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To evaluate apoptotic and proliferative indices in premalignant and malignant squamous epithelial lesions of uterine cervix on light microscopy

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Abstract
Introduction: *The term apoptosis was first recognized in 1972 for its distinct morphology and named after the Greek term meaning "falling off". Kerr, at University of Queensland was first to distinguish apoptosis (Greek: apo- from, ptosis – falling) from traumatic cell death and suggested the term apoptosis. Apoptosis is seen in various physiological and pathological conditions.*
Material and Methods: *Haematoxylin and Eosin stained sections were used for morphology of the lesion and for evaluation of apoptotic and mitotic counts. Apoptotic index (AI) was calculated as the number of apoptotic cells and apoptotic bodies, expressed as a percentage of the total number of non- apoptotic tumor cells counted in each case.*
Results: *Premalignant cases formed 46.29 % and malignant cases were 53.7 % of all lesions. Out of total malignant lesions, majority (69%) were of KSCC, and (31%) were of NKSCC.*
Discussion: *In the present study, it was observed that there is an increase in mean AI, MI and TI with increasing grade of the lesion. AI, MI and TI progressively increased from CIN1 to CIN2 to CIN3 to SCC.*
Conclusion: *It can be concluded that this study is based purely on morphology as it is fairly reliable, easily practiced and inexpensive method for detection of both apoptosis and mitosis.*
Keywords: *Apoptosis, CIN, Squamous cell carcinoma (SCC), Apoptotic and Proliferative indices.*

Introduction

The term apoptosis was first recognized in 1972 for its distinct morphology and named after the Greek term meaning "falling off". Kerr, at University of Queensland was first to distinguish apoptosis (Greek: apo- from, ptosis – falling) from traumatic cell death and suggested the term apoptosis.[1] Apoptosis is seen in various physiological and pathological conditions.

Apoptosis in pathological conditions

Cell death produced by a variety of injurious stimuli, such as radiation and cytotoxic drugs.

1. Cell injury in certain viral diseases, such as, viral hepatitis.
2. Pathological atrophy in parenchymal organs after duct obstruction, such as in the pancreas, parotid glands and kidney.
3. Cell death in tumors.[2]

One biological reason for apoptosis in tumor cells is the increased sensitivity to apoptosis of cells that have lost their matrix attachment or cell-cell contacts. This could be due, to a loss of an expression of cell adhesion molecules, especially integrin and cadherin molecules from the surface of the neoplastic cells.[3]

As the mitotic index increases with increasing grade of the tumors, 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 induces selective accumulation of cell that can survive (i.e not apoptose) in a hypoxic environment.

The beneficial anti-cancer effects of chemotherapy are primarily mediated through induction of apoptosis in tumor 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 tumors which exhibit apoptosis may be more sensitive to chemotherapy and hence likely to have a better prognosis. [4]

The study was conducted in the Department of Pathology, over a period of 12 months. Cervical biopsies received from the Department of Gynecology and Obstetrics were included in the study. The samples were fixed in 10% formalin and processed in the conventional manner. Haematoxylin and Eosin stained sections were used for morphology of the lesion and for evaluation of apoptotic and mitotic counts. The sections were screened under 40X objectives and 10X eye piece, but the final count of the apoptotic cells was carried out under high magnification (oil immersion, 100X lens). From each section, 10 fields devoid of any preservation or fixation artifact, inflammation and necrosis were selected.

In each section, 1000 tumor cells were evaluated for the presence of apoptotic cells and apoptotic bodies and Apoptotic index (AI) was calculated as the number of apoptotic cells and apoptotic bodies, expressed as a percentage of the total number of non- apoptotic tumor cells counted in each case.[5] Similarly Mitotic index (MI) was calculated by counting the mitoses among 1000 tumor cells or from dysplastic cells in CIN or from metaplastic lesions in cervical biopsy specimen.

Turnover index (TI) was obtained from sum of AI and MI i.e. (TI= AI+MI).

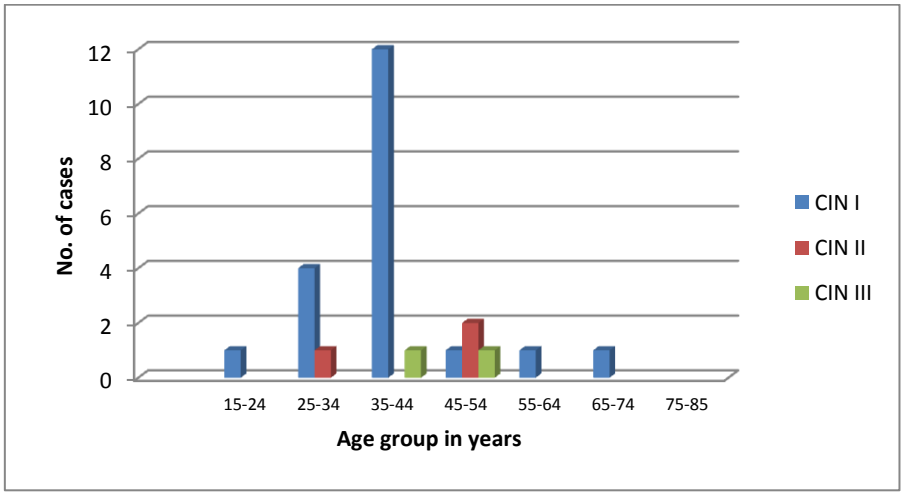
Results

A total of 54 cases of premalignant and malignant squamous epithelial lesions of uterine cervix were evaluated during the study period, out of which 25 were premalignant and 29 were malignant.

Table 1- Distribution of premalignant and malignant squamous epithelial lesions of uterine cervix

Nature Of Lesion	No. Of Cases	Percentage
Premalignant	25	46.29
Malignant	29	53.7
Total	54	

Graph 1: Age wise distribution of premalignant squamous epithelial lesions of uterine cervix

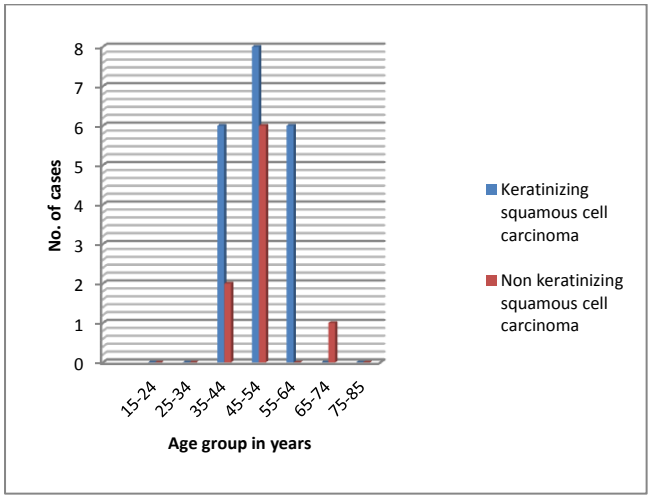


The age wise distribution of premalignant squamous epithelial lesions of uterine cervix is shown in Graph 1.

The maximum number of cases i.e 13 (52%) belonged to the age group 35-44 yrs, followed by 5 cases (20%) of 25-34 yrs age group. 4 cases (16%)

belonged to the age group of 45-54 yrs and 1 case (4%) each of age groups- 15-24 yr, 55-64yrs, 65-74 yrs.

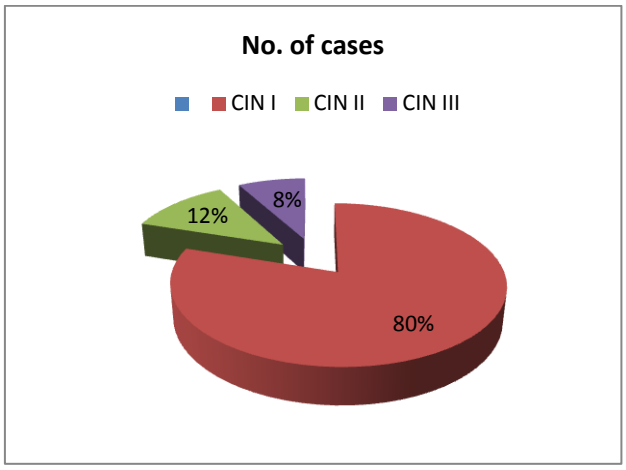
Graph 2: Age wise distribution of malignant squamous epithelial lesions of uterine cervix



The age wise distribution of malignant squamous epithelial lesions of uterine cervix is shown in Graph 2.

The maximum number of cases i.e 14 (48.27%) belonged to the age group 45-54 yrs, followed by 8 cases (27.5%) of 35-44 yrs age group. 6 cases (20.68%) belonged to 55-64 yrs age group, and 1case (3.44%) of 65-75 yrs age group.

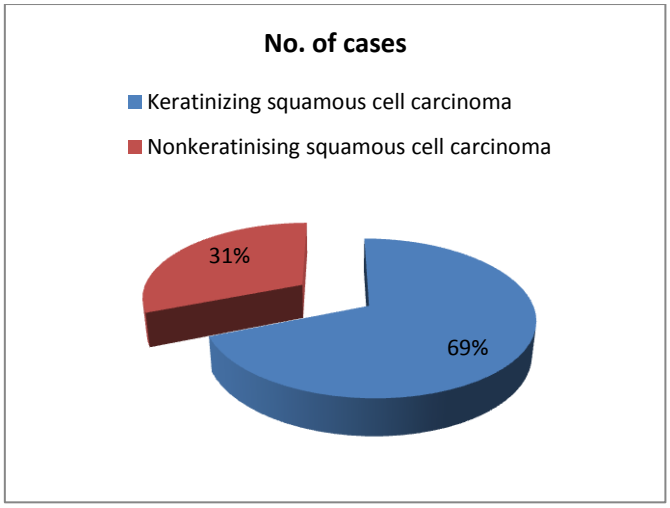
Graph 3: Histological types of premalignant squamous epithelial lesions of uterine cervix



The histological types of premalignant squamous epithelial lesions of uterine cervix are shown in Graph 3.

Out of the total 25 cases of premalignant cervical squamous epithelial lesions, majority i.e 20 cases (80%) were CIN I, followed by 3 cases (12%) of CIN II and 2 cases (8%) of CIN III.

Graph 4: Histological types of malignant squamous epithelial lesions of uterine cervix



The histological types of malignant squamous epithelial lesions of uterine cervix are shown in Graph 4.

Out of total 29 cases of malignant cervical squamous epithelial lesions, majority i.e 20 cases (69%) were of KSCC, and 9 cases (31%) were of NKSCC.

Table 2: Apoptotic indices, mitotic indices, and turnover indices in the various histological groups of cervical squamous epithelial lesions

Diagnosis	No.of cases	Apoptotic index(mean ±SD)	Mitotic index (mean ±SD)	Turnover index(mean ±SD)
CIN I	20	0.875±0.263	0.050±0.060	0.925±0.317
CIN II	3	1.266±0.057	0.233±0.057	1.500±0.100
CIN III	2	1.600±0.282	0.30±0.00	1.900±0.282
KSCCC	20	3.345±0.370	0.815±0.227	4.160±0.513
NKSCC	9	3.055±0.320	0.711±0.190	3.766±0.370

Discussion

Squamous cell carcinomas irrespective of the site, evolve through a sequence of changes occurring in the normal squamous epithelium, ranging from dysplasia of varying degree to frank malignancy.[6] Various risk factors associated with the development of cervical cancer in developing countries include poor sexual hygiene, socioeconomic factors (education & income), viruses (herpes simplex virus, human immunodeficiency virus) and others (smoking, diet, oral contraceptives, hormones etc.) [7]

In the present study, 25 cases were premalignant (46.29%), 29 cases (53.7%) were malignant (Table 1).

Mysorekar VV et al[8], (2008) reported 39 premalignant cases (33.91%) and 76 malignant cases (66.08%). Our study matches with study by Mysorekar VV as we also found higher number of malignant cases than premalignant cases.

Majority of premalignant lesions were also in 35-45 yrs age group (52%) and maximum number of malignant lesions were in between the age group of 45-54 yrs (48.27%). This finding in our study is similar to the study of Mysorekar VV et al[8] (2008) who reported maximum number of premalignant lesions between 35-45 yrs (28.2%) and maximum number of malignant lesions between the age group 35-45 yrs (27.63%) closely followed by age group 45-54yrs (26.3%).

Out of 25 premalignant lesions, CIN I was found to have the maximum number of cases i.e 20 (80%). This is consistent with the study of Mysorekar VV et al [8] (2008) who also reported highest number of CIN I cases (14) out of all premalignant cases .

In the present study the most common type of malignant lesion was keratinizing squamous cell carcinoma (69%) followed by non-keratinising squamous cell carcinoma (31%). Mysorekar VV et al[8] (2008), also reported the most common type of malignant lesion as keratinizing squamous cell carcinoma (89.5%), followed by non-keratinising squamous cell carcinoma (10.5%). Therefore our study matches this study as both studies show majority of keratinizing SCC and much lesser number of non keratinizing SCC.

In the present study, it was observed that there is an increase in mean AI, MI and TI with increasing grade of the lesion. AI, MI and TI progressively increased from CIN1 to CIN2 to CIN3 to SCC (Table 2). Sagol O et al[9] (1999) also reported that the squamous cell carcinoma cervix group showed significantly higher mitotic and apoptotic cell counts when compared with preneoplastic lesions. They concluded that both apoptosis and mitosis are markedly increased in progression towards malignancy in cervical epithelium.

Mysorekar VV et al [8](2008) also reported increase in mean AI and MI with increasing grades of lesion from dysplasia to carcinoma cervix.

Dey P et al[10] (2000) reported that mean AI , MI and TI increases from lower to higher grades of CIN to carcinoma of cervix.

Therefore, our study, is in agreement with study by Sagol O et al, Mysorekar VV et al, Dey P et al as all the three indices show progressive increase with increasing severity of the lesion in all studies.

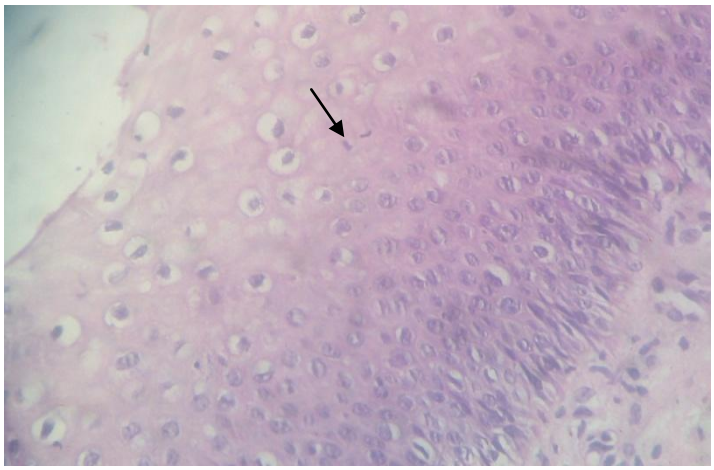


Figure 1 CIN-I showing many apoptotic cells (thin arrows). (H &E, 100X)

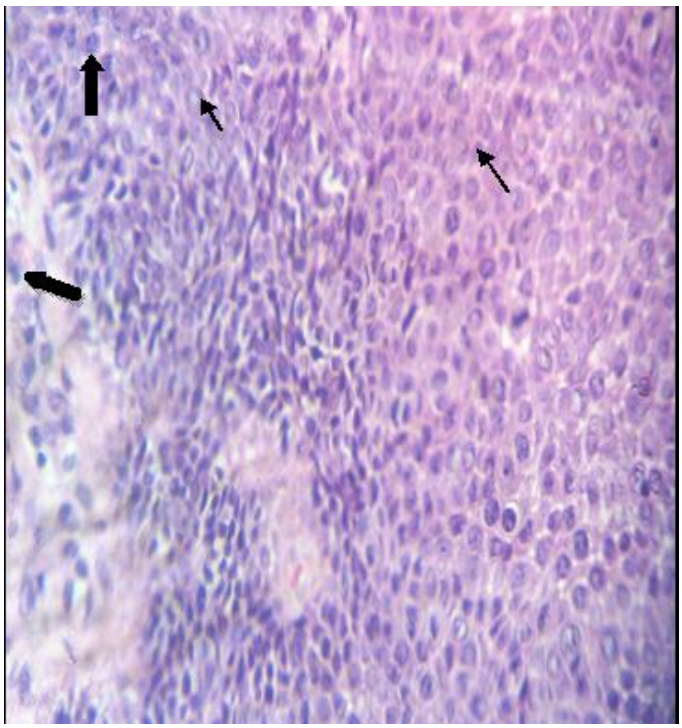


Figure 2 CIN-II showing mitotic figures (thick arrows) and apoptotic cells (thin arrows). (H & E, 400X)

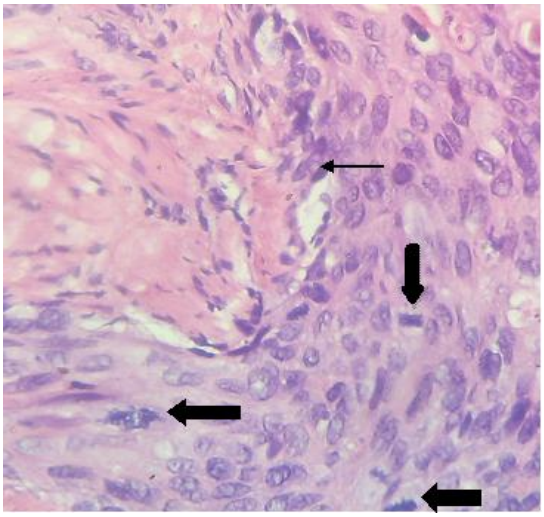


Figure 3- CIN-III showing abnormal mitotic figures (thick arrows) and apoptosis (thin arrows). (H&E, 400X).

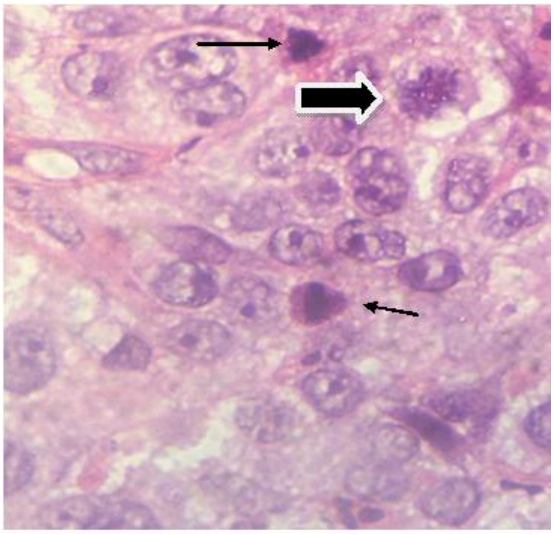


Figure 4 Keratinising squamous cell carcinoma cervix showing abnormal mitotic figure(thick arrow) and apoptotic cells (thin arrows). (H & E, 1000X)

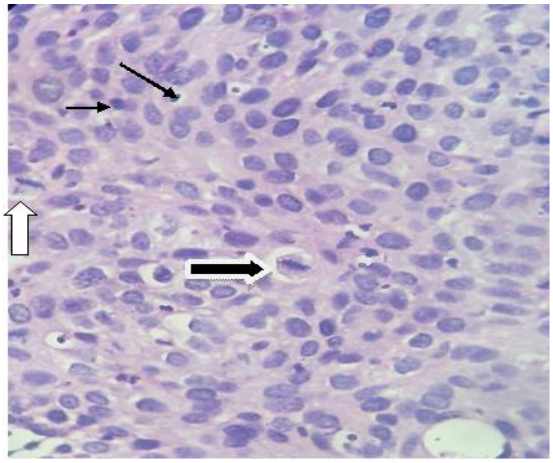


Figure 5 Non Keratinising squamous cell carcinoma showing many mitotic figures (thick arrows) and apoptotic cells (thin arrows). (H & E, 400X)

Conclusion

Most of the premalignant lesions were in the age group of 35-44 yrs. Maximum number of malignant lesions were in the age group of 45-54 yrs.

Out of 25 premalignant lesions, majority i.e 20 cases (80%) were of CIN I.. Out of 29 malignant lesions, majority i.e 29 cases (69%) were of keratinizing squamous cell carcinoma.

AI, MI and TI were evaluated across squamous epithelial lesions of uterine cervix ranging from dysplasias to carcinoma.

Comparison of AI, MI and TI between the malignant group of lesions with the premalignant group revealed that these indices increased highly as the nature of the lesion changed from premalignant to malignant.

It can be concluded that this study is based purely on morphology as it is fairly reliable, easily practiced and inexpensive method for detection of both apoptosis and mitosis.

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