Original Article

Evaluation of Apoptotic Index in Various Grades of Oral Epithelial Dysplasia at a Tertiary Care Hospital

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ABSTRACT
Background: Oral cancer is the 11th most common cancer in the world, with an estimated 300,000 new cases and 145,000 deaths in 2012. In India, 20/100,000 population are affected by oral cancer, which accounts for around 30% of all types of cancer. Most cases of oral cancer are associated with habits (tobacco/areca nut) and are preceded by asymptomatic clinical lesions collectively referred to as oral potentially malignant disorder (OPMD). OPMDs include leukoplakia, erythroplakia, reverse smoker's palate, erosive lichen planus, oral submucous fibrosis, lupus erythematosus and actinic keratosis. Hence; the present study was conducted for assessing apoptotic index in various grades of oral epithelial dysplasia.
Materials & Methods: A total of 100 cases of oral epithelial dysplasia were enrolled. Complete demographic and clinical details were obtained from all the cases. A diagnosis was made with formalin-fixed, paraffin-embedded, and histopathology. Slides were examined using microscope at both 10 x and 40× magnification. Calculation of AI was done as several apoptotic bodies/a total number of cells in one field which is expressed in percentage. Apoptotic bodies appeared as a round oval mass of intensely eosinophilic cytoplasm with fragments of dense nuclear chromatin.
Results: Mean apoptotic index among cases with mild, moderate and severe dysplasia was 1.51, 2.13, and 3.74 respectively. Significant results were obtained while comparing the apoptotic index among cases with different histological dysplasia grading. **Conclusion:** Apoptotic index could be used as an adjuvant for histological grading of oral epithelial dysplasia.
Key words: Oral Epithelium, Dysplasia.

INTRODUCTION

Oral cancer is the 11th most common cancer in the world, with an estimated 300,000 new cases and 145,000 deaths in 2012. In India, 20/100,000 population are affected by oral cancer, which accounts for around 30% of all types of cancer. Most cases of oral cancer are associated with habits (tobacco/areca nut) and are preceded by asymptomatic clinical lesions collectively referred to as oral potentially malignant disorder (OPMD). OPMDs include leukoplakia, erythroplakia, reverse smoker's palate, erosive lichen planus, oral submucous fibrosis, lupus erythematosus and actinic keratosis.[1-3]

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.[6-9] Another useful parameter is the assessment of cellular proliferation. Proliferating cells (cells in G1, S, G2 and M phases of the cell cycle) express Ki-67 antigen, which is a non-histone nuclear protein. These proliferating cells can be visualized by staining with monoclonal Ki-67 antibody. 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.[4-6] Hence; the present study was conducted for assessing apoptotic index in various grades of oral epithelial dysplasia.

MATERIALS & METHODS
The present study was conducted for assessing apoptotic index in various grades of oral epithelial dysplasia. A total of 100 cases of oral epithelial dysplasia were enrolled. Complete demographic and clinical details were obtained from all the cases. A diagnosis was made with formalin-fixed, paraffin-embedded, and histopathology. Slides were examined using microscope at both 10 x and 40× magnification. Random examination of four fields was done and the number of epithelial cells was counted. The apoptotic bodies were counted and assessed by using the Leica application suite. Calculation of AI was done as several apoptotic bodies/a total number of cells in one field which is expressed in percentage. Apoptotic bodies appeared as a round oval mass of intensely eosinophilic cytoplasm with fragments of dense nuclear chromatin. All the results were recorded on a Microsoft excel sheet and were analyzed using SPSS software.

RESULTS
Out of 100 cases, 33 cases were of mild, 41 cases were of moderate and remaining 26 cases were of severe dysplasia. Mean apoptotic index among cases with mild, moderate and severe dysplasia was 1.51, 2.13, and 3.74 respectively. Significant results were obtained while comparing the apoptotic index among cases with different histological dysplasia grading.

Table 1: Distribution of cases according to histopathologic grading

Histological grading	Number	Percentage
Mild	33	33
Moderate	41	41
Severe	26	26
Total	100	100

Table 2: Apoptotic index

Histological grading	Mean	SD	p- value
Mild	1.51	0.21	0.001 (Significant)
Moderate	2.13	0.54	
Severe	3.74	0.76	
Total	2.65	0.61	

DISCUSSION
Oral epithelial dysplasia is a spectrum of architectural and cytological epithelial changes caused by accumulation of genetic changes and is associated with an increased risk of progression to squamous cell carcinoma. It is a microscopic diagnosis of immense clinical importance. The initial reports of oral potentially malignant disorders with oral epithelial dysplasia transforming to oral cancer helped in understanding the nature of oral malignancies. Since then, clinical studies on oral potentially malignant disorders have combined microscopic findings of oral epithelial dysplasia to assess the malignant transformation potential of different grades of epithelial dysplasia.[7-9]
Out of 100 cases, 33 cases were of mild, 41 cases were of moderate and the remaining 26 cases were of severe dysplasia. Mean apoptotic index among cases with mild, moderate and severe dysplasia was 1.51, 2.13, and 3.74

respectively. Viswanathan, Vidya et al evaluated the role and significance of apoptotic indices (AI) and proliferative indices (PI) in premalignant and malignant squamous cell lesions of the oral cavity. A total of 62 histologically proven cases of premalignant and malignant oral squamous cell lesions were analyzed. AI was found to increase gradually from normal to dysplasia to carcinoma. The highest AI was seen in well-differentiated squamous cell carcinomas (SCCs). PI also was found to increase significantly from normal to dysplasia to carcinoma. The highest PI was seen in poorly differentiated SCC. AI in conjunction with the PI offers an accurate idea as to the nature and course of the lesion and may help to plan timely surgical intervention that results in better clinical prognosis and outcome.[11]
Significant results were obtained while comparing the apoptotic index among cases with different histological dysplasia grading. Singh A et al evaluate apoptotic index in patients with oral epithelial dysplasia. 58 cases of oral epithelial dysplasia were included. Slides were examined using microscope 40× magnification. 4 fields were selected, and the number of epithelial cells was counted. The apoptotic bodies were counted and assessed by using the Leica application suite. Grading of oral epithelial dysplasia was mild in 12, moderate in 20 and severe in 26 cases. The difference was significant (P< 0.05). AI in mild grade was 1.31, in moderate was 2.49 and in severe was 3.17. The difference was significant (P< 0.05). Apoptotic index can be used as a marker in assessment of oral epithelial dysplasia.[12]

CONCLUSION
Apoptotic index could be used as an adjuvant for histological grading of oral epithelial dysplasia.

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