Apoptosis: Significance as a Prognostic Indicator in Oral Dyplastic Lesions

Vidya Vishwanathan*, Shruti Vimal**, Arpana Dharwadker**

*Assistant Professor **Associate Professor, Dept. of Pathology, DPU, Dr. D. Y. Patil Medical College, Hospital & Research Centre, Pimpri, Pune, Maharashtra 411018, India.

Abstract

Background: Oral cavity lesions are usually squamous cell in nature. These lesions can present as both malignant and premalignant conditions. Premalignant lesions are usually dysplastic lesions which can be mild, moderate and severe, leukoplakias or erythroplakia. 10-20% of premalignant lesions have a tendency to progress into malignancy. Hence it becomes important to identify these lesions to prevent progression. Apoptotic index can be used as a simple tool to assess the progression of the disease and identify individuals who have a tendency to development malignancy. *Aims*: The aim of this study is to assess the apoptotic index in premalignant oral squamous cell lesions and evaluate its usefulness in assessing disease progression. *Material and methods*: 33 cases were studied. 5 were normal and used as control and the rest of 28 from varying degrees of dysplasia's. Apoptotic index was assessed on H&E stained sections using oil immersion lens. Apoptotic bodies were counted in 10 oil immersion fields and expressed as a percentage. *Results*: The results showed that apoptotic index increased with the grade of dysplasia's, showing a peak in moderate dysplasia. *Conclusion*: From this study we can conclude that apoptotic index can be used as a simple tool to evaluate patients with oral premalignant lesion. It can also help in identifying patients who will progress to malignancy and can be used as a prognostic marker.

Keywords: Dysplasia; Apoptotic Bodies; Apoptotic Index.

Introduction

Squamous cell lesions in the oral cavity can be premalignant or malignant in nature. Premalignant lesions are benign lesions which show a high predilection for malignant transformation. The most common premalignant lesions encountered in the oral cavity are leukoplakias followed by erythroplakia, which have a tendency to present as hyperplasia, dysplasia or carcinoma in situ. Oral leukoplakia was defined by WHO as any white patch or plaque that cannot be characterised clinically or pathologically as any other disease. They account for 85% of premalignant lesions. Erythroplakia is a red patch that does not fall under any particular pathology. Almost all erythroplakias show some amount of dysplasia, carcinoma in situ or invasive squamous cell

carcinoma [1,2]. Clinical studies have shown that approximately 10-20% of all oral dysplastic lesions progress to carcinoma and 20-30% show progression in the disease over a period of 10yrs. Hence early diagnosis becomes important leading to early cure and minimal deformity. Histological methods that assess factors such as apoptosis, help in identifying individuals who are at great risk to develop carcinoma and may also act as a prognostic indicator [1,2].

Materials and Methods

The study was carried out in Department of Pathology of Dr. D.Y. Patil Medical College and Research Centre, Pimpri, Pune, Maharashtra. A retrospective and prospective study was conducted in both male and female patients presenting with lesions in the oral cavity. Histologically proven cases of dysplasia were also included in the study. Patients who had immunological disease or were immunocompromised were excluded from the study as were other oral lesions which were non squamous

Corresponding Author: Shruti Vimal, Associate Professor, Dept. of Pathology, DPU, Dr. D. Y. Patil Medical College, Hospital & Research Centre, Pimpri, Pune, Maharashtra 411018, India.
E-mail : shruti.vimal@gmail.com

cell in nature. The current study was conducted over duration of 6months and included 33 cases. Clinical histories along with all other relevant investigations were conducted. Excisional/incisional biopsy were taken and stained with haematoxylin and eosin stains. Oil immersion lens were used to assess the apoptotic bodies in the H&E stained sections. 10 fields devoid of artefacts are selected and 1000 cells were counted for the presence of apoptotic bodies and cells The apoptotic index was expressed as a percentage of total number of non-apoptotic cells counted [3,4].

Statistical Analysis

SPSS for Windows computer program (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Student t test was used and statistical significance was assessed by p value.

Results

A total of 33 cases of dysplastic lesions were studied. 5 cases were from normal oral mucosa and were used as control. A preliminary diagnosis was made on all the biopsies. Apoptotic index was assessed on the remaining 28 cases. The distributions of premalignant lesions are shown in Table 1. The most common lesion seen was those of mild dysplasia. The age group included in this study were 25-80 yrs. Normal oral mucosa was encountered mostly in the younger age group whereas dysplastic lesions were more frequent in the older individuals, with severity increasing with age of the individual. As the age of the individual increased the chances of dysplasia progressing to carcinoma also increased. Of the 28 cases 25 were males and 3 were females. The most common site was found to be the tongue followed by the lip as shown in Table 2. 5 cases of leukoplakia were studied with the mean apoptotic index being 0.11+/- 0.05%. One out of the five patients was a female and one patient had history of tobacco smoking. All of them presented with a grey white patch. Mild dysplasia's presented with a grey white or an erythematous patch, most of the patients were male with history of tobacco smoking. The mean apoptotic index was found to be 0.14+/- 0.09%, which was higher than the leukoplakic lesions. Moderate dysplasias were encountered as slightly elevated lesion with a mean apoptotic index slightly higher than mild lesions. The mean was found to be 0.17+/-0.17% which was significantly higher than the previous lesions studied. Sever dysplastic lesions presented with a markedly elevated lesion with the apoptotic index dipping to 0.14+/-0.14% which was found to be similar to mild dysplasia. This lowering of value which was seen in severe dysplasia is not statistically significant (p>0.4).

The mean apoptotic index seen in all 33 cases are shown in Table 3. The mean apoptotic index is seen to increase with the severity of dysplasia, with a peak seen in moderate dysplasia. No significant increase was seen between moderate and severe dysplasia.
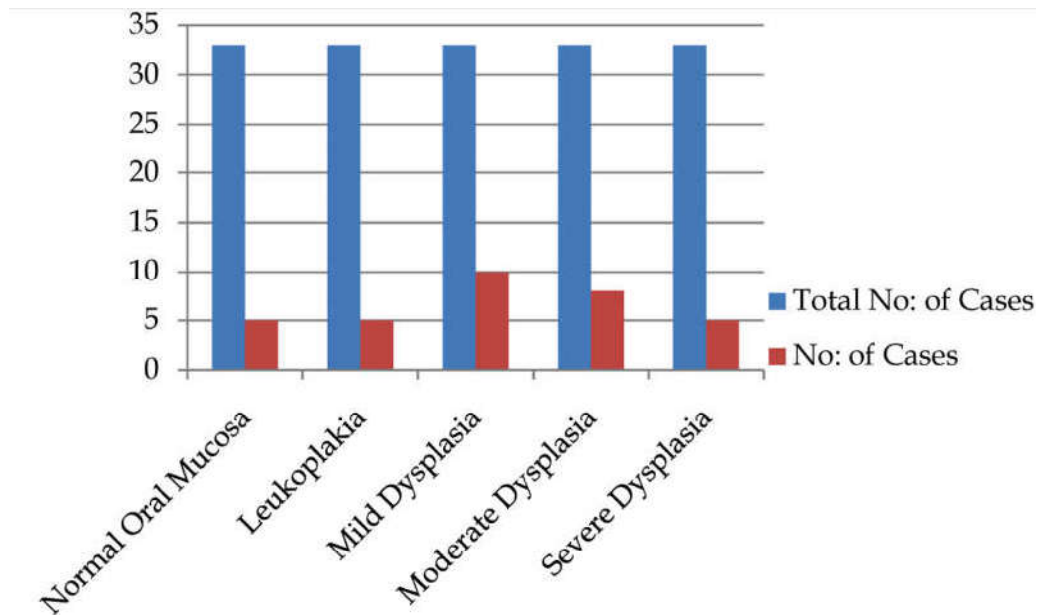
Graph 1: Distribution of dysplastic lesions

Total No: of Cases

■ Tongue
■ Lip
■ Gingiva
■ Buccal Mucosa
■ Floor of the Mouth
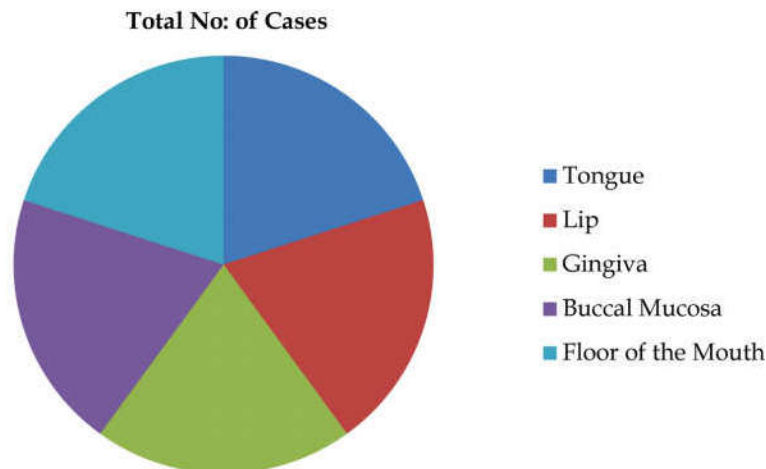
Graph 2: Site distribution for dysplastic lesions in our study

Table 1: Mean apoptotic index in all grades of dysplastic lesions

Histological Diagnosis	Apoptotic Index	Mean Apoptotic Index
Normal Oral Mucosa	0.05% - 0.10%	0.09%
Leukoplakia	0.05% - 0.20%	0.11%
Mild Dysplasia	0% - 0.20%	0.14%
Moderate Dysplasia	0% - 0.40%	0.17%
Severe Dysplasia	0.10% - 0.20%	0.14%

Discussion

Apoptosis is known to have an important role in tumour progression and development. In our study we have evaluated premalignant lesions arising from the oral cavity. Out of the 33 cases evaluated 5 cases were normal mucosa which was obtained from tonsillectomy specimens. 5 cases were from a grey white patch in the oral mucosa which were diagnosed as leukoplakia. These 5 cases did not show any significant evidence of dysplasia. The remaining 23 cases showed varying degrees of dysplasia's. 10 out of 23 cases showed features of mild dysplasia, 8 showed moderate dysplasia and 5 showed severe dysplasia. From the 28 cases studied 25 were male and 3 were female. The dysplastic lesions were found to be more common in males and also those males who had a history of tobacco smoking. The youngest patient was 25yrs old and the oldest was 79. Normal oral mucosa was encountered in the younger age group whereas dysplastic lesions were more common in the older age group [5,6,7]. Tobacco is one of the important etiological factors in the development of dysplasias [4]. Cases from all areas of the oral cavity were included such as tongue, lip, buccal mucosa, gingiva and floor of the mouth. The most common location was found to be the tongue followed by lip and gingiva. Variable presentations were seen, with leukoplakia or erythroplakic plaque being the most frequent [2,8].

Our study is based on the assessment of apoptotic index in 28 oral premalignant or dysplastic lesions using the light microscope. Apoptotic index was calculated using oil immersion lens as conducted by Soini et al. During this study we concluded that light microscopy provides a fairly accurate assessment of apoptosis [5]. Apoptotic bodies were seen in the basal and suprabasal regions in the normal mucosa and mild dysplasia whereas as the severity increased they became generalised [9,10]. This lead us to believe that as the severity of the lesion increased the apoptosis became generalised. Mitotic figures were also evident which could be differentiated from apoptosis. Apoptotic cells which were seen in the areas of necrosis and inflammation were not counted. We observed an increase in the apoptotic index as the lesion progressed from mild to severe dysplasia which peaked at moderate dysplasia, the possible reason for this could be the less number of severe dysplastic cases [5,6]. However, the fall in apoptotic index is not statistically significant ($p > 0.4$) in comparison with moderate dysplasia.

Conclusion

In our study we evaluated the apoptotic index in dysplastic lesions involving the oral cavity. Most of the cases were male and had a history of tobacco use.

We found that there was an increase in apoptotic index with the degree of dysplasia. Evaluation of apoptosis gives us a fairly good idea regarding the grade of dysplasia. Lower apoptotic index corresponds to lower grade of dysplasia and higher index corresponds to higher grades. The location of the apoptotic bodies also gives us an idea about the grade of dysplasia. Low apoptotic index acts as a good prognostic marker. Hence we can conclude that apoptotic index can be used as a reliable and easy marker to assess the grade of dysplasia and also it can act as a prognostic indicator in the progression of dysplasia to carcinoma.

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