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Proliferative and Apoptotic Indices in Squamous Epithelial Lesions of the Cervix

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Background: Cell proliferation is an important feature of dysplasia and carcinoma. Cell death (apoptosis) is another phenomenon which is responsible for the control of cell number in normal and neoplastic tissue. Simple indices can be used to measure cell proliferation and apoptosis.

Objective: To compare the proliferative and apoptotic indices in various cervical squamous epithelial lesions, and to determine their prognostic value.

Method: Two hundred lesions were evaluated for their morphology, apoptotic index (AI), mitotic index (MI), and argyrophilic nucleolar organiser region (AgNOR) counts.

Result: Mean AI, MI and AgNOR counts significantly increased from benign lesions, to cervical intraepithelial neoplasia (CIN), to invasive carcinoma.

Conclusion: AI, MI and AgNOR counts are useful cell kinetic analysis because they reflect the frequency of two important events, namely mitosis and cell death. These counts can be useful as prognostic markers and aid patient management decisions.

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The cervix is commonly affected with various conditions including disorders of epithelial maturation, hyperplasia, benign tumors, dysplasia, and malignant neoplasms. Squamous cell carcinomas at all sites, evolve through a sequence of changes occurring in the normal squamous epithelium, ranging from dysplasia of varying degree to frank malignancy. Cell proliferation is an important feature of dysplasia and carcinoma. Simple indices can be used to measure cell proliferation[1]. These include: 1) Mitotic index: This is the percentage of mitoses in the lesion. The mitotic index is the oldest method of measuring proliferative activity and can be very useful to diagnose malignancy. 2) Argyrophilic nucleolar organiser region (AgNOR) index: This is a count of the nucleolar organiser regions (NORs). NORs are loops of deoxyribonucleic acid (DNA) that encode ribosomal ribonucleic acid (RNA) and are located in the nuclei of all cells. The proteins of NORs are argyrophilic and can be stained by a silver staining method (AgNOR technique)[2]. The number of silver binding dots is a valuable marker of proliferative activity as it reflects the extent of ribosomal biogenesis.

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However, net growth of a tumor not only depends on proliferative activity but also on the number of cells undergoing death either by apoptosis or by ischaemic necrosis[1]. To date, very few studies provide information about cell death by apoptosis in relation to cell proliferation. Hence, the present study was conducted to investigate the correlation between proliferative and apoptotic indices in the spectrum of squamous epithelial lesions in the cervix and to establish the prognostic significance of these indices.

METHOD

Two hundred specimens of benign and malignant squamous epithelial lesions of the cervix were reviewed. These included cervical biopsies done for diagnostic evaluation, as well as surgical specimens resected as a therapeutic measure. Biopsies showing extensive ulceration of the cervical squamous epithelium were excluded from the study or adequate epithelial cells were not available for performing the various counts. Similarly, tumors showing extensive necrosis with scanty viable tissue, and tumors with radiation effect in the cells, were also were excluded.

The samples were fixed in 10% formalin and processed in the conventional manner. Haematoxylin and Eosin (H&E) stained slides were studied for the morphology of the lesion, and the apoptotic and mitotic counts were done using the 40X objective. Apoptotic cells were identified by their shrunken appearance, condensed hyperchromatic nuclear chromatin, deeply eosinophilic cytoplasm, and the clear halo which separated them from the adjacent tumor cells (Figure 1). The apoptotic index (AI) was defined as the number of apoptotic cells among 100 squamous cells. The mitotic index (MI) was defined as the number of mitoses among 100 squamous cells. AgNOR staining was done on 3μm thick paraffin sections by the method described by Smith and Crocker[2]. AgNORs, which were identified as black dots in the nuclei of the cells (Figure 2), were counted in 100 squamous cells under the oil immersion lens. The mean number of AgNOR dots in each nucleus was calculated in each case. When AgNORs were aggregated into clusters, each cluster was counted as a single structure.

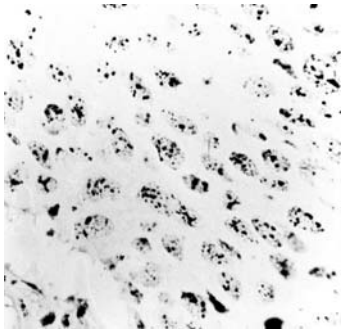

Figure 1: Photomicrograph Showing an Apoptotic Cell in Squamous Cell Carcinoma of the Cervix (Hematoxylin & Eosin, x400)

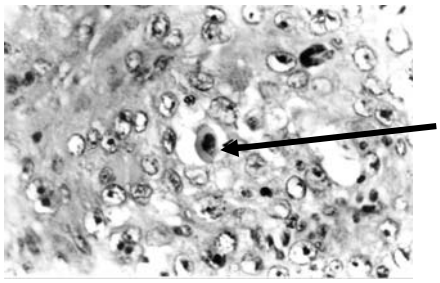

Figure 2: Photomicrograph Showing a High AgNOR Count in Squamous Cell Carcinoma of the Cervix (Silver Stain for AgNOR, x400)

The relationship between the various indices in the spectrum of cervical squamous epithelial lesions was statistically analysed using the analysis of variance (ANOVA) technique.

RESULT

Two hundred cases were studied, 67 (33.5%) aged 35-44. Table 1 shows the age distribution of patients and their diagnoses. Table 2 shows the apoptotic indices, mitotic indices and AgNOR counts in the various histological groups of cervical squamous epithelial lesions. It was found that all the three values increased significantly, from normal epithelium to hyperplasia, to cervical intraepithelial neoplasia (CIN) and to invasive carcinoma. Normal squamous epithelium showed no apoptosis and only a few AgNORs which were confined to the parabasal layers. Metaplastic squamous epithelium revealed counts parallel to those in the adjacent hyperplastic areas.

Table 1: Age Distribution of the Various Squamous Epithelial Lesions of the Cervix

Age group (years)	Hyperplasia/ squamous metaplasia in Chronic cervicitis	Condyloma acuminatum	Cervical intraepithelial neoplasia (CIN)			Carcinoma -in-situ	Invasive squamous cell carcinoma	Total
			I	II	III			
15-24	2	0	2	2	0	0	0	6 (3.0%)
25-34	12	1	2	2	1	1	4	23 (11.5%)
35-44	34	1	3	3	3	2	21	67 (33.5%)
45-54	22	0	2	2	2	2	20	50 (25.0%)
55-64	5	0	2	2	1	0	20	30 (15.0%)
65-74	6	0	2	2	0	0	11	21 (10.5%)
75-84	2	0	1	0	0	0	0	3 (1.5%)
Total	83(41.5%)	2(1.0%)	14(7.0%)	13(6.5%)	7(3.5%)	5(2.5%)	76(38.0%)	200(100%)

Table 2: Apoptotic Indices, Mitotic Indices and AgNOR Counts in the Various Histological Groups of Cervical Squamous Epithelial Lesions

Group	Apoptotic index Mean ± SD	Mitotic index Mean ± SD	AgNOR count Mean ± SD
I. Normal squamous epithelium	0	0.20±0.12	1.25±0.34
II. Hyperplasia /squamous metaplasia in chronic cervicitis	1.09±0.78	0.80±0.48	1.91±0.72
III. Condyloma acuminatum	2.14±0.70	1.67±0.20	2.68±0.31
IV. CIN I	2.13±1.06	1.70±0.25	2.86±0.50
V. CIN II	2.60±1.61	1.96±0.91	3.26±1.50
VI. CIN III / Carcinoma-in-situ	3.15±1.62	2.82±1.24	4.77±1.07
VII. Invasive squamous cell carcinoma	4.49±2.23	4.70±2.03	6.95±1.87

Values are expressed as Mean ± Standard deviation.
For AI, MI and AgNOR: I versus II, II versus III, II versus IV, IV versus V, V versus VI and VI versus VII – significant ($p<0.01$ in each case). III versus IV – not significant ($p>0.05$ in each case).

The two cases of condyloma acuminatum showed values which were not significantly different from those in CIN I. In high grades of dysplasia, AgNORs were seen throughout the thickness of the epithelium, and apoptotic cells were found in moderate numbers. Maximum counts were obtained in invasive carcinoma; however, as the majority of the squamous cell carcinomas in the present study (68 out of 76, i.e. 89.5%) were moderately differentiated (large cell, non-keratinising), the relationship between the various indices and the degree of differentiation of squamous cell carcinoma could not be accurately determined. Nevertheless, it was observed that the two cases of poorly differentiated squamous cell carcinoma of the cervix (small cell carcinoma) had the highest AgNOR counts.

DISCUSSION

Proliferative activity of a tumor is an indicator of its biological behaviour. We used two easily available and relatively inexpensive indices, namely mitotic count and AgNOR staining to measure proliferative activity, and obtain reliable results. Previous studies using AgNOR stainingand Ki-67 immunostaining have shown low proliferative activity in normal epithelium and progressively higher degrees of proliferative activity associated with increasing grades of CIN and carcinoma[1,3-7]. Mean parabasal cell AgNORs have been described to be higher as compared to basal layers, as in the present study; it supports the view that parabasal cells are the main source for cell renewal in ectocervical squamous epithelium, while basal cells serve as reserve cells[4,5]. Pahuja et al noted that highest mean values of Ki-67 and AgNOR counts were found in poorly differentiated squamous cell carcinoma of the cervix, similarly it was observed in the present study[4].

Apoptosis is programmed cell death which is responsible for deletions of cells in normal and in tumor tissues[1]. The cell number in normal and neoplastic tissue depends on factors influencing the balance between cell growth and death. In the normal uterine cervix, epithelial stem cells are stimulated by estrogen to undergo proliferation, maturation, and

desquamation, so that the cell number is controlled, and the epithelium is completely replaced by a new population of cells every 4-5 days. This explains the absence of detectable evidence of apoptosis in normal cervical epithelium[5]. The observed increase in apoptosis with the grade of the lesion, suggests a mechanism whereby apoptosis helps to eliminate cells that have been produced in excess, that have developed improperly, undergone abnormal mitosis or sustained genetic damage[8,9]. It has been suggested that an increasing imbalance between cell proliferation and apoptosis contributes to the progression of CIN[10]. Mandal et al suggested that tumors of large size and higher growth rate outgrow their blood supply, and exhibit greater degrees of apoptosis induced by hypoxic injury[11]. An inverse relationship between histological grade and apoptotic count has been found in squamous cell carcinomas of the oral cavity; however, in the present study of cervical squamous cell carcinomas, no such relationship could be established[11]. Other authors have demonstrated a positive correlation between the frequency of apoptotic bodies and Gleason's grade in prostate cancer[12]. Our finding of mean apoptotic counts increasing from lower to higher grades of CIN to carcinomas of the cervix correlates with those of Dey et al, although our values are slightly higher in comparison[1]. Dey et al propose the use of the turnover index (TI) which is taken as the sum of the mitotic index and apoptotic index, as an indicator of biological behaviour of tumors[1].

Feng et al found that the proliferation marker Ki-67 was markedly increased, and the senescence markers p15 (INK4b), p16 (INK4a) and p14 (ARF) were overexpressed in both cervical dysplasia and carcinoma, in comparison to normal cervical tissue[7]. Lee et al showed that angiogenesis along with proliferation and apoptosis showed progressive increases from CIN to invasive carcinoma of the cervix[13]. Besides squamous epithelial lesions of the cervix, proliferative and apoptotic indices have also been found to be useful to distinguish between benign and malignant lesions of the endocervix and other organs[14-17].

CONCLUSION

The proliferative activity of a lesion is a reliable indicator of its malignant potential, together with the apoptotic count, gives an idea about the net growth of a tumor. Hence, both proliferative and apoptotic indices combined are useful to differentiate between benign, preinvasive and invasive squamous epithelial lesions of the cervix. Mitotic count, AgNOR count and apoptotic count are the simplest techniques that can be employed in any laboratory. These will help to determine the prognosis and plan the management in cases of cervical lesions.

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