Original Research Paper

Pathology

APOPTOTIC INDEX- A SIGNIFICANT BIOMARKER IN SPECTRUM OF CERVICAL LESIONS ON PAP SMEAR EXAMINATION

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ABSTRACT

BACKGROUND- Cervical cancer is second most common malignancy. The Pap test, a significant screening tool, has certain subjective limitations. Specific biomarkers are needed to aid diagnosis. Apoptosis is easily discernible in Pap smears in form of karyorrhexis, karyolysis and condensation of chromatin. This study was done to evaluate significance of apoptosis in cervical lesions.

METHODS- The study was conducted on Pap stained smears. Apoptotic index was calculated in each case. The lesions were divided into different categories and mean apoptotic index was calculated for each category. Statistical evaluation was carried out using Student t test.

RESULTS- Total 426 smears, the most common lesion was inflammatory (43.7%) and least common was invasive carcinoma (4.2%). Invasive tumours had a significantly higher mean apoptotic index.

CONCLUSION- Apoptotic index increases with increasing grade of dysplasia. Apoptotic index is a useful biomarker to evaluate proliferative activity in cervical lesions.

KEYWORDS : Cervical Cancer, PAP smear, Apoptosis

INTRODUCTION

Cervical cancers are the second most frequent type of female cancer, responsible for about 5% of cancer deaths in females worldwide[1]. Approximately 20 per 100,000 Indian women are likely to suffer from carcinoma of cervix [2].Screening for cervical cancer by cytologic examination (the Papanicolaou [Pap] test) has reduced the incidence of invasive cervical cancer in many countries, yet cervical cancer remains a leading cause of death and illness in women[3].

Due to subjective limitations of the Pap test, there is a need for the identification of specific biomarkers for dysplastic epithelial cells to aid in primary screening and lesion diagnosis [4].

Apoptosis is genetically controlled death which enables elimination of the cells that have been damaged[5]. Apoptosis is a complex and finely regulated process linked to cellular proliferative activity. It has a definite bearing on tumour progression. Apoptosis and its relationship with growth and progression of tumour have been studied in numerous papers and "apoptotic index" is included among the parameters used to measure tumour growth in various types of neoplasms including cervical cancer[6].

Apoptotic index is defined as a measure of the rate of death of cancer cells within a tumour. This is estimated by the proportion of dying or apoptotic cells per 1000 cancerous cells[7]. Apoptosis is easily discernible in cervical Pap smears in the form of karyorrhexis, karyolysis and pyknosis. Their frequency of occurence increases with increase in the severity of the premalignant and malignant lesions of cervix[8,9]. As apoptotic tumour cells can be identified and counted by light microscopy, there has been interest in the application of the enumeration of apoptosis in malignant growths as a putative prognostic marker[10].

AIM

The aim of the study was to evaluate the role of apoptotic index as a proliferative marker in various cervical lesions on PAP smear

MATERIAL & METHODS

The present study is a retrospective study and was performed on Pap stained cervical smears obtained in the department of pathology, Combined Medical Institute, Dehradun over a period of 4 years, from June 2013 to June 2017. A detailed clinical history and examination was carried out along with routine investigations

We evaluated a total of 426 smears, which included 123 normal smears, 186 inflammatory smears, 45 smears of Atypical squamous cells of undetermined significance, 32 smears of Low grade squamous intraepithelial neoplasm, 22 smears of high grade squamous intraepithelial neoplasm, and 18 smears of invasive carcinoma.

In each section, 1000 tumour cells were evaluated for the presence of apoptotic cells and apoptotic bodies under 40x; and apoptotic index was calculated as the number of apoptotic cells and apoptotic bodies expressed as a percentage of total number of tumour cells counted in each case [11].

The apoptotic cells were recognized by certain well defined features like cell shrinkage, condensation and deep eosinophilia of cytoplasm and pyknotic, round to crescentric or irregular nucleus .Areas of artefactual change, preservation or fixation artefact, necrosis and inflammatory exudates were excluded[12]

The mean apoptotic index was calculated for all the categories of cervical lesions. Statistical evaluation was carried out using the Student t test, with P<0.05 being significant[13].

RESULTS

The present study was conducted in the Department of Pathology over a period of 4 years on a total of 426 smears. Age of the patients in the present study ranged from 18 to 74 yrs. Mean age in the present study was 51 yrs.

We evaluated a total of 426 smears,which were divided into 6 categories on basis of The Papanicolaou classification in the Bethesda system (FIG/TABLE 1). The smears included 123 (28.9%) normal smears, 186 (43.6%) inflammatory smears, 45 (10.6%) smears of Atypical squamous cells of undetermined significance, 32 (7.5%) smears of Low grade squamous intraepithelial neoplasm, 22 (5.2%) smears of high grade squamous intraepithelial neoplasm, and 18 (4.2%) smears of invasive carcinoma.

Apoptotic index was calculated for all the cases in percentage. Mean apoptotic index was calculated for all the categories along with the standard deviation (TABLE/FIG 2). There was increase in mean apoptotic index with increasing grade of dysplasia. Mean apoptotic index was maximum for invasive carcinomas i.e. 2.38 ±0.69 while it was minimum for inflammatory smears i.e. 0.02± 0.009 and the difference was found to be statistically significant.

Statistical analysis showed that inflammatory lesions and smears of ASCUS had same apoptotic index (p-value =0.072, insignificant) but HSIL and invasive carcinoma smears had a significantly higher apoptotic index (p-value =0.001, significant). ASCUS and LSIL lesions did not show statistically significant difference in mean apoptotic index. (p-value =0.062, insignificant)

DISCUSSION

About one million deaths amongst world's women population are attributed to cancer cervix every year. Cervical cancer has taken a second place amongst malignancies that affects women [14]. If diagnosed and treated early, morbidity may be reduced by 70% and mortality by 80%. The Pap test is a screening method used to detect potentially precancerous and cancerous processes in the cervix[15].

The incidence of cervical cancer has diminished mainly in developed countries but more than 80% of woman dying from cervical cancer live in developing countries[16].

Apoptosis is genetically regulated cell death which permits elimination of damaged cells. Apoptosis is a physiological process for controlling cell death and its disturbance is thought to cause carcinogenesis. Many studies have assessed apoptosis by light microscopy in different types of human tumours[17].

Apoptosis can be identified morphologically by both light and electron microscopy. Apoptosis and its relationship with growth and progression of tumor have been studied and "apoptotic index" is included among the parameters used to measure tumor growth in various types of neoplasms including cervical cancer. "Apoptotic index" is measured in by calculating percentage of apoptotic cells and apoptotic bodies from atleast 1000 tumour cells at high magnification (400X)[18].

In our study, we observed an increase in apoptotic index as the nature of cervical lesion progressed from inflammatory to invasive carcinoma. In a study done by Bhardwaj S and Wani FA, the mean AI and standard deviation of premalignant group of lesions were: mild dysplasia- 0.62 ± 0.6, moderate dysplasia- 1 ± 0.44 and severe dysplasia- 1 ± 0.6; and for malignant group of tumours, i.e., keratinizing squamous cell carcinoma- 3.38 ± 1.15, non- keratinizing squamous cell carcinoma- 2.92 ± 1.41. Statistically significant difference was noted between premalignant and malignant group of lesions[19].

In a study done by Dey P.et al, there was an increase in mean apoptotic index with greater degree of dysplasia and statistical comparison between CIN group and carcinoma showed a highly significant difference[20]. In a study done by Nam et al., the apoptotic index significantly increased as the grade of cervical neoplasia increased[21].

Similar results were noted in studies done by Shoji et al.[09] and Mysorekar VV et al.[22] showing increase in mean apoptotic index with greater degree of dysplasia, which was statistically significant.

CONCLUSION

Thus, we come to a conclusion that apoptotic index increases with increasing grade of dysplasia. It is a useful biomarker to evaluate proliferative activity and also an important diagnostic and prognostic tool in cervical neoplastic lesions. It helps to distinguish cervical intraepithelial neoplasia from invasive cervical neoplasia and also determines the progressive potential of dysplastic and neoplastic changes.

Apoptotic index, being a simple and inexpensive technique, can also help in planning the management in cases of cervical lesions.

TABLE/FIG 1- CATEGORIZATION OF CERVICAL LESIONS (ON BASIS OF THE PAPANICOLAOU CLASSIFICATION IN THE BETHESDA SYSTEM)

S.No	DIAGNOSIS	CASES	PERCENTAGE (%)
1	Normal Smears	123	28.9
2	Inflammatory Smears	186	43.6
3	Atypical squamous cells of undetermined significance	45	10.6
4	Low grade squamous intraepithelial neoplasm	32	7.5
5	High grade squamous intraepithelial neoplasm	22	5.2
6	Invasive carcinoma.	18	4.2
	TOTAL	426	100

TABLE/FIG 2- GRADE Vs APOPTOTIC INDEX

DIAGNOSIS	CASES	MEAN APOPTOTIC INDEX (AI)- %	STANDARD DEVIATION (SD)	RANGE (Min-Max)
Normal Smears	123	0.0001	0.0004	0-0.1
Inflammatory Smears	186	0.021	0.009	0-0.5
Atypical squamous cells of undetermined significance	45	0.988	0.112	0-1.1
Low grade squamous intraepithelial neoplasm	32	1.096	0.223	0.1-1.5
High grade squamous intraepithelial neoplasm	22	1.791	0.476	0.2-2.1
Invasive carcinoma	18	2.381	0.691	0.4-2.5

TABLE/FIG 3- APOPTOSIS IN FORM OF KARYOLYSIS
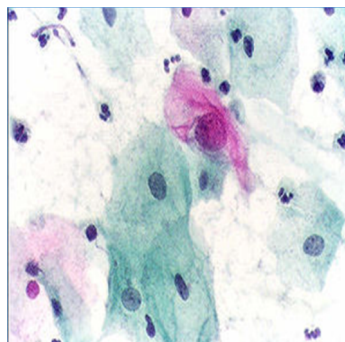
ABBREVIATIONS-
PAP test- Papanicolaou test
AI- Apoptotic Index
ASCUS- Atypical squamous cells of undetermined significancw
LSIL- Low Grade Squamous intraepithelial neoplasm
HSIL- High Grade Squamous intraepithelial neoplasm
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REFERENCE
1. HPV Information Centre (2010) ICO Information Centre on HPV and Cervical Cancer
2. Gandhi G, Kaur B. Elevated frequency of Micronuclei in uterine smears of cervix cancer patients. Caryologia. 2003;56:217–22.
3. Cancer incidence in Sweden 2005. Stockholm: National Board of Health and Welfare (Socialstyrelsen), 2007.
4. Srivastava S (2010) P16INK4A and MIB-1: an immunohistochemical expression in preneoplasia and neoplasia of the cervix. Indian J Pathol Microbiol 53: 518- 524.
5. Dlamini Z, Mbita Z, Zungu M (2004) Genealogy, expression, and molecular mechanisms in apoptosis. Pharmacol Ther 101: 1-15.
6. Soini Y, P. Paa¨kko¨, and Lehto V P. Histopathological Evaluation of Apoptosis in Cancer. American Journal of Pathology. 1998;153(4).
7. Ansari HA, Mehdi G, Maheshwari V, Siddiqui SA. Cytohistologcal evaluation of Apoptotic Index in Breast Tumours: Journal of Cytology 2005;22(4):184-188
8. Aires G.M.A., Meireles J.R.C., Oliveira P.C, Oliveira J.L., Araújo E.L., Pires B.C., Cruz E.S.A., JesusN.F,. PereiraC.A.B and Cerqueira E.M.M. Micronuclei as biomarkers for evaluating the risk of malignant transformation in the uterine cervix. Genetics and Molecular Research 2011; 10 (3): 1558-64 .
9. Shoji Y, Saegusa M, Takano Y, Ohbu M, Okayasu I. Correlation of apoptosis with tumour cell differentiation, progression, and HPV infection in cervical carcinoma. J. Clin Pathol. 1996;49:134-8
10. Apoptotic Index and Mib-1 Antibody Expression in Premalignant and Malignant Lesions of Uterine Cervix. Gynecol Obstet 2013, 3:5
11. Vijaya V Mysorekar, Saritha David, Saraswati G Rao (2008) Proliferative and Apoptotic Indices in Squamous Epithelial Lesions of the Cervix, Bahrain Medical Bulletin 30: 327-332.
12. Aihara M, Scardino PT, Truong LD, Wheeler TM, Goad JR, Yang G, Thompson TC. The frequency of apoptosis correlates with the prognosis of Gleason grade 3 adenocarcinoma of the prostate. Cancer 1995;75(4):522-29
13. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 1998;11(2):155-68
14. Dr.Samar Ghazal/Musmar, Pattern and factors affecting Pap smear test in Nablus, a retrospective study. Middle east Journal of family medicine. 2004;4:1-11.
15. Pap smear. By Healthline medical review team. www.healthline.com/health/pap-smear#Overview1/2015/11
16. Devilee, Fattaneh A, Tavassoli and Peter. WHO Breast and Female Genital OrganPathology and Genetics of Tumours LYON: IARC PRESS; 2003.
17. Dlamini Z, Mbita Z, Zungu M. Genealogy, expression, and molecular mechanisms in apoptosis. Pharmacol Ther. 2004;101:1-15.
18. Soini Y, P. Paakko, and Lehto V P. Histopathological Evaluation of Apoptosis in Cancer. American Journal of Pathology. 1998;153(4).
19. Bhardwaj S and Wani FA. Evaluating the Importance of Apoptotic Index, Mitotic Index and Turnover Index in Premalignant and Malignant Lesions of cervix. Open Journal of Pathology. 2015;5:29-37.
20. Dey P, Das R and Sabuddin. Correlations between Apoptotic and Proliferative Indices in Cervical Intraepithelial. Indian Journal of Pathology and Microbiology. 2000;43:271-275.
21. Nam JH, Kim JH, Huh J, Koh CW, Na JH. Correlation of lesion grade in cervical neoplasia with cell proliferation and apoptosis. International Journal of Gynecological Cancer. 1998;8(4).
22. Mysorekar VV, David S, Rao SG. Proliferative and Apoptotic Indices in Squamous Epithelial Lesions of the Cervix. Bahrain Medical Bulletin. 2008;30(4).