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Research Article

The Expression of Apoptosis in Normal Epithelium and its Clinical Relevance

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I N F O

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A B S T R A C T

Apoptosis is a programmed cell death by which cells are systematically deleted in a multi-cellular organism. It is important in organogenesis during development, physiological atrophy of the tissues, and the removal of the cells with genetic defects. It is altered in various cellular changes like hyperplasia, dysplasia, and neoplasia of oral epithelium. We studied apoptosis by TUNEL assay in 100 normal epithelium, dysplastic areas of premalignant lesions (n=31) and squamous cell carcinoma of oro-pharyngeal region (n=100). We found an increase in apoptosis in dysplastic areas of oral epithelium and a slight decrease in squamous cell carcinoma compared to normal epithelium. On conclusion, we found that apoptosis play an important role in the maintenance of the normal number of cells in normal and dysplastic oral epithelium. Its possible deregulation/ inhibition plays an important role in the unhindered growth of the malignant cells.

Keywords: Apoptosis, Dysplasia, Neoplasia

Introduction

Apoptosis is programmed cell death. It is a systematic means of cell death within an organism. Apoptosis is normally observed in morphogenesis during development, removal of auto-reactive immune cells, hormonal or age-related tissue atrophy & removal of the cells with genetic abrasion.[1] Thus it plays an important role whereby unnecessary cells may undergo cell death and allow the growth and differentiation of cells better geared to deal with changing environmental demands.[2]

The cell undergoing apoptosis can be easily recognised by light microscopy. It detaches from the neighbouring cells and shrinks in size. The chromatin aggregates peripherally, and the nucleus breaks into two or more fragments. There is extensive surface blebbing and the cell undergoes fragmentation into a number of membrane-bound apoptotic bodies composed of cytoplasm and tightly packed organelles with or without nuclear fragments.[3]

The apoptosis is important for tissue with high cell turnover like mucosa of the gastrointestinal tract.[4] It is altered in various cellular changes like hyperplasia, dysplasia and neoplasia.[5] The aim of our study was to investigate the apoptosis in normal epithelium and its alteration in various disorders of the oro-pharynx e.g. pre cancer conditions like leukoplakia, oral sub mucous fibrosis and lichen planus, and squamous cell carcinoma.

Methods

The cases were collected from the department of pathology and the study was done in the department of physiology and pathology, Maulana Azad Medical College, New Delhi.

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The cases that were clinically diagnosed with mild non-specific changes, but with no pathological alterations were used as normal mucosa (n=100).

The pre-malignant lesions of the oral cavity were studied in three groups:

1. Leukoplakia (n=90)
2. Oral sub mucous fibrosis (n=65)
3. Lichen planus (n=20)

One hundred cases of squamous cell carcinoma of oro-pharynx region were included in to study of apoptosis in neoplastic cells (n=100).

All the biopsies were fixed in 10% buffered formalin and embedded in paraffin wax. The categorising cases as normal, preneoplastic and neoplastic was based on the light microscopical examination using hematoxylin & eosin stained slides.

Apoptosis (TUNEL Staining)

The apoptotic cells were detected by in situ labelling of 3'-end of DNA fragments generated by apoptosis-associated endonucleases. The sections were dewaxed in xylene and re-hydrated in different grades of acetone. The tissues were then treated with terminal deoxynucleotidyl transferase enzyme and biotin-labelled nucleotide. The slides were washed with phosphate-buffered saline (pH 7) and were then treated with avidin-peroxidase complex. The brown colour was developed with di diaminobenzidine, after which the slides were counterstained with hematoxylin.

Calculation

Cells were defined as apoptotic if the completely nuclear area of the cell had taken up brown colour. The apoptotic index (percentage of cells showing apoptosis) was calculated by counting at least five hundred cells.

Statistical Analysis

To find the difference in means apoptotic index of normal, premalignant and malignant lesions student-t test was used. The P-value of <0.05 was considered as significant.

Results

The apoptotic index (AI %) was studied in normal epithelium (n=100), dysplastic areas of pre-cancerous lesions and in cases of squamous cell carcinoma (n=100). Dysplasia was seen in 31 cases of preneoplastic lesions, which were further divided into, leukoplakia (22), oral sub mucous fibrosis (8), and lichen planus (1).

The mean expression of apoptosis in the normal epithelium (22.7 ± 27.3, mean ± SD, Figure 2) was lower than in the dysplastic areas of pre-cancerous lesions (Table1, Figure 3), but the significant difference was observed only with leucoplakia (53.6 ± 26.1, mean ± SD, p<0.001). However a decrease in the mean apoptotic index was observed in squamous cell carcinoma (17.6 ± 25.6, mean ± SD, Figure 4) as compared to normal epithelium though the difference between them was not statistically significant (p=0.2). The mean apoptotic index was significantly lower in squamous cell carcinoma as compared to dysplastic areas of preneoplastic conditions (P < 0.001).

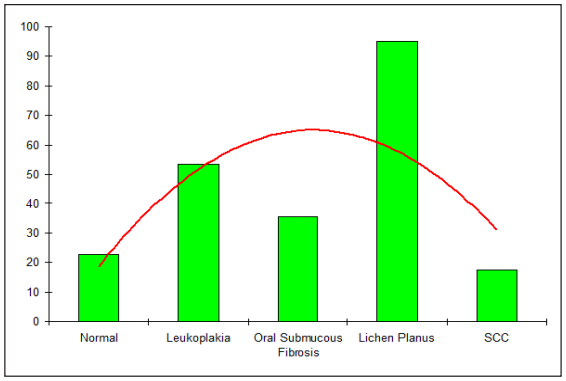

Figure 1.Bar Diagram and Trend Line Showing Mean Apoptotic Index of Normal, Preneoplastic and Squamous Cell Carcinoma

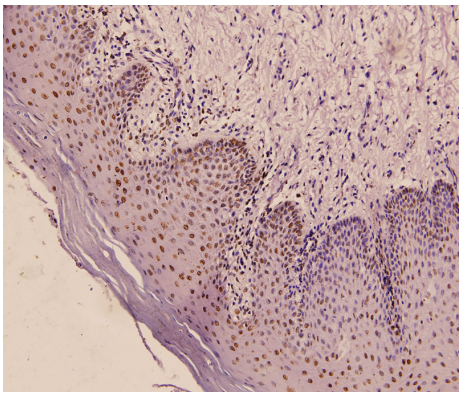

Figure 2.Expression of Apoptosis in Normal Epithelium (TUNEL, 100X)

Table 1.Apoptotic Index in Normal oral Mucosa, Dysplastic Areas of Premalignant Condition and in Squamous Cell Carcinoma

	Squamous cell carcinoma Mean±S.D 17.4±25.6	Leukoplakia Mean±S.D 53.6±26.1	Oral sub mucous fibrosis Mean±S.D 35.6±24.3	Lichenplanus Mean±S.D 95.0±0
Normal Mucosa Mean ± S.D 22.6 ± 27.3	p=0.2	p<0.001	P=0.5	NA*

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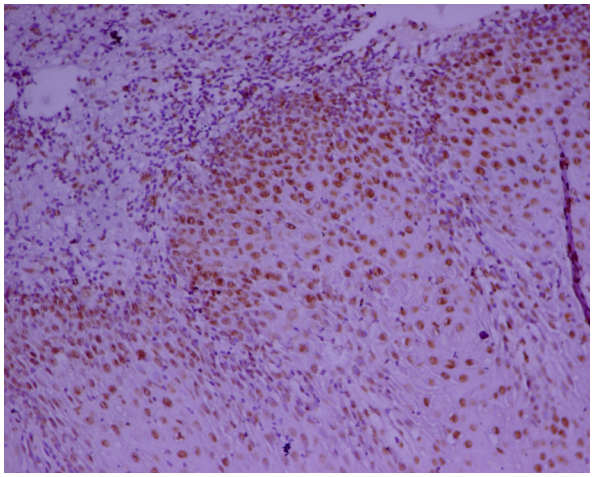

Figure 3.Expression of Apoptosis in Dysplastic Area of Leukoplakia (TUNEL, 200X)

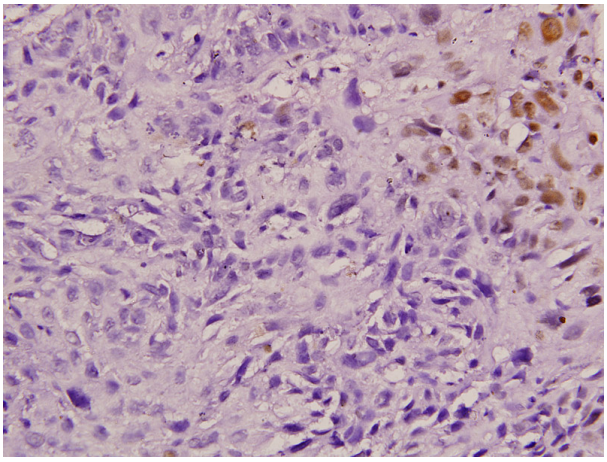

Figure 4.Expression of Apoptosis in Squamous cell Carcinoma (TUNEL, 400X)

Discussion

Apoptosis (programmed cell death) plays an important role in the maintenance of steady state in continuously renewing tissues.[4] In stratified squamous epithelium (e.g. of oral cavity) the cells are removed by apoptosis as well as by desquamation (terminal differentiation of the proliferative cells).[6]

The expression of apoptosis in normal epithelium has been studied previously. Tormane et al observed very little apoptosis in the bronchial epithelium.[7] However; a study conducted by Birchall MA et al.[8]

Observed high number of cells undergoing apoptosis and their position was confined to suprabasal region. Various in situ studies also show a similar result.[9] In our study, we observed the apoptotic cells in basal and parabasal region. The apoptosis was high (mean apoptotic index 22.6%), about one fifth of the cells are deleted by apoptosis. Thus in the normal epithelium the apoptosis is a significant mean of elimination of unnecessary or the unwanted cells.[7]

A study conducted by Birchall M et al.[8] observed an increase in apoptosis in dysplasia, Tormane U et al. also observed a similar increase in precancerous lesions as compared to normal mucosa.[7] In our study, we also observed a similar increase in apoptosis in the dysplastic cells as compared to normal mucosa.

Due to various environmental factors (like smoking, tobacco chewing and the use of betel nut) cell genome undergoes certain changes, and the cell morphologically appear dysplastic. Due to these genetic changes, the apoptotic machinery gets stimulated.[8] Thus, apoptosis acts as a means by which the genomically defective cells can be removed in premalignant lesions of oral cavity.

We have observed an increase in apoptosis from normal epithelium through dysplasia, however with the onset of malignant change apoptosis decreases. The malignant cells of squamous cell carcinoma are genetically abnormal hence an increase in apoptosis was expected however we found that apoptosis was slightly lower in squamous cell carcinoma as compared to control cases (although this difference was not statistically significant). More over we found apoptosis significantly lower in squamous cell carcinoma as compared to premalignant lesions. This can be explained by possible defect in regulatory system of apoptosis whereby apoptosis does not get stimulated in malignant cells or the inhibitory component is more active. On similar lines, Birchall M et al. suggested that a change in apoptosis accompanies the on set of invasion in the pre-malignant lesion of the oral cavity and oro-pharynx.[8] They also found a decrease in apoptotic index in squamous cell carcinoma as compared to the pre-neoplastic epithelium. Thus, inhibition of apoptosis may be an important part of final step from premalignant to malignant transformation of cell.

On conclusion in this study we found that apoptosis is an important mechanism of removal of cells in actively proliferating squamous cells of normal oral epithelium. We also found that apoptosis play an important role in containment of dysplastic cells in oral premalignant lesions and its possible inhibition have a role in uncontrolled growth of malignant cell in squamous cell carcinoma.

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