

Assessment of Apoptotic Index in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma: A Light Microscopic Study.

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ABSTRACT

Background: In India, oral cancers are the most common cancer in males and the third most common in females. Apoptosis means programmed cell death. In general, the onset and development of cancer has been linked to a reduction in the rate of apoptosis.

Aim: To assess apoptotic index in oral epithelial dysplasia and oral squamous cell carcinoma and to evaluate its prognostic role in oral cancers and to correlate apoptotic index and histological grading of oral epithelial dysplasia and oral squamous cell carcinoma.

Materials and Methods: Study sample includes diagnosed 60 paraffin wax blocks of oral epithelial dysplasia, oral squamous cell carcinoma (OSCC) retrieved from the department of Oral Pathology and Microbiology. Assessment of apoptotic index (AI) using light microscopy was performed on haematoxylin and eosin stained tissue sections of each samples. Statistical Analysis Used was Students 't' test was performed.

Results: The mean AI increased progressively with increasing grades of dysplasia, with the maximum AI in well-differentiated (WD) OSCC, and a less was noted with progression toward higher grades. Statistical significance was not seen between different grades of dysplasia. The difference between WD OSCC and poorly-differentiated OSCC was statistically significant ($P < 0.05$) in our study.

Conclusion: Apoptosis can be accurately assessed using light microscopy. Tumours that exhibit lesser apoptosis index tend to show aggressive behaviour.

Key words: Apoptotic index, dysplasia, Oral squamous cell carcinoma

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INTRODUCTION

Oral cancer is one of the major health issues in south East Asia. The development of OSCC is a molecular and histological multistep process. Its progression includes sequential histopathological alterations ranging from hyperplasia through dysplasia to carcinoma in situ and invasive carcinoma.¹

Apoptosis is a complex, inherently programmed cell death. Discordantly, any deregulation of the apoptotic pathway can pave way to the immortalization of replicating cells, resulting in the accumulation of sequential genetic damage that can induce cell death.² As counting of apoptotic bodies using light microscopy is feasible and there has been interest in the enumeration of apoptosis in malignant growths as a putative prognostic marker.^{3,4} Here we performed the evaluation of AI using light microscopy to assess the significance of AI as a proliferation marker in premalignant and malignant lesions of the oral cavity.

MATERIALS AND METHODS

Study sample includes 60 tissue specimens embedded in paraffin wax blocks of reported cases of oral epithelial dysplasia and OSCC retrieved from the Department of Oral Pathology and Microbiology. One section of 5 microns thickness was sectioned from each block and stained with haematoxylin and eosin stains. Apoptotic bodies were counted using binocular light microscope under a magnification of x40 in a stepladder fashion. The area selected for apoptotic bodies counting include the most invasive

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part and the most cellular part of the tissue section. In each section, 1000 tumour/dysplastic epithelial cells were evaluated from ten fields for the presence of apoptotic cells and apoptotic bodies. The areas showing necrosis, tissue folds, inflammation and calcifications were not considered for counting. Two observers were there in this study, and the common results were formulated to avoid interobserver variability. Counting of apoptotic bodies were done as per the morphological criteria proposed by Kerr et al and van Diest et al.. Apoptotic cells present in normal human tissues and cancers can be identified by well-

established criteria.^{3,4}

These include:

Cell shrinkage (reduction in size, eosinophilic cytoplasm with round and smooth margin clearly separating from neighbouring cells).

Chromatin condensation (hyper basophilic in colour and irregular in shape).

Nuclear fragmentation (round shaped one or more chromatin pieces with variability in size).

Absence of inflammation in field, Apoptotic bodies, which appeared as tiny, round and pyknotic nuclear fragments, were seen scattered among tumour cells and occasionally forming a cluster.^{3,4}

AI was calculated as the number of apoptotic cells and bodies, expressed as a percentage of the total number of non-apoptotic

tumour cells counted. Statistical evaluation was carried out using the Student's 't' test, considering $P < 0.05$ being significant. Statistical analysis was performed using SPSS 12.0 for Windows (Microsoft, WA, USA).

RESULTS

The apoptotic cells showed cell shrinkage, condensation, deep eosinophilia of the cytoplasm and pyknotic, round to crescentic or irregular nucleus (Fig-1& Fig-2A). Karyorrhexis was observed frequently. Apoptotic bodies seen as tiny, round and pyknotic nuclear fragments, which were scattered among tumour cells forming occasional clusters. (Fig-2B) Main pitfalls included the presence of mononuclear inflammatory cells, densely stained mitotic figures, nuclear debris and necrotic cells.

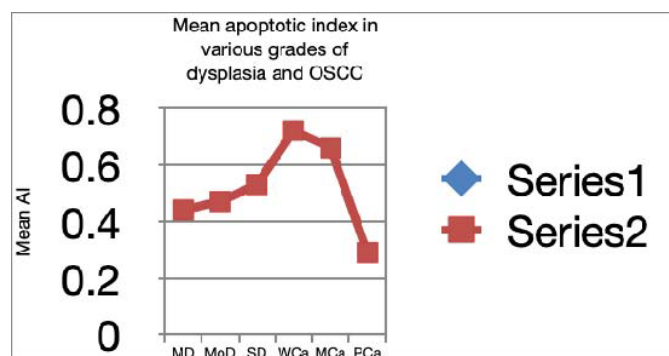
AI was evaluated in 60 cases, including 30 cases of oral epithelial dysplasia (mild = 10, moderate = 10, severe = 10), and 30 cases of OSCC (well differentiated [WD] = 10, moderately differentiated [MD] = 10, poorly differentiated [PD] = 10).

The mean AI increased progressively with increasing dysplasia, with the maximum AI was seen in WD squamous cell carcinoma. There was a fall in AI noted with progression towards higher grades. (Table:1, Graph :1)

There was no statistical significance between different grades of epithelial dysplasia. The difference between WD SCC and poorly differentiated SCC was statistically significant ($P < 0.05$).

Table: 1 Mean Apoptotic index in different grades of Oral Epithelial Dysplasia and OSCC

HISTOLOGICAL GRADE	MEAN AI%
Epithelial Dysplasia	0.48±0.047
Mild dysplasia	0.44±0.045
Moderate dysplasia	0.47±0.025
Severe dysplasia	0.53±0.046
Oral Squamous Cell Carcinoma (OSCC)	0.56±0.047
Well Differentiated OSCC	0.72±0.027
Moderately Differentiated OSCC	0.66±0.045
Poorly Differentiated OSCC	0.29±0.023



Graph 1: Apoptotic index (AI) in different grades of Oral Epithelial Dysplasia and OSCC. MD-mild dysplasia, MoD-moderate dysplasia, SD-severe dysplasia, WCa- well differentiated OSCC, MCa- moderately differentiated OSCC, PCa- poorly differentiated OSCC.

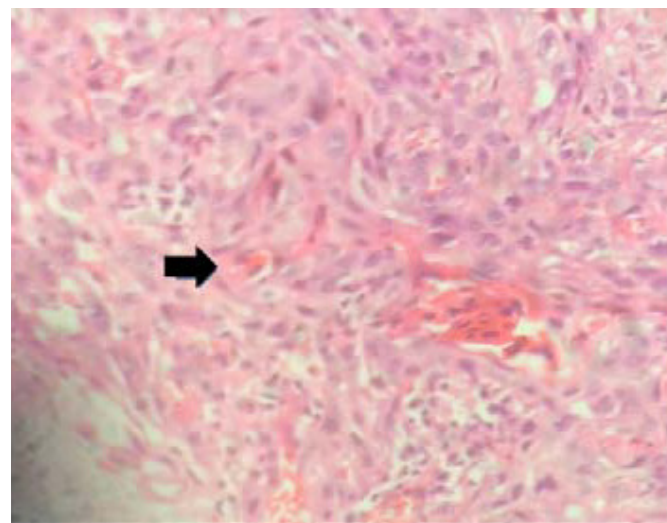


Fig. 1: Apoptotic cells in OSCC (H&E, 10X)

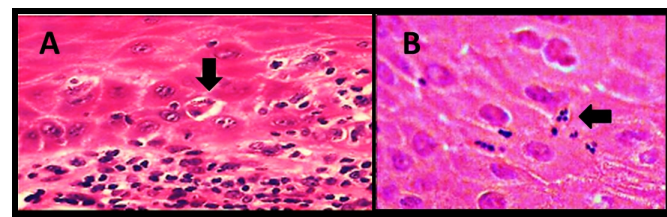


Fig. 2 A and B: A. apoptotic cells B. apoptotic bodies in dysplastic epithelium (H&E, 40X)

DISCUSSION

Clinical behaviour of tumours is difficult to be practiced on histopathological parameters alone. Most human tumours are characterized by an imbalance of regulatory mechanisms controlling cell cycle programme as well as apoptosis. The mechanism of carcinogenesis of OSCC involves the activation of oncogenes and inactivation of tumour suppressor genes, outcome of which is a cellular imbalance between cell growth and cell death.⁵ The three principle antiproliferative pathways on carcinogenesis include inhibition of cell growth, Induction of cell differentiation and programmed cell death.⁶

Apoptosis is an imperative phase in tissue homeostasis, cell differentiation and turn over.⁶ Any deregulation of apoptotic pathway can pave to the immortalization of replicating cells, resulting in the accumulation of sequential genetic damage that can induce cell death.²

Our study was focused on evaluation of AI in 60 oral premalignant and malignant squamous cells lesions on light microscopy. Apoptotic bodies were counted using ×40 magnification which was similar to the views of Soini et al.³ We observed that a fairly accurate assessment of apoptosis is possible by light microscopy and tumours that exhibit less apoptosis tend to show aggressive behaviour. This result was in accordance with study conducted by Jain et al.⁷

To avoid human errors, various other advanced and better methods have been developed to study apoptosis, i.e. electron microscopy, flow cytometry, electrophoresis, in situ end labelling of fragmented DNA and terminal deoxynucleotidyl transferase-mediated d-UTP biotin nick end labelling technique (TUNEL). Although it is accepted that electron microscopy is the best way to identify apoptotic cells, this method is impractical. The TUNEL technique is now the most commonly used method for evaluating apoptosis but it is not economically feasible. We have used light microscopic evaluation because it is the simplest and economically feasible technique of assessment of AI.

When normal cells are damaged beyond repair, they are removed by apoptosis. But the cancer cells are resistant to apoptosis and they continue to multiply in an unregulated manner.⁸ Thus the beneficial anticancer effects of chemotherapy are predominantly

mediated through induction of apoptosis in tumour cells de novo or as a result of chemotherapy-induced damage of cellular metabolic processes or cell cycle control mechanisms. Thus, it is possible that tumours that exhibit apoptosis may be more sensitive to chemotherapy and likely to have a better prognosis.⁹

CONCLUSION

A decrease in the rate of apoptosis has been linked to the onset and development of cancer. This counting of apoptotic bodies using light microscopy is feasible, there has been interest in the enumeration of apoptosis in malignant growths as a putative prognostic marker. However there appear to be valid biological reasons for a relationship between low AI and poor prognosis, other factors in tumour progression such as mitotic rate and invasive capability have a confounding influence on tumour behaviour than the AI alone.

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