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Research Article

ROLE OF AGNOR IN CERVICAL LESIONS

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ABSTRACT

Carcinoma cervix is one of the leading causes of death of female population in developing countries. By virtue of its accessibility, cancer cervix can be readily diagnosed even in its preinvasive stage. If treated in the earlier stages the patient can often be cured of the disease. In the recent past, there has been emergence of various newer techniques to assess the proliferative capacity of cells. The definitive diagnosis of the patient's condition rests on the follow up histologic evidence from the biopsy material. The aim of the study is to evaluate the role of Argyrophilic staining of nucleolar organizer regions in Pap smear and histopathological section of biopsy cervix.

INTRODUCTION

For each case of cancer of body of the uterus, there are 25 cases of cancer cervix in India. A close quarters observation on the social behavior of our society reveals that most of the women have their marriages at very early part of their life leading to early age at first intercourse and poor sexual hygiene which are considered to be important etiological factors for cervical carcinoma.

George Papanicolaou M.D., Ph.D. after whom the Pap smear is named laid the foundation for preventive medicine at its best. He correctly estimated cytology as an infant science destined to become a titan. In the same way there were so many modifications made in the method of collection of specimen, techniques, methods of staining, interpretation and follow up[1,2]. The primary purpose of cervical cytology is to screen the population and identify the patients who have an abnormal pap smear. It has been the corner stone of cancer cervix screening for almost 50 years. In the recent past, there has been emergence of various newer techniques to assess the proliferative capacity of cells. This has been an important criterion to assess the malignant behaviour of cells. Various techniques are available like DNA content analysis, 'S' phase fraction calculation by means of DNA cytometry, proliferating antigens like C3, F10, DNA polymerase-2, Ki67 and PCNA.

A simple and inexpensive method is the staining and counting of the Nucleolar Organizer Regions. This is based on RNA transcription activity. Silver colloidal solutions of high concentration have been used for this purpose and this is called AgNOR stain. The number of AgNORS in a cell nucleus reflects the proliferative activity of the cell with progressive increase in number from normal cells to dysplastic and carcinomatous cells. AgNOR in cervical cytology has also been studied. AgNOR though expensive, the single step technique and ease, which it can be done, is very impressive.

Along with routine haemotoxylin and eosin staining in histopathological sections, AgNOR stain can be used. Studying the number, shape and distribution of AgNOR dots in the cell gives information not only about the morphology, but also about the behaviour of the cells. It is useful in differentiating doubtful cases of CIN.

AgNOR count also has prognostic significance. CIN lesions with low AgNOR counts are more likely to regress in comparison to CIN lesions with high AgNOR count. So it can be used as an adjunct to routine cytology and histopathology for diagnosis of cervical lesions in doubtful cases.

MATERIALS AND METHODS

The present study has been carried out in the Department of Pathology, Madurai Medical College, Madurai, India for a period of 2 years after obtaining ethical committee permission.

The cytological materials obtained in the form of smears were fixed in 95% alcohol. Details of the patients such as age at marriage, parity, contraception and symptoms were recorded in the working proforma after getting informed consent. The smears were stained by PAP stain[3]. The smears were analyzed and the cellular details were evaluated under

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light microscopy. For the abnormal smears, biopsy cervix was advised. Each of the samples was then subjected to an argyrophilic staining for the nucleolar organizer region according to the modified colloidal silver technique of Crocker et al[4].

The histopathology specimens of cervix biopsy were fixed in 10% formalin. The tissue slices were processed, paraffin, blocked, 5 microns thin sections were cut and stained by Hematoxylin and Eosin as described by Bancroft in Theory and Practice of Histological Techniques[3] and AgNOR stain.[4]

Observation and Results

3842 pap smears were received from Government Rajaji Hospital, Madurai in the two year study period. Among this, abnormal smears were identified and biopsy was advised. Totally 220 abnormal smears had follow up biopsy material.

The cytology reports were categorized under The Bethesda System[5,6]. 80 (36.4%) patients had reactive cellular changes. The cells had cyanophilic cytoplasm and round to oval nucleus with fine chromatin in the background of degenerated basal cells and polymorpho nuclear leukocytes.

ASCUS cases were 53(24.1%).The criteria for ASCUS were the nuclear enlargement with evenly distributed chromatin and superficial or intermediate type cytoplasm. LSIL were 2 (0.09%) with enlarged hyperchromatic nucleus, intermediate type cytoplasm and well defined cell borders in cytology and HSIL were 33 (15%) in which there were increased number of cells with high nuclear cytoplasmic ratio, irregular nuclear outline and nuclear chromatin distribution .

44 (20%) cases were squamous cell carcinoma, in which cells were arranged as syncytial masses. The cells had indistinct cell boundaries with large irregular hyperchromatic nuclei. One case (0.5%) of atypical endocervical cells, 5 (2.3%) cases of atypical endocervical cells favour neoplastic. There are 2 cases (0.9%) of adenocarcinoma with pallisading of columnar tumour cells in cytology.

AgNOR stained slides were viewed under oil immersion and intra nuclear silver dots are hand counted making use of light microscope. After counting at least 100 cells, AgNOR score was calculated; i.e., mean number of AgNOR dots per nucleus. The count was repeated by another person to minimize observer error. AgNOR dots on pap smears were analysed and the Table 1 shows lesion wise count of AgNOR in the cytological smears.

Table 1 AgNOR in Cytology

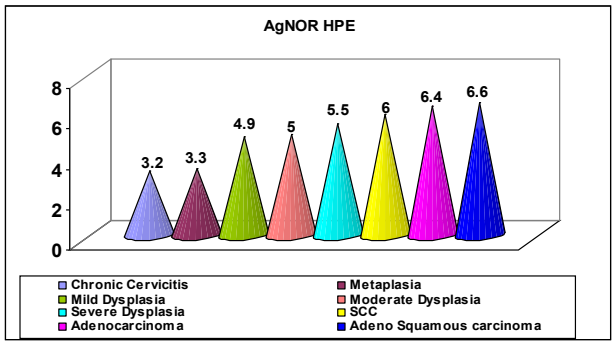
PAP Smear results	AGNOR – PAP
	Mean
Reactive cellular changes	3.1
ASCUS	4.6
LSIL	4.8
HSIL	4.9
Squamous cell carcinoma	5.8
Atypical endocervical cells	4.8
Atypical endocervical cells favour neoplastic	5.5
Endocervical adeno carcinoma in situ	7.1

In the histopathological examination of biopsy cervix, non neoplastic lesions were 97 (44.1%), pre malignant lesions were 40 (18.2%) and malignant lesions were 83 (37.7%).

Among these 97 non neoplastic lesions, 91 (94%) were chronic cervicitis. One (1%) was endocervical polyp, 4(4%) were metaplasia and one (1%) was TB cervicitis. Among 40 premalignant lesions, 14 (35%) mild dysplasia, one (3%) moderate dysplasia and 25 (62%) severe dysplasia with carcinoma in situ were detected.

The total number of malignant lesions was 83. Out of these, well differentiated squamous cell carcinoma were 20 (24.5%), moderately differentiated SCC were 45 (54.5%) poorly differentiated SCC were 6 (7%) adenosquamous were 5 (6%) and adenocarcinoma 7 (8%) .

When the AgNOR dots were analysed in silver colloidal solution stained 220 histopathological sections, the AgNOR count of 1.1 to 2 category were 3(1.4%) and 2.1 to 3 were 24 (10.9%), 3.1 to 4 were 64(29.10%). 4.1 to 5 were 42 (19.1%). 5.1 to 6 were 28(12.7%) 6.1 to 7 were 34(15.4%) and more than 7 were 25(11.40%). Fig 1 shows lesion wise AgNOR count in the histopathological sections.



DISCUSSION

The aim of the study was to find the Ag NOR counts in various lesions of cervix and to know the usefulness and prognostic significance of AgNOR in the detection of cervical neoplastic lesions.

AgNOR Staining in pap Smear

In this study, mean AgNOR score for reactive lesions is 3.1. ASCUS is 4.6, LSIL is 4.8, HSIL is 4.9, SCC is 5.8, atypicalendocervical cells is 4.8, atypical endocervical cells favour neoplastic is 5.5 and endocervical adenocarcinoma in situ is 7.1. Malignant cases have higher AgNOR than reactive cases.

In a study by J.S.Misra et al, there was a progressive increase in AgNOR count when the severity of the lesion was increased. The statistical analysis shows p value <0.05 between normal and inflammatory lesions. Highly significant difference between inflammatory and LSIL cases, between LSIL and HSIL and between severe dysplasia and frankly malignant cases were noted. ($p<0.01$)[7].

Eagan et al observed that mean AgNOR count increased steadily whereas the mean size of AgNORs decreased from CIN I to CIN II[8].

Cardillo studied AgNOR counts in cervical smears of squamous metaplasia and cervical intra epithelial neoplasia. The smears previously stained with Papanicolaou technique were destained and restained with AgNOR silver. He found statistically significant difference ($p < 0.05$) in AgNOR counts in squamous metaplasia and various grades of CIN[9].

An Indian study done by Prathiba and Kuruvilla (1995) on the role of AgNOR in diagnosis of premalignant and malignant lesions of the cervix, showed that mean AgNOR count progressively increased from normal to CIN I, CIN II, CIN III and invasive carcinoma. The difference between counts in CIN I and CIN II and invasive carcinoma was statistically significant[10].

Crocker et al in 1990 also showed statistically significant difference in AgNOR counts between CIN I, CIN II and CIN III[11].

Agnor Staining In HPE

In our study there is no significant difference in AgNOR counts between squamous metaplasia and chronic cervicitis. The mean number of AgNORs per nucleus is significantly higher in dysplasia (mild 4.9, moderate 5, severe 5.5) and malignant lesions (squamous cell carcinoma 6, adenocarcinoma 6.4) as compared to metaplasia 3.3 and chronic cervicitis 3.2.

All carcinomas and severe dysplasia have significantly higher AgNOR counts per nucleus compared to mild dysplasia. NOR counts are higher in adenocarcinoma when compared to squamous cell carcinoma. Statistical analysis reveals significant difference between mean AgNOR counts of chronic cervicitis and dysplasia; mild dysplasia and severe dysplasia, severe dysplasia and invasive carcinoma, squamous cell carcinoma and adeno carcinoma.

In Jyomita Agarwal, JK Gupta study (1997) AgNORs was counted in biopsies from 202 cases of various lesions and cervix. The mean number of AgNORs per nucleus was significantly higher in CIN (4.05±0.04) and malignancy (5.50±0.65) as compared to squamous metaplasia (1.74±0.32) and chronic cervicitis (1.54±0.42). Adeno carcinomas had higher AgNOR counts as compared to other carcinomas. It concluded that the estimation of AgNORs can be helpful in distinguishing benign lesions from CIN and malignancy of the cervix[12].

In our study also, it is noted that the size of AgNOR dots decrease with increase in AgNOR count. This is in accordance with the study reported by Eagan[8] who noted an inverse relationship between AgNOR numbers and sizes, and proved that severe dysplasia could be distinguished from mild dysplasia on the basis of AgNOR size.

CONCLUSION

Histopathological examination of the cervix is the gold standard against which the accuracy of Pap smear can be obtained. Among the various newer techniques to assess the proliferative behavior of the cells, a simple and inexpensive method is the staining and counting of the Nucleolar Organizer Regions.

AgNOR count also has prognostic significance. CIN lesions with low AgNOR counts are more likely to regress in comparison to CIN lesions with high AgNOR count. So it can be used as an adjunct to routine cytology and histopathology for diagnosis of cervical lesions in doubtful cases.

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