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Original Research Article.

Utility of AgNOR Count in Non-Neoplastic and Neoplastic Lesions of the Uterine Cervix

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ABSTRACT

Background: Uterine cervix is the most common site for occurrence of non-neoplastic and neoplastic lesions in female genital tract. Squamous cell neoplasia of the cervix is the most common malignant tumour in most developing countries. The aim of study was to evaluate the incidence of benign & malignant lesions of the uterine cervix and determining the grade of malignancy by AgNOR staining technique.

Material & Methods: This study was carried out in the Department of Pathology, on material received from attached hospitals of SMS Medical College, Jaipur. A total of 210 cases of non-neoplastic and neoplastic lesions of uterine cervix were included. After processing, paraffin embedded sections of each case were stained with hematoxylin and eosin and separate sections from same block were utilized for AgNOR staining and results were recorded.

Results: Our study showed that maximum 29 (82.85%) cases of chronic cervicitis were between 31-50 years of age and squamous hyperplasia (58.33%), squamous metaplasia (40%) & 50% cases in cervical intraepithelial neoplasia belonged to 41-50 years of age group. Majority of malignant cases (34.31%) were found between 41-50 years of age group. The moderately differentiated keratinizing SCC showed the range of AgNOR count 4.9-5.8 and mean AgNOR count was 5.20±0.42 with predominant type III dots. Statistical analysis revealed significant difference between the mean AgNOR count of Group A and Group B (P<0.001), group A and group D (P<0.001) and group B and group D (P<0.001).

Conclusion: It was concluded that AgNOR staining is a very useful method as predictor of progression, but should be supplemented with other parameters.

Key Words: Benign Lesions, Malignant Lesions, AgNOR Staining, SCC.

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INTRODUCTION

Uterine cervix is the most common site for occurrence of non-neoplastic and neoplastic lesions in female genital tract. In recent years, the understanding of cervical pathology had progressed and the differential diagnosis of cervical carcinoma has expended to include possible pre-malignant and malignant lesions. Some degree of inflammation may be found in virtually all multiparous and in many nulliparous adult women.[1]

Squamous cell neoplasia of the cervix is the most common malignant tumour in most developing countries. If tumor detected earlier, the treatment would be more effective than the one adopted in the usual times of diagnosis and the life expectancy will be increased. Theoretically the mortality rate of cervical carcinoma can be reduced by the prevention programs and by early diagnosis and it is certainly an easier and less expensive strategy to achieve the some objectives.[2]

As the biological behavior of tumor differs from individual to individual and from one type of tumor to another, so with the increasing incidence of tumor the efforts of pathologist have increased equally to establish certain common variables in determining the biological behavior of tumor and its potential for metastasis, recurrence, survival and overall mortality.

It has been established that a strong correlation exists between proliferative activity and poor outcome of the tumor, so there is an increasing focus on various markers of proliferative potential as an independent indices of malignancy, such as thymidin labeling index, flow cytometry, Ki-67 immunostaining, AgNOR staining and proliferative cell nuclear antigen. Of greater interest is the possibility that measurement of proliferative activity may be of prognostic significance with tumor grade and this may provide information in addition to that provided by routine histopathology.[3]

Any one of the methods can be taken in use for determining the biological behavior and prognosis of tumor, but most are highly sophisticated requiring specialized instruments and techniques, the overall cost of procedure amounting very high. AgNOR is one method which being economic, rapid, simple and easy to perform. Moreover it can be performed on paraffin embedded sections and the results are comparable to other more sophisticated techniques.[4] AgNORs count is valuable in differentiating normal cervical squamous epithelium from CIN III. The squamous cell carcinoma can be subtyped with keratinizing SCC having a worse prognosis. On the other side, NORs is of no practical use in discriminating between the histological types of cervical adenocarcinoma. The aim of study was to evaluate the incidence of benign & malignant lesions of the uterine cervix and determining the grade of malignancy by AgNOR staining technique.

MATERIALS & METHODS

This study was carried out in the Department of Pathology, on material received from attached hospitals of SMS Medical College, Jaipur. A total of 210 cases of non-neoplastic and neoplastic lesions of uterine cervix were included.

After processing, paraffin embedded sections of each case were stained with hematoxylin and eosin and separate sections from same block were utilized for AgNOR staining and results were recorded. The case selection was at random.

Hematoxylin and Eosin stains were prepared as follows:

I. Mayers's Hematoxylin stain: Hematoxylin crystals (1 gm), Distilled water (100ml), Sodium iodate (0.2gm), Ammonium or potassium alum (50gms),Citric acid (1gm) and Chloral hydrate (50gms).
   Preparation: Alum was dissolved in water without heat. Hematoxylin crystals were added and shaked until all components were in complete solution. The sodium iodate, citric acid and chloral hydrate were added and shaked until all components were dissolved. The final colour of the stain was reddish violet.

II. Eosin stain: 1% stock solution (Eosin Y, water soluble (1gm), Distilled water (20ml) & 95% alcohol (80ml). Working solution was prepared by mixing 1 part of stock solution with 3 parts of 80% alcohol.

H & E Staining Procedure:

1. Slides were deparaffinized and hydrated to water.
2. Mayer's haematoxylin was pour on the slide and kept for 15 minutes.
3. Washed in running water for 20 minutes.
4. Counterstained with eosin for 2 minutes.
5. Dehydrate in 95% absolute alcohol, 2 changes of 2 minutes each.
6. Cleared in xylene, 2 changes of 2 minutes each.
7. Slides were mounted in DPX.

Argyrophil Nucleolar Organizer Region Staining (AgNOR Staining)

i. 20% gelatin in 10% aqueous formic acid (2 gm gelatin, 100 ml Deionized water & 1 ml Formic acid)
   Mix gelatin in deionized water and pure formic acid. Keep it in a hot water bath and stir constantly till gelatin dissolves and solution becomes clear.

ii. 50% Silver nitrate solution (4 gms Silver nitrate, 8 ml Deionized water)
   Dissolve silver nitrate in deionized water, solution should be freshly prepared each time.

Staining procedure: Paraffin embedded sections

1. Cut sections at 4µm thickness
2. Deparaffinize, hydrate to deionized water.
3. Mix one volume of 2% gelatin solution with two volumes of 50% silver nitrate solution and pour over the slide to cover it completely.
4. Keep the slides in incubator at 37^0c for 15 minutes.
5. Wash off the silver colloid with deionized water.
6. Dehydrate the sections to xylene and mount.

All sections were examined under a X100 oil immersion lens, 100 cells were examined and number of AgNOR dots were counted.

AgNOR staining patterns (AgNOR stain X100)

Fig 1: Large size, centrally placed – Type I dots, often seen in resting cells.

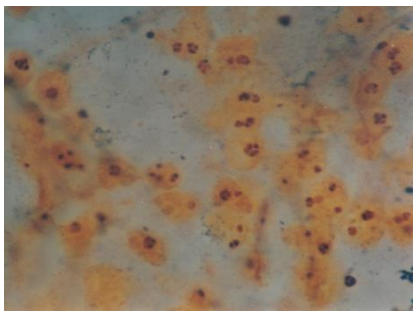

Fig 2: Dots with subdots – Type II dots, common in proliferating cells.

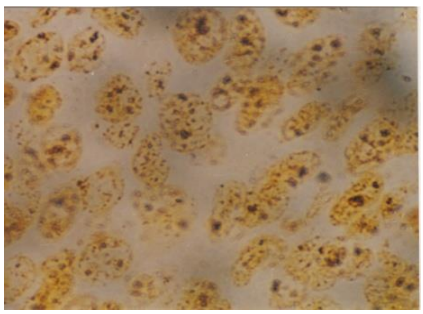

Fig 3: Fine and medium sized, scattered complex pattern – Type III dots, frequently observed in malignant cells.

RESULTS

Our study showed that maximum 29 (82.85%) cases of chronic cervicitis were between 31-50 years of age and squamous hyperplasia (58.33%), squamous metaplasia (40%) & 50% cases in cervical intraepithelial neoplasia belonged to 41-50 years of age group. Majority of malignant cases (34.31%) were found between 41-50 years of age group (table 1). In squamous hyperplasia the range of AgNOR count per cell was 2.0-3.5 with mean AgNOR count of 2.78±0.29 (table 2) and showed predominantly type I dots.

In common the cervical intraepithelial neoplasia presented with the range of AgNOR count 2.0-5.2 and mean AgNOR count per cell was 3.46±1.05 (table 3). In our study the moderately differentiated keratinizing SCC showed the range of AgNOR count

4.9-5.8 and mean AgNOR count was 5.20±0.42 with predominant type III dots. In one case of Anaplastic carcinoma the mean AgNOR count was 5.80 (table 4). Statistical analysis revealed significant difference between the mean AgNOR count of Group A and Group B (P<0.001), group A and group D (P<0.001) and group B and group D (P<0.001) (table 5).

Table 1: Age distribution of patients in different uterine cervical lesions

Type of lesion	Age distribution (yrs)					
	21-30 yrs	31-40 yrs	41-50 yrs	51-60 yrs	61-70 yrs	Total
Chronic cervicitis	3	14	15	3	0	35
Cervicitis with squamous hyperplasia	1	8	14	1	0	24
Cervicitis with squamous metaplesia	2	7	8	3	0	20
Cervicitis intraepithelial neoplasia	2	4	10	3	1	20
Cervical polyp	0	4	2	0	0	6
TB cervix	0	0	0	2	0	2
Acute cervicitis	0	0	1	0	0	1
Malignant cases	7	28	35	27	5	102
Total	15	65	85	39	6	210

Table 2: The range of AgNOR count and mean AgNOR count per cell in different benign lesions of uterine cervix

Type of lesions	No. of cases	No. of AgNOR dots	Range of AgNOR count	Mean	SD
Chronic cervicitis	35	1-4	1.5-3.2	1.93	0.405
Cervicitis with squamous hyperplasia	24	1-5	2.0-3.5	2.78	0.293
Cervicitis with squamous metaplesia	20	1-4	2.0-3.0	2.28	0.261
Cervical polyp	6	1-5	1.4-3.2	2.52	0.767
TB cervix	2	1-3	1.5-1.6	1.55	0.05
Acute cervicitis	1	1-5	2.8	2.80	-

Table 3: AgNOR counts in different grade of cervical intraepithelial neoplasia

Grade	No. of cases	Range of AgNOR count	Mean	SD
CIN I	7	2.0-2.5	2.20	0.207
CIN II	3	3.0-3.6	3.33	0.249
CIN III	8	4.0-4.8	4.21	0.262
In situ	2	5.0-5.2	5.10	0.100

Table 4: The range of AgNOR count and mean AgNOR count per cell in different malignant lesions of uterine cervix

S.No.	Type of lesions	No. of cases	No. of AgNOR dots	Range of AgNOR count	Mean	SD
1.	Squamous cell carcinoma					
	Well differentiated keratinizing SCC	3	2-8	4.0-5.0	4.53	0.411
	Moderately differentiated keratinizing SCC	3	2-8	4.9-5.8	5.20	0.424
	Moderately differentiated non-keratinizing SCC	50	2-8	2.5-6.0	4.45	0.592
	Poorly differentiated SCC	40	2-8	4.0-5.5	5.01	0.361
2.	Adenocarcinoma	5	1-7	3.5-5.6	4.32	0.803
3.	Anaplastic carcinoma	1	3-8	5.80	5.80	-

Table 5: Uterine cervical lesions studied in different groups

Group	Histopathological diagnosis	No. of cases	Mean	SD
Group A	Benign lesions	88	2.28	0.053
Group B	Cervical intraepithelial neoplasia	20	3.46	1.05
Group C	Keratinizing SCC	6	4.83	1.88
Group D	Non-Keratinizing SCC	90	4.70	0.576

DISCUSSION

AgNOR staining has emerged as a simple and quick method to measure the proliferation rate of a given cell, on light microscopy comparable in results to that of Ki-67 monoclonal antibody. The silver staining method for necleolar organizer regions has been claimed to be highly significant in differentiating malignant lesions from normal, reactive or benign neoplastic cells.

In malignancy, the AgNORs become dispersed throughout the nucleus to a varying extent due to dispersion of relatively large number of AgNORs in the nucleus. This technique thus may prove of great use is diagnostic histopathology.

In the present study amongst the benign lesions chronic non-specific cervicitis was the commonest one (16.66%). Cervical intraepithelial neoplasia was noticed in 9.52% cases. The moderately differentiated squamous cell carcinoma accounted 51.96% of malignant cases. Thus the present findings correspond to the findings of Solapurkar ML (1998).[5]

Most of the patients in the study belonged to age group 41-50 years, in contrast to the study of Solapurkar ML in 1998[5], who found most patients within the age group 31-40 years.

The mean AgNOR count increased from chronic cervicitis 1.93±0.405 to cervical intraepithelial neoplasia 3.46±1.05. These results are comparable to the study of Lakshmi S et al in 1993.[6] They suggested that AgNOR counts may be of significance in the evaluation of cervical carcinogenesis and could elaborate histopathological diagnosis of cervical lesion.

Leopardi O et al in 1992[7] observed a progressive increase in the mean AgNOR values from normal tissue to CIN III. In their study the range of variation on the counts was associated with overlapping between the various cases.

A progressive increase in mean AgNOR count was also observed by the Marbaix E et al in 1989.[8] They also noted that total area of AgNOR per nucleus increases with the differentiation of the cells or with its carcinomatous transformation.

Prathiba D & Kuruvilla S in 1995[9], found a progressive increasing mean AgNOR count from normal cervix to CIN III.

In the present study the mean AgNOR count showed a progressive increase from CIN I (2.20), CIN II (3.33), CIN III (4.21) to in situ lesion (5.10). These observations are comparable with figures reported by Egan M et al in 1990[10], who reported a count 2.3 in CIN I, 3.5 in CIN II and 4.7 in CIN III. Another study done by Egan M, Freeth M & Croker J in 1988[11] noted an increased mean AgNOR count from CIN I to CIN III. It was suggested that this simple technique is diagnostically useful and has considerable clinicopathological potential in cervical pathology and cytology.

Our study showed that the statistical analysis revealed significant difference between the mean AgNOR count of Group A and Group B ($P<0.001$), group A and group D ($P<0.001$) and group B and group D ($P<0.001$). This compares well with study of Prathiba D in 1995.[9]

As the group C is small, statistical significant difference could not be calculated between group B and C and Group C & D.

The size of AgNOR dots followed a decreasing trend from benign to malignant, 48.57% of chronic cervicitis, 66.66% of squamous hyperplasia had medium and large size dots. These findings were comparable with the findings of Marbaix E et al in 1989[8] and Prathiba D, Kuruvilla B in 1995.[9]

The configuration of AgNOR dots had a well-marked demarcation between benign and malignant uterine cervical lesions. Most of the benign lesions had simple configurations, while 45% of cervical intraepithelial neoplasia & 50% of malignant cases exhibited complex configuration.

CONCLUSION

It was concluded that AgNOR staining is a very useful method as predictor of progression, but should be supplemented with other parameters. It can serve as a useful adjunct to routine histopathology and other clinical parameters for evaluating the biological behavior and thus prognosis of the neoplasm.

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