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Original Article

Apoptotic Count as a Guide for Histological Grading of Carcinoma Esophagus: A Light Microscopic Study

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ABSTRACT

Background: Many studies have been done in the past on the correlation between apoptotic count and histological grading of different tumors.

Aims: The study aims to find out if a correlation between apoptotic count and histological grading exists in squamous cell carcinoma of the esophagus, and also to review the literature on such a relationship in the context of some other tumors.

Settings and Design: Cases of squamous cell carcinoma of the esophagus who presented at a tertiary care center over a period of one year were reviewed.

Materials and Methods: The endoscopic biopsy specimens of 56 patients of squamous cell carcinoma of esophagus were fixed in 10% buffered formalin, processed for routine paraffin sections, sections taken, stained by hematoxylin and eosin and examined under light microscope, using 40x objective and 10x eyepiece. Apoptotic bodies were counted in each high-power field (HPF).

Statistical Analysis Used: Standard error of difference in apoptotic count in different tumor groups found and *P* value calculated, using Student's t test.

Results: An inverse correlation of the apoptotic count per HPF with the histological grade of the tumor was found.

Conclusions: Grading of squamous cell carcinoma of esophagus, solely on the basis of apoptotic count can be used in the first place or to corroborate conventional histological grading done on the basis of morphology.

Keywords: Apoptotic count, histological grading, squamous cell carcinoma esophagus

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INTRODUCTION

Apoptosis is a complex, tightly regulated and active cellular process whereby individual cells are triggered to undergo self-destruction in a manner that will neither injure the neighboring cells nor elicit any inflammatory reaction.[1] There have been various studies in the past in an attempt to find the correlation between apoptotic count and histological grade of different neoplastic lesions and also, its effect on survival of patients, with supposedly contradictory conclusions. However, the common conclusion of most studies is that apoptotic count can be used in predicting the grade, and indirectly, prognosis of tumors. This study aims to find out if such a correlation exists in the cases of carcinoma esophagus so that grading done on the basis of apoptotic count could be used in the first place or to corroborate findings of morphological classification. A review of the literature of relationship between apoptotic count and histological grading in various neoplastic lesions is also presented.

MATERIALS AND METHODS

In the study a total of 56 patients were included. The selection of cases of carcinoma esophagus was strictly based on clinical examination and investigations followed by confirmation by histopathology. The cases which could not be confirmed histopathologically or whose records were incomplete or missing were excluded.

The biopsy specimens were fixed in 10% buffered formalin and processed for routine paraffin sections. The 5-micron sections of uniform thickness were taken and stained by hematoxylin and eosin. By light

microscopy on hematoxylin-eosin stained slides, with a 40x objective and 10x eyepiece, apoptotic bodies were counted per high-power field (HPF). Apoptotic bodies were morphologically identified as shrunken cells with compact, segregated and sharply delineated mass of chromatin with a deeply eosinophilic cytoplasm. Maximum HPFs were examined in each case and average taken to guarantee representativeness.

RESULTS

Out of 56 patients, 39 were males and 17 females, male to female ratio being 2.3: 1. Age of the patients varied from 30 years to 73 years. Most of the patients (50%) were in the 51-60-year age group. The average age of male patients was 54.4 years, and of females, 56.9 years. On the basis of conventional grading of tumors, using morphology of tumor cells, keratinization and mitotic figures, 12 cases (21.4%) were graded as well-differentiated, 36 cases (64.3%) as moderately differentiated and eight cases (14.3%) as poorly differentiated squamous cell carcinomas. The apoptotic count/HPF was calculated in these three histological groups: the first group (well-differentiated), having apoptotic count/ HPF ranging from 3.47 to 3.53 (mean = 3.50); the second group (moderately differentiated), having apoptotic count / HPF ranging from 2.19 to 2.23 (mean 2.21); and the third group (poorly differentiated), having apoptotic count / HPF ranging from 0.83 to 0.89 (mean = 0.86) [Table 1]. Student's t-test was used to analyze results in different tumor groups. The differences in the mean apoptotic count / HPF were found to be statistically significant between well and moderately differentiated carcinoma (two-tailed $P < 0.0001$), moderately and poorly differentiated carcinoma (two-tailed $P < 0.0001$) and well and poorly differentiated carcinoma (two-tailed $P < 0.0001$). Thus an inverse correlation of the apoptotic count / HPF with the histological grade of the tumor was found.

Table 1: Relationship of mean apoptotic count per high-power field with degree of histological differentiation in the cases of carcinoma esophagus

Histological grade	Number of cases	Range of apoptotic count / HPF	Mean apoptotic count / HPF
*†Well-differentiated	12	3.47 to 3.53	3.50
*‡Moderately differentiated	36	2.19 to 2.23	2.21
‡†Poorly differentiated	08	0.83 to 0.89	0.86

*Two-tailed *P* value < 0.0001 (Student's t-test), ‡Two-tailed *P* value < 0.0001 (Student's t-test), †Two-tailed *P* value < 0.0001 (Student's t-test)

DISCUSSION

The term "apoptosis" was proposed by Wyllie for the observed morphological findings of what appeared to be controlled cell deletion.[2] Morphological features of apoptosis include compaction of nuclear chromatin which then becomes marginated against the nuclear envelope and subsequently, there is nuclear fragmentation. Meanwhile, the cytoplasm condenses, any microvilli disappear and blunt blebs appear on the plasma membrane. Cells separate from their neighbors, and desmosome complexes are fragmented. While this is occurring, the cytoplasm continues to condense and apoptotic bodies, which contain membrane-enclosed fragments of the nucleus, bud from the cell. Lastly, the apoptotic bodies are engulfed by neighboring cells and macrophages, without an associated inflammatory response.

Although it is accepted that electron microscopy is the best way to identify apoptotic cells,[3] this method is not practical in most histological studies of specimens. Detection of apoptotic cells in formalin-fixed tissue sections of tumors is possible because of characteristic morphological features (as described above) that are manifest even in the routinely stained sections.[1,2] Another feature supporting their identification is that the apoptotic process typically involves a small number of individual cells surrounded by adjacent surviving cells, often producing a "halo" effect. The results obtained by "plain" morphology show good correlation with deoxyribonucleic acid (DNA) end-labeling methods[4] and immunohistochemistry[5] to detect apoptotic bodies. Thus, morphology alone although less sensitive, is a fairly reliable and inexpensive method for the detection of apoptosis.

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 machinery. It is obvious that apoptosis is generally increased in cancers. Part of the explanation probably involves participation of many oncogene and tumor suppressor gene products in the regulation and execution of apoptosis. Among them are *p53, Rb, ras, raf, and myc, p53* being the most important. It monitors the state of DNA, and, in case of DNA damage, stalls the cell cycle. This takes place through the induction of a protein that prevents phosphorylation of cyclin-dependent kinases, the well-known positive regulators of the cell cycle. In the absence of phosphorylated active cyclin-dependent kinases, *Rb* protein, another regulator of the cell cycle, remains unphosphorylated (inactive), and, hence, the cell cycle halts.[6] This then leads to activation of

DNA repair machinery. If the DNA repair fails, p53 takes over again and triggers apoptosis in a process that involves upregulation of the apoptosis-inducing bax and down-regulation of the anti-apoptotic bcl-2 which ultimately triggers apoptosis through activation of caspases. Loss of cell adhesion[7] and hypoxia-induced apoptosis[8] are other possible explanations for increased rate of apoptosis in cancers.

In various studies to find a correlation between apoptotic count and histological grade in different tumors and also, its effect on survival of patients, high variability in the apoptotic count has been reported by different authors for the same type of tumors. Still some generalizations can be made. In lymphomas[9,10] and hormone-dependent epithelial tumors such as breast,[11] endometrial[12] and thyroid carcinomas,[13] a higher extent of apoptosis is associated with tumors of a higher grade. Similar observations have been made in carcinoma prostate,[4] cervix[14] and urinary bladder.[15] This is in contrast with other epithelial tumors in which association with tumor grade is inverse, as in oral squamous cell carcinoma.[16] Similarly, apoptotic count is increased in non-small cell carcinoma of lung[17] and also in spermatocytic seminoma[18] (compared to the usual seminoma) that may provide some insight into the excellent prognosis of these tumors.

As is evident from the mechanism of apoptosis described above, cells with chromosomal lesions or mutated DNA may be eliminated by apoptosis; for example, by the p53 pathway which detects DNA lesions and initiates repair or induces apoptosis if the repair process is defective as in neoplasia. Thus, apoptosis may have a role in suppressing malignant transformation by eliminating cells with lesions in the genome. Apoptosis also may have a role in preventing the development of aneuploidy and other genetic abnormalities which are commonly associated with cancer cells and progression of neoplasia. In fact, inhibition of apoptosis is associated with the transformation of normal colorectal epithelium into the malignant phenotype.[19] So, what could be reasons for the apparent paradox of a higher apoptotic count in a rapidly growing tumor of high grade? In such cases, an association between poor outcome and high apoptotic indices may be a consequence of the relationship between proliferation and apoptosis. As the mitotic index increases with grade in certain cancers, it is possible that a high apoptotic index indirectly reflects a biologically aggressive tumor. Also, hypoxia of tumor cells within the growing neoplastic mass may induce apoptosis but at the same time induce selective accumulation of cells that can survive (i.e. not apoptose) in a hypoxic environment. Thus, a paradoxical situation may occur where a tumor demonstrates florid apoptosis, but at the same time progresses to a more malignant phenotype.[20]

CONCLUSION

As evident from above, variable, or even contradictory results have been obtained in different studies to correlate apoptotic count and histological grade in tumors. We conclude that if we adhere to the established criteria for recognition and counting of apoptotic bodies, the apoptotic cells can be readily and accurately demonstrated on routine hematoxylin and eosin-stained sections of various malignant lesions. The present study is an endeavor in that direction with special reference to squamous cell carcinoma of esophagus and suggests that grading of squamous cell carcinoma of esophagus, solely on the basis of apoptotic count (highest for well-differentiated and lowest for poorly differentiated carcinoma) can be used in the first place or to corroborate conventional histological grading done on the basis of morphology. In fact, the former could be even better as the latter is more subjective, and has problems of poor reproducibility due to high intra and inter-observer variations and subsequent overlapping of different histological groups which is very important in assessing the correct grade of the tumors as the treatment and prognosis of patients rests on it. However, care must be taken to count a sufficient number of cells and account must be taken of tumor heterogeneity. Non-neoplastic apoptotic cells such as apoptotic T lymphocytes and macrophages may also pose a problem for the estimation of the apoptotic count. This limits the usefulness of the study.

Although there appear to be valid biological reasons for a relationship between a high apoptotic count and good prognosis (due to lower histological grade) of tumors (including carcinoma esophagus), this has not been substantiated in published studies of some other tumors. It is likely that other factors in tumor progression such as mitotic rate and invasive capability have a confounding and possibly greater influence on tumor behavior than the apoptotic index alone. Further research is necessary to address and analyze these points.

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