study. All the H &E stained slides were analysed under the light microscope under x400 magnification.

In the study sample, 1000 dysplastic cells/tumor cells were evaluated for the presence of apoptotic bodies/cells. A particular area was selected in the slide and total number

[1]Tutor, Department of Oral Pathology, Government Dental College & Hospital, Srinagar, [2]Professor, Department of Oral Pathology, Government Dental College & Hospital, Srinagar, [3]Lecturer, Department of Oral Pathology, Government Dental College & Hospital, Srinagar

Corresponding author: Dr. Sonia Gupta, Tutor, Department of Oral Pathology, Government Dental College & Hospital, Srinagar

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of epithelial cells were first counted and noted. Then, the number of apoptotic cells in that particular area was also counted and 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 assessed as the percentage of apoptotic cells/bodies, among the total number of non-apoptotic cells that were counted in each case.

Morphologically, apoptosis is characterized by a series of morphological changes, which can be appreciated by light microscopy. On histological 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. Nuclei show various stages of chromatin condensation as well as aggregation and ultimately, karyorrhexis.

All the data were statistically analysed with the help of statistical package for social sciences (SPSS) software version 21.0 using mean, standard deviation and Post hoc Bonferroni test. A probability value of <0.05 was considered to be statistically significant.

RESULTS

In mild to moderate oral epithelial dysplasia, apoptotic bodies were most commonly seen in the basal and suprabasal layers while in severe dysplasia and OSCC, they were randomly distributed (Figure 1). The mean apoptotic index increased progressively with increasing grades of OED and decreased

Group	No. of cases	Apoptotic index (Mean±SD)	P-value
OED	15	0.4628±0.1778	0.005
OSCC	15	0.6217±0.1289	
SD- Standard deviation, OED-Oral epithelial dysplasia, OSCC-Oral squamous cell carcinoma			

Table-1: Mean apoptotic index in oral epithelial dysplasia and oral squamous cell carcinoma

Group	No. of cases	Apoptotic index (Mean±SD)	P-value
Mild OED	05	0.2258±0.0923	0.005
Moderate OED	05	0.4631±0.0981	
Severe OED	05	0.5815±0.0742	
WDSCC	05	0.6936±0.1328	0.005
MDSCC	05	0.5467±0.0527	
PDSCC	05	0.4618±0.0487	
SD- Standard deviation, OED-Oral epithelial dysplasia, WDSCC-Well-differentiated oral squamous cell carcinoma, MDSCC-Moderately-differentiated oral squamous cell carcinoma, PDSCC-Poorly-differentiated oral squamous cell carcinoma			

Table-2: Mean apoptotic index in different grades of oral epithelial dysplasia and oral squamous cell carcinoma

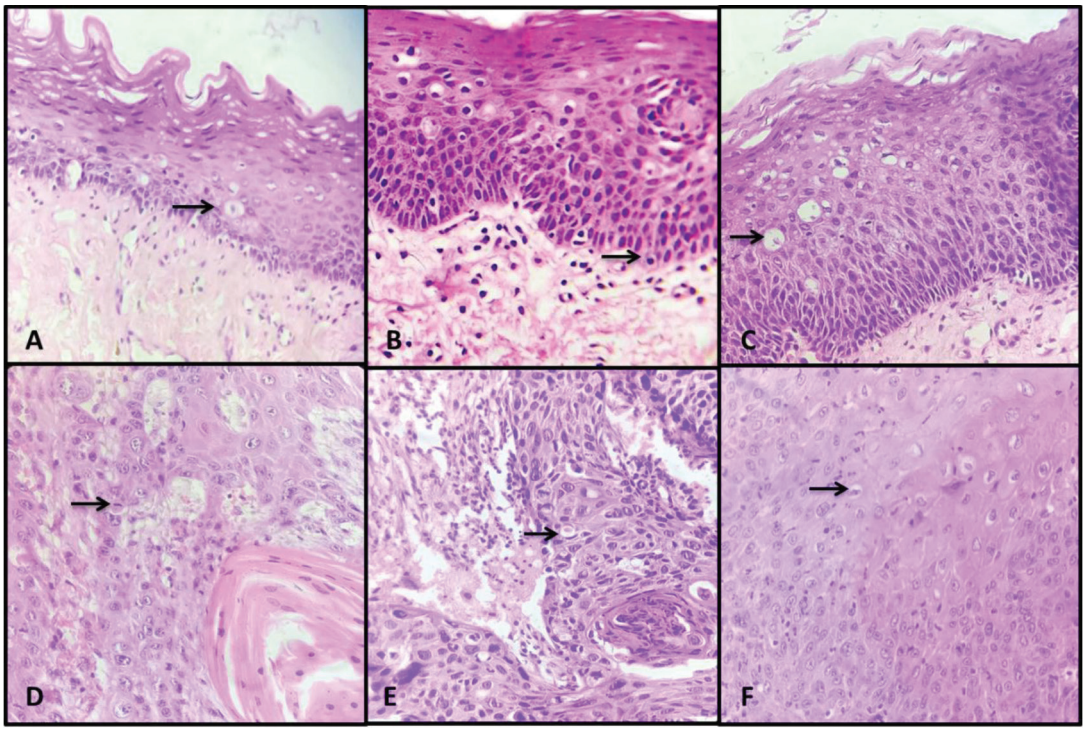

Figure-1: Photomicrograph showing apoptotic bodies in A) Mild oral epithelial dysplasia, B) Moderate oral epithelial dysplasia, C) Severe oral epithelial dysplasia, D) Well-differentiated OSCC, E) Moderately -differentiated OSCC and F) Poorly-differentiated OSCC

Group	Apoptotic index [Post hoc Bonferroni (p – value)]
Mild OED vs Moderate OED	0.001
Mild OED vs Severe OED	0.001
Moderate OED vs Severe OED	0.001
WDSCC vs MDSCC	0.001
WDSCC vs PDSCC	0.001
MDSCC vs PDSCC	0.001
OED-Oral epithelial dysplasia, WDSCC-Well-differentiated oral squamous cell carcinoma, MDSCC-Moderately-differentiated oral squamous cell carcinoma, PDSCC-Poorly-differentiated oral squamous cell carcinoma	

Table-3: Comparison of apoptotic index between different grades of oral epithelial dysplasia and oral squamous cell carcinoma

with increasing grades of OSCC.

The mean apoptotic index in oral epithelial dysplasia and oral squamous cell carcinoma was 0.4628±0.1778 and 0.6217±0.1289 respectively with a statistically significant p-value (Table 1). The mean apoptotic index in mild, moderate and severe oral epithelial dysplasia was 0.2258±0.0923, 0.4631±0.0981 and 0.5815±0.0742 respectively with a statistically significant p-value. The mean apoptotic index in well-differentiated, moderately-differentiated and poorly-differentiated oral squamous cell carcinoma was 0.6936±0.1328, 0.5467±0.0527 and 0.4618±0.0487 respectively with a statistically significant p-value (Table 2). On comparing the mean AI of different grades of oral epithelial dysplasia, the results were highly significant. Apoptotic index was significantly higher on comparing well-differentiated oral squamous carcinoma (WDSCC) with moderately-differentiated oral squamous carcinoma (MDSCC), well-differentiated oral squamous carcinoma with poorly-differentiated oral squamous carcinoma and moderately-differentiated oral squamous carcinoma with poorly- differentiated oral squamous carcinoma (PDSCC) (Table 3).

DISCUSSION

Apoptosis is an ordered and orchestrated cellular process that occurs in physiological and pathological conditions. A large number of stimuli can induce apoptosis in a cell. Multiple signaling pathways lead to activation of the apoptosis depending on the triggering factor and the cell type. Apoptosis prevents the development of aneuploidy and other genetic aberrations that are associated with the development and progression of OPMD.[10] At certain stages, during the development of a tumor, the equilibrium between the cell proliferation and its apoptosis is interrupted, resulting in dysregulation of cell proliferation. Thus, a dysfunction in the apoptotic system can lead to a wide variety of diseases including oral cancers.[11] The present study was conducted to evaluate the apoptotic index in patients with oral epithelial dysplasia and oral squamous cell carcinoma.

In the present study, apoptotic cells were most commonly seen in the basal and suprabasal regions of early dysplastic lesions, but as the severity of the lesion increased the apoptosis becomes more generalized. In the case of carcinomas, the apoptotic bodies were counted in the substance of the tumor. These results were in accordance with the study done by Gupta K et al in 2017.[12]

In this study, it was observed that there was an increase in apoptotic index with increasing grades of OED. A decrease in apoptotic index with the increasing severity of OSCC was observed with a maximum value in WDSCC. These results were similar to study carried out by Gupta I et al, Gupta K et al and Jain A et al.[11-13] Various authors have suggested that increase in apoptosis occurs with disease progression, gradually up to carcinoma in situ but falls again in OSCC.[13,14] Tumor growth is a summation of mitosis and cell production as well as cell loss and death. Thus, a high apoptotic index in WDSCC was observed in our study suggests that tumors that exhibit more apoptosis may be slower growing and therefore may be less biologically aggressive.[15] Analysis of apoptotic index showed a progressive increase in apoptosis in parallel with biological aggressiveness indicating increased synthetic activity of proteins during neoplastic progression. As the tumor grows, there is increase in cell proliferation and probably due to the large tumor size and high growth rate potential as well as the tumor outgrows its blood supply leading to hypoxic injury-causing increased apoptosis.[16]

CONCLUSION

Apoptosis can be used as a prognostic marker in oral epithelial dysplasia and oral squamous cell carcinoma. In near future, it will be better if the histopathology reports of all oral potentially malignant disorders and malignant lesions of the oral cavity are submitted with their apoptotic index. Oral cancers that exhibit less apoptosis tend to show aggressive behaviour and have a greater potential for metastasis. This will be helpful in providing timely surgical intervention and less deformity, thus helping in prognosis and its outcome. Recently, researches are being carried out to modify the apoptotic programme as potential target in the treatment of the diseases.

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