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ORIGINAL ARTICLE

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AgNOR count and its diagnostic significance in cervical intraepithelial neoplasia.

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OBJECTIVE(S) : To study AgNOR counts in cervical intracepithelial neoplasia CIN I, CIN II and CIN III lesions.

METHOD(S) : AgNOR counts were done in histology specimens in 43 histologically proven cases of CIN.

RESULTS : AgNOR count in CIN I was 1.64, in CIN II 2.68 and in CIN III 4.3. The differences in AgNOR counts between CIN I and CIN II, between CIN II and III, and between CIN I and CIN III were statistically significant.

CONCLUSION(S) : AgNOR count is a simple and inexpensive technic which can be used as an adjunct to histology for diagnosing CIN especially in doubtful cases.

Key words : AgNor count, cervical intraepithelial neoplasia

Introduction

The misery of women due to cervical cancer, which is the commonest cancer in women in a developing country like ours is a scourge of humanity. Early detection and management of squamous intraepithelial lesions (SIL) is the best approach to achieve control over cancer cervix. Cytological screening by Pap smear has brought down the incidence of cervical cancer in developed countries [1]. Unfortunately cytology fails to identify high risk low grade SIL (LSIL) and high grade SIL (HSIL) which would progress to invasive cancers. Such information can be provided by a molecular tumor marker [2]. One such molecular tumor marker is AgNOR which stands for silver stained (Ag) nucleolar organizer regions (NORs). NORs are loops of DNA present in nucleus of cell on acrocentric chromosomes 13, 14, 15, 21 and 22. NORs are associated with argyrophilic proteins (having affinity for silver) like polymerase C_{23} and B_{23}. Simple silver staining technic can recognize these argyrophil associated proteins. They appear as black dots after silver staining in nucleolar and extranucleolar regions [5]. In a normal cell 20 black dots of AgNORs should be seen (2 per arm of chromosome i.e. 2 x 10 = 20) but only one or two dots are seen as the dots are tightly packed [4]. As we move from normal cells towards the dysplastic cells and malignant cells, the amount of DNA increases, and the number of AgNOR dots (AgNOR count) also increases. With this knowledge in background, this study was undertaken to determine AgNOR counts in CIN I, CIN II and CIN III lesions and to establish their diagnostic role in preinvasive lesions in the setup of a developing nation like ours.

Method

During the one year study period 20680 patients attended our gynecological outpatient from whom 2060 were selected for study as they belonged to either high risk group or suspect group for developing cervical cancer. The criteria for selecting the women to be classified under the aforesaid groups were –

High risk group – age at consummation less than 20 years, five or more children, history of smoking or tobacco

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chewing, history of STD, unexplained persistent vaginal discharge, poor hygiene, low socioeconomic status, use of oral contraceptives, diverse number of sexual partners of husband or wife, and remarriage.

Suspect group – postcoital bleeding, postmenopausal bleeding, intermenstrual bleeding, menstrual irregularity of any sort, any naked eye abnormality of cervix like hypertrophic, eroded or ulcerated cervix, and cervix bleeding on touch.

Selected patients were screened for cervical cancer by Pap smear and those with LSIL or HSIL on cervical cytology were subjected to colposcopy directed biopsy. The biopsy specimens were subjected to histopathological study for confirmation of diagnosis of CIN I, CIN II and CIN III. Thereafter the specimens were studied for AgNor counts seen as black dots inside the cell. The number of AgNor dots per cell was then calculated and expressed as AgNor count according to recommendation of Crocker et al [5].

AgNOR dots seen both inside and outside the nucleolus were counted. The mean of AgNOR dots per cell was calculated and expressed as AgNOR count.

Results

Histopathological examination confirmed CIN in 43 cases which were taken up for AgNOR counts. 80.87% of these had CIN I, 16.52% CIN II, and 2.61% CIN III.

The distribution of cases of CIN in different age group showed that 4.35% were in < 25 years of age group, 52.4% in 26 to 35 years age group, 26.09% in 36 to 45 years age group, and 17.39% in > 45 years of age group. The distribution of cases according to religion showed that maximum patients were Hindus (83.48%) followed by Muslims (13.9%) and Sikhs (2.60%). 67.83% belonged to low socioeconomic group and 32.17% to middle income group. There was no one belonging to upper socioeconomic group. The distribution of cases according to parity showed that 6.74% were P_0, 65.22% were P_1 to P_3, and 27.82% were P_4 or more. Eighty-four percent had consummation of marriage before 20 years of age and the remaining 16% between 21-30 years. Only 1.74% had multiple sexual partners while 98.26% had one partner only. The leading symptom was excessive vaginal discharge (45.22%) followed by lower abdominal pain (26.09%), and intermenstrual bleeding (19.13%). 6.09% had history of postcoital bleeding. On speculum examination 48.70% had hypertrophied cervix, 27.83% had cervical erosion, and 27% bled on touch. 10.43% did not have any abnormality on speculum inspection of the cervix.

The mean AgNOR count in our study in CIN I was 1.64 (Rage 1.4 – 2.5), in CIN II 2.68 (Range 2.0 – 4.0), and in CIN III 4.3 (Range 4.0 – 4.6). True sample t test was applied to find the standard error difference between the mean AgNOR counts of CIN I, II and III. The difference was significant (P<0.001) between the mean AgNOR counts of CIN I, II and III (Table 1). It was also noted that the size of AgNOR dots decreased with the increase in AgNOR count.

Table 1. AgNOR count in different grades of CIN.

	Number of patients	AgNOR count Mean ± SD	Range
CIN I	21	1.64 ± 0.36 [a]	1.4 – 2.5
CIN II	19	2.68 ± 0.68 [b]	2.0 – 4.0
CIN III	3	4.3 ± 0.3 [c]	4.0 – 4.6

[a] P values between a and b, b and c, and c and a – < 0.001.

Discussion

The expression AgNOR designates the silver stained NOR proteins. The argyrophil AgNOR technic is remarkably specific for detection of NORs by virtue of silver binding to a wide array of NOR associated proteins (NOR APs). These include RNA polymerase I, C_{23} protein (nucleolin) and B_{23} protein [3]. The AgNOR technic stains the NORs as black dots in the nucleus. The number and size of NOR dots in malignant cells is significantly different from that in normal or benign cells, and reflects the current phase of transcription of cells. Marked cellular atypia is present in repair and regeneration of squamous and columnar epithelia.Cells from epithelial repair have enlarged nuclei which vary in size and shape. Parts of nuclei are hyperchromatic due to unevenly distributed chromatin, nuclear membrane is irregular, and nucleoli are prominent and irregular in size and shape. These changes are sometimes mistaken for cancer. The AgNOR technic provides an index of cell proliferation. The number, shape and distribution of AgNOR dots counted in the cell gives information not only about the morphology but also about the behaviour of the cell. There is progressive increase in the mean AgNOR count in cells with squamous metaplasia and in cells undergoing repair upto the various grades of CIN demonstrating ongoing proliferation [6]. In the present study it was observed that mean AgNOR count per cell was more in CIN II than that in CIN I and more CIN III than that in CIN II.

The differences in AgNOR count between CIN I and CIN II and between CIN II and III, and between CIN III and

I were statistically significant (P<0.001). Egan, et al [2] observed that mean AgNOR count increased steadily whereas the mean size of AgNORs decreased from CIN I to CIN III. Cardillo [6] studied AgNOR counts in cervical smears of squamous metaplasia and cervical intraepithelial neoplasia. The smears previously stained with Papanicolaou technic 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 [6].

An Indian study done by Pratibha and Kuruvilla [7] 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 II and in normal cervix and between counts in CIN III and in invasive carcinoma was statistically significant [7]. However Rowland [3]. in a study on nucleolar organizing regions in cervical intraepithelial neoplasia, did not find any significant difference in AgNOR count in squamous epithelium of normal cervix, CIN I and CIN II but there was a small but significant increase in CIN III group. Table 2 shows mean AgNOR counts in various grades of CIN in different studies. Although there is variation in AgNOR counts in different studies, the difference is statistically signficant in various grades of CIN in all studies except in the study reported by Rowlands [3].

Table 2. Reported mean AgNOR counts in different grades of CIN.

	AgNOR count		
	CIN I	**CIN II**	**CIN III**
Crocker et al [5] (1990)	2.3	3.5	4.7
Rowlands [3] (1988)	2.11	2.27	2.86
Pratibha and Karuvilla [7] (1995)	1.5	3.0	4.3
Present study	1.64	2.68	4.3

In our study it was also noted that the size of AgNOR dots decreased with increase in AgNOR count. This is in accordance with the study reported by Egan [2] who noted an inverse relationship between AgNOR numbers and sizes, and proved that CIN III could be distinguished from CIN I and II on the basis of AgNOR sizes. AgNOR counts can thus be useful in differentiating doubtful cases of CIN. Another advantage of AgNOR counts lies in retrospective study [6]. The samples can be destained and restained with silvers when unstained slides are unavailable and also in doubtful cases whose corresponding histologic specimens are not available.

AgNOR counts also have prognostic significance. It has been noted that CIN lesions with low AgNOR counts are more likely to regress in comparison to CIN lesions with high AgNOR counts [2].

Conclusion

AgNOR count is a reproducible simple efficient and inexpensive method which can be used as an adjunct to routine cytology and histopathology for diagnosis of cervical intraepithelial neoplasia especially in doubtful cases. Studies have reported that dysplasia with low AgNOR counts are more likely to regress as compared to those with high AgNOR counts which on the other hand are likely to progress to invasive cancer showing its prognostic significance. Although more studies are necessary, our preliminary study indicates the potential diagnostic importance of AgNOR counts in CIN.

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