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Silver Binding of Nucleolar Organizer Regions (AgNORs) in Premalignant & Malignant Lesions of Cervix

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Abstract

Cancer of the cervix is one of the most common and important of the neoplasm among Indian women. In western communities, cervical carcinoma is about twice as frequent as carcinoma of the corpus uteri. For last so many years cytogenetists are trying to lay down certain criteria in order to diagnosis and grade different malignancies so that a simple yet reliable method can be brought into routine practice. The aim of present study the role of AgNOR in differentiating benign, premalignant and malignant lesion of cervix.

Keywords: Cancer, Cervix, AgNORs, Histopathological, H&E

Introduction

Tumours of the cervix are relatively common as causes both morbidity and mortality in members of the female population and are of particular pathological and clinical importance because of high frequency with which in situ carcinoma can be identified by cytological examination of cells scraped from the accessible cervix.

Cancer of the cervix is one of the most common and important of the neoplasm among Indian women [1]. Cervical cancer is seen most commonly in subjects between 48 and 58 years of age. In recent years there has been an increasing number of cases in younger women, these are often of high malignancy and may be seen in patients who have previously had negative cervical smears. The most common site affected is squamo-columnar junction[2].

Cancer cervix seems to follow a progressive course from epithelial dysplasia to carcinoma in situ to invasive carcinoma. There is evidence pointing to human papilloma virus (HPV) 16,18,31,33 & 35, sexually transmitted as the cause of cervical cancer in 85% cases other are HSV-2 past and present occurrence of clinical genital warts has been found to be an important risk factors. Early marriage, early coitus, early child bearing and repeated child birth have been associated with increasing risk. A recent WHO study finds an increased risk with increased duration of pill use and with the use of oral contraceptives high in oestrogen[3].

The cervical carcinoma is of great interest as it is preceded by cervical intraepithelial neoplasia at which stage diagnosis affords a very favourable outcome, a lot of

histological procedure are tried again and again for early diagnosis. Other than PAP's smear, histopathological examination newer and advanced technique are available for example the immunohistochemistry, electron microscopy, flow cytometry, nucleic acid hybridization analysis of cells, proliferation indices like thymidine labelling PCNA, Ki-67, but most of these procedure are expensive and not feasible every where[4]. The study of the argyrophilic nucleolar organizer regions (AgNORs), which was used extensively in cytogenetics, has been identified as a reliable indicator of cell proliferation and in turn, the malignant potential of a lesion.

In all recent works on the role of AGNOR count indicates that invariably there is high count in cases of carcinoma cervix due to proliferative activity of tumours. In the present study an attempt has been made to study the AgNOR count in premalignant and malignant lesions.

Materials and methods

This study has been conducted in the department of pathology, S.P. Medical College, Bikaner, Rajasthan. The study was conducted on surgical biopsies from 100 cases of various cervical lesions, operated in the associated group of hospitals in Bikaner. These cases were selected randomly, the biopsies were subjected to routine paraffin sectioning. Histopathological diagnosis was first established on these sections using the routine Haematoxylin and Eosin (H&E) stains. Based on routine histopathological diagnosis, the cervical tumours were classified according to WHO grading system proposed by Poulson (1975)[5]. Further sections were cut from the prepared paraffin blocks. The paraffin sections were subjected to AgNOR staining technique.

Following information was obtained for every case:

Paraffin Sections[6]:

- The tissue was fixed in 10% formalin.
- Pieces of the fixed tissue were subjected to the procedures of dehydration, clearing and embedding in an automatic tissue processor.
- The dehydrated, cleared tissue pieces were further impregnated with paraffin wax by immersion in a succession of wax bath on the automatic tissue processor.
- The treated tissue was embedded in paraffin wax. The blocks were made using L shaped metallic modules. The embedded tissue blocks allow to cool.
- The blocks were fixed on a rotator microtone and sections of 3 microne thickness were cut and cut sections were transferred to a water bath and thereby picked on glass slides.
- The sections were fixed to the slides by keeping them in a incubator at 37^{o} c overnight.
- The sections were then subjected to the H&E and AgNOR stains.

Haematoxylin and Eosin stain (H&E stain)[6]:

- The paraffin sections were dewaxed in xylene, hydrated through various grades of alcohol.
- Sections were rinsed in running water for 1 minute and then briefly in distilled water and then stained in Harris's Alum haematoxylin for 8 minutes.
- Differentiation was done by dipping the stained sections in 1% acid alcohol Then rinsed well in water.
- Sections were dehydrated through different grades of alcohol and then passed through two baths of xylene.
- Sections were dried and mounted with Distrene 80 Dibutylpthlate xylene (DPX) mountant.

AgNOR staining Technique[6]:

- Paraffin sections were incubated at 37^{o}c overnight, further dewaxed in xylene, hydrated through various grades of

ethanol and washed well with triple distilled water. The sections were dried thoroughly and subjected to AgNOR stains.

- The AgNOR stain prepared was poured over the tissue sections and left for 60 minutes at room temperature.
- The silver colloid was washed off with triple distilled water thoroughly and sections were counter stained with 0.5% saffranine.
- Stained sections were mounted with DPX mountant.

Counting Procedure[6]:

- AgNOR were counted as black dots in the nuclei of cells using of 100x oil immersion objective.
- 100 cells were studied in each case and the mean AgNOR per nucleus was calculated.
- AgNOR were counted in cells of control tissue, cells of benign and malignant tumours. The cells in all above cases were chosen randomly.
- The final score in control and various cervical lesions was subsequently compared with the histopathological diagnosis, nature of the tumour and WHO grades of Tumours.

Results

The study was conducted on surgical biopsies from hundrade cases of various cervical lesions operated in the associated group of hospital, S.P. Medical coilege, Bikaner, Rajasthan and observed that mean AgNOR score in benign lesions considered together (53 cases) was 1.93±0.53, in premalignant lesions (16 cases) it was 3.25±0.51 and in malignant lesions (31 cases) was 4.81±0.92. The difference in mean AgNOR score in benign, premalignant and malignant lesions found to be a statistical significant ($p<0.001$). In this way it is useful in differentiating benign, premalignant and malignant lesions.

Table 1: Number of cases of different cervical lesions studied.

Sr. No.	Group	Type of Lesion	No. Of Cases
1	Benign	CNSC	10
2		CNSC with N.Cyst	10
3		Squamous Metaplasia	10
4		Mucous Polyp	10
5		Leiomyomatous Polyp	7
6		Adenomatous Polyp	6
7	Pre-Malignant	Dysplasia	8
8		Carcinoma in situ	8
9	Malignant	Sq. C.C. well differentiate	10
10		Sq. C.C. poorly differentiate	4
11		Adeno carcinoma	10
12		Adeno Sq. Carcinoma	3
13		Leiomyosarcoma	4
Total			100

Table 2: Mean AgNOR count in different benign lesions of cervix.

Lesion	No. of cases	Mean AgNOR count/cell	SD
Chronic Non Specific Cervicitis	10	1.81	0.34
Nabothian Cyst	10	1.12	0.15
Squamous Metaplasia	10	2.38	0.22
Mucous Polyp	10	1.90	0.37
Leiomyomatous Polyp	7	2.10	0.36
Adenomatous Polyp	6	2.50	0.36
Total	53	1.96±0.53	

Table 3: Mean AgNOR count in different pre-malignant lesions of cervix.

Lesion	No. of cases	Mean AgNOR count/cell	SD
Dysplasia	8	3.00	0.54
Carcinoma in situ	8	3.50	0.37
Total	16	3.25±0.51	

Table 4: Mean AgNOR count in different malignant lesions of cervix.

Lesion	No. of cases	Mean AgNOR count/cell	SD
Sq. C.C. well differentiate	14	4.05	0.52
Adeno carcinoma	10	5.0	0.40
Adeno Sq. Carcinoma	3	5.5	0.50
Leiomyosarcoma	4	6.2	0.64
Total	31	4.81±0.92	

Table 5: Statistical analysis of AgNOR counts between benign, pre-malignant and malignant cervical lesions.

Group	No. of cases	Mean AgNOR per cell	SD	SE	Difference of mean	T	P value	Remark
Benign	53	1.93	0.53	0.07	---	---	---	
Pre-malignant	16	3.25	0.51	0.12	1.32	9.16	<0.001	**
Malignant	31	4.81	0.92	0.16	2.88	16.17	<0.001	**

Discussion

The present study a total number of 100 cases were examined histopathologically and later on AgNOR count.Out of 100 cases 53 were benign, 16 were premalignant and 31 were malignant cases.Premalignant & malignant lesions showed a significant difference in AgNOR counts. This means increase cervical intraepithelial neoplasia (CIN) and invasive carcinoma. Similar results found that Egan et al (1988)[7], yokoyama et al (1990)[8] , Pratibha et al (1995)[9] and Agarwal et al (1997)[10] but lower than the Wistuba et al (1993)[11].

In normal cell the AgNOR are tightly packed in the nucleus and are indiscernable.

In rapidly proliferating cells like neoplastic cells necleolar disaggregation may take place resulting in dispersion of individual AgNORs. In this study the mean AgNOR count was found to increase progressively from cervicitis to CIN and invasive carcinoma. On comparing CIN with Non specific cervicitis and CIN with invasive carcinoma there was a statistically significantbdifference in both the sets. These findings strongly support the view that proliferative activity and malignant potential of intraepithelial neoplastic lesion of cervix increase progressively as the grade of lesion become higher. Increase in cell ploidy and increased transcriptional activity may also result in higher AgNOR count.

Some problems faced in this study were due to the number of cases because old sections which were older than one year did not stain properly so the study done on fresh section only. Small nucleus such as in benign lesions the entense affinity of the nucleus for silver staining obscure individual AgNOR.

The above findings denotes that AgNOR count can be used as diagnostic tool together with routine H&E staining to differentiate benign and malignant lesions and is of value in borderline cases. It also helps to assess the various types of malignancy in the increasing trends for the purpose of prognostic aspect of cancer. This method being simple, reproducible and cost effective adds to its benefits and must be used in adjunct to other method, thus rendering earlier diagnosed and better prognosis.

Conclusion

The findings of this study indicate that the AgNOR can be demonstrated by means of argyrophilia of their associated proteins using a simple silver staining method and technique can be used as adjunct to routine histopathological examination of cervical lesions especially for grading cervical intraepithelial neoplasia thus rendering earlier diagnosis and better prognosis.

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