EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 12
762-764
Year: 2017

HISTOPATHOLOGICAL EVALUATION OF APOPTOSIS IN ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA

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Article Received on 17/10/2017 **Article Revised on 06/11/2017** **Article Accepted on 27/11/2017**

ABSTRACT

Introduction- In India, oral cancer is the most common cancer in males and the third most common cancer in females. 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. Thus, the aim of this study is to assess the significance of apoptotic index (AI) as a 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). 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-** Further studies need to be undertaken to detect and understand the apoptotic mechanisms in the progression from OED to OSCC.

KEYWORD: OED, OSCC, AI, WDSCC.

INTRODUCTION

Oral squamous cell carcinomas (SCCs) are usually preceded by a premalignant lesion mostly presenting clinically as leukoplakia, erythroplakia, oral submucous fibrosis and histologically as hyperplasia, dysplasia or carcinoma *in situ*. Leukoplakia does not necessarily correlates with histological dysplasia.

Early diagnosis of these lesions greatly increases the chance of cure and significantly reduces deformity in the patients. In the recent past, histological techniques that identify the parameters such as cell proliferation and cell death have become quite significant. Measuring these parameters may not only help in identifying the individuals who are at a greater risk of developing carcinomas, but they also carry a significant prognostic value and also represent a good model of tumor development.[1,2]

A technique of counting of apoptotic cells and apoptotic bodies has been discussed by various authors. [3, 4, 5] 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, Faculty of Dental Sciences, IMS,BHU. 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.

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 and aggregation and, ultimately, karyorrhexis.

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 (0.7559±0.0856) [Table 1].

Table 1: Mean AI and SD among all groups,

Histological grades	Total number of cases	Apoptotic index Mean±SD
OED	30	0.4430±0.1803
Mild OED	12	0.2425±0.0893
Moderate OED	10	0.4725±0.0979
Severe dysplasia	8	0.6125±0.0760
OSCC	30	0.5800±0.1379
WDSCC	10	0.7559±0.0856
MDSCC	12	0.5320±0.0589
PDSCC	8	0.4492±0.0493

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].

Table 2: Comparisons between different histological grades of OED.

Histological grades	Mean difference	t value	P value
OED OSCC	0.1928	4.432	<0.001*
Mild OED Moderate OED	0.2382	5.849	<0.001*
Mild OED Severe OED	0.3770	9.600	<0.001*
Moderate OED Severe OED	0.1429	3.413	0.005

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

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].

Table 3: Comparisons between different histological grades of OSCC.

Histological grades	Mean difference	t value	P value
WDSCC MDSCC	0.2092	6.256	<0.001*
WDSCC PDSCC	0.3088	8.092	<0.001*
MDSCC PDSCC	0.0887	3.263	0.004

*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.

DISCUSSION

Individuals with primary dysplastic lesions in the oral cavity are at risk for developing OSCC.[6] A large number of stimuli can induce apoptosis in a cell type dependent manner. Depending on the triggering factor and the cell type, there are multiple signaling pathways that lead to activation of the apoptotic reliable results and are an inexpensive method for detection of apoptotic cells, hence used in this study.[6]

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.[7,8] 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.[1,9] 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.[1,2,10] In this study, AI increased progressively from dysplasia to carcinoma but decreased with decreasing differentiation of the tumor.

Analysis of AI showed a progressive increase in apoptosis in parallel with biological aggressiveness, indicating increased synthetic activity of these 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, the tumor outgrows its blood supply leading to hypoxic injury‑causing increased apoptosis.[11]

Increased apoptosis with increasing grades of neoplastic lesions is associated with large tumor size and with a shortened disease‑free survival period. Compelling evidence indicates that oncogenic changes promote apoptosis during multistage carcinogenesis.[2]

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.[12] 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.[13]

CONCLUSION

We have seen in this study that apoptosis can be used as a prognostic marker in OED lesions and OSCC. 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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