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A COMPARISON OF KI67 AND AGNORS STAINING IN SQUAMOUS CELL CARCINOMA OF THE CERVIX

Pathology

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ABSTRACT

Background: Carcinoma cervix is a major burden on the population and the government especially in developing countries like India. Biopsy cervix is definitive in establishing a diagnosis in cervical lesions. However, it has its own limitations such as the study of morphological parameters alone with no information as to the fate of the lesion. Hence to overcome these draw backs and to increase the accuracy in deciding the magnitude of progression, cytokinetic markers come into role.Both Ki67 and AgNORs staining have been extensively studied as cytokinetic markers in many tumours including genital tract cancers.Significant relationships between Ki67 score and AgNORs count has been found in a variety of cancers. **Objectives:** To analyse Ki67 and AgNOR as markers of proliferation in squamous cell carcinoma of cervix and establish a correlation between the two markers. **Summary:** 100 cervical biopsy specimens of clinically evaluated and histopathologically diagnosed cases of squamous cell Carcinoma of Cervix were included in this study. Majority of our cases (35%) showed high Ki67 positivity. We established a significant correlation ($p<0.05$) between histopathological type of tumor and Ki-67 score.No significant correlation could be established between Ki67 score and clinical stage of tumor. We were able to establish a significant correlation between histological type and mAgNOR count ($p=0.003$).We also found in our study, a significant correlation between size of the AgNOR dots and their dispersion with histological type ($p=0.0122$).The correction between of AgNOR and stage of tumor was also found to be non significant ($p=0.2$). We established a significant correlation between KLI and mean AgNOR count in squamous cell carcinoma of cervix. ($p=0.0061$).

KEYWORDS

Carcinoma Cervix, Squamous cell carcinoma, Cytokinetic markers.

INTRODUCTION:

Carcinoma cervix is a major burden on the population and the government especially in developing countries like India, although its incidence has been controlled in developed nations. This decrease may be attributed to the efficacy of cervical cytology screening programs which are done in a categorical manner.

Carcinoma cervix stands next only to breast cancers that affect the female population being responsible for about 5% of deaths due to malignancies worldwide.[1]

The estimated incidence of cervical cancer is 570,000 and remains as a leading cause of morbidity and mortality worldwide. Approximately 311,000 women die each year from cervical cancer[2]. The contribution is roughly around 25% by the Indian women to the total occurrence in the world.[3]

Biopsy cervix is definitive in establishing a diagnosis in cervical lesions. However, it has its own limitations such as the study of morphological parameters alone with no information as to the fate of the lesion (progression/regression).

Further, interobserver variability needs special mention.[4,5]

Hence to overcome these draw backs and to increase the accuracy in deciding the magnitude of progression, cytokinetic markers come into role.

There has been increasing interest in the study of cytokinetic characteristics of tumors and in particular, the application of this information to clinical practice. [6]

Both Ki67 and AgNORs staining have been extensively studied as cytokinetic markers in many tumours including genital tract cancers.

Uncontrolled proliferation is a driver for cancer and is one of the hallmarks of this disease. Among proliferation markers, Ki67 is the most studied marker in cancer research. It is a nuclear protein expressed in proliferating cells during the G1, S, G2 and M cell cycle phases and is absent in quiescent cells (phase G0) [7] AgNOR staining is a technique to detect the argyrophilia of nuclear organiser regions (NORs). These are loops of DNA which encode for ribosomal RNA and are associated with non-histone nuclear proteins which can be stained in silver with one step technique. The resultant silver stained black dots can be counted (AgNORs count), this reflects the cellular proliferative activity and is elevated in a number of malignant tumours when compared to benign tissue.[8,9] In a rapidly proliferating cell, the chromosomal and AgNOR distribution remains disorganized with the resultant formation of multiple, small and dispersed nucleoli.

Significant relationships between Ki67 score and AgNORs count has been found in a variety of cancers. Through this study we aim to determine role of Ki67 and AgNOR as proliferation markers in squamous cell carcinoma of cervix and to establish a correlation between these two markers.

METHODOLOGY:

This study was conducted in Department Of Pathology, Gandhi Medical College, Bhopal, Madhya Pradesh.

Study Design:
Prospective study

Study Period
18 Months (1 January 2020 to 30 June 2021)

Study Setting:
Histology Section Of Department Of Pathology Gandhi Medical College, Bhopal.

Sampling Specimens:
All cervix biopsies diagnosed as squamous as carcinoma of cervix on routine histopathology in Department of Pathology, Gandhi Medical College Bhopal.

Inclusion Criteria:
All Patients presenting with diagnosed squamous cell carcinoma of cervix on biopsy sample.

Exclusion Criteria:

- Patients with cancers other than squamous cell carcinoma of cervix.

- Patients undergoing treatment (radiotherapy/chemotherapy).
- Patient with benign lesions of cervix or cervical dysplasia. (LSIL/HSIL)

Study Methods:
- Proforma based data collection of case history.
- Histopathological reporting of samples
- Ki-67 IHC staining and AgNOR staining on adjacent samples

Statistical Analysis:
- Data was entered in Microsoft office excel work sheets.
- Then data was analysed using appropriate statistical tests using software

Ethical Consideration:
The study was approved by Institutional Ethics Committee of Gandhi Medical College, Bhopal(M.P.) with letter No. 432/MCEC/2020

Sample Processing:
- All samples under study received in 10% formalin were routinely processed according to standard techniques after grossing.
- The wax blocks of tissue were cut using microtome and mounted on slides followed staining procedure.

RESULTS:
The prospective study included 100 cervical biopsy specimens of clinically evaluated and diagnosed cases of squamous cell Carcinoma of Cervix. The clinical, histopathological data, Ki67 IHC study and AgNOR study conducted in cases are listed in the master chart. A peak is seen at 46-55 years group followed by 56-65 years and 35-45 years. Youngest female was aged 30 years and oldest 77 years. Post-menopausal bleeding was most common complaint (39/100) of patients followed by foul smelling white discharge (23/100). Most cases belong to stage II followed by stage III. Out of total 100 cases 88 cases were keratinizing squamous cell carcinoma type and remaining 12 were non-keratinizing squamous cell carcinoma type. Out of 88 cases of KSCC, moderately differentiated squamous cell carcinoma was most common histological subtype (69/88) in present study followed by well differentiated squamous cell carcinoma (10/88). Out of total 100 cases of SCC, majority cases were Moderately differentiated squamous cell carcinoma (69/100) followed by non-keratinising squamous cell carcinoma (12/100). 100 cases of SCC included in present study as per their Ki-67 score, Majority cases (35/100) show score 3. 100 cases of SCC included in present study as per their Ki-67 labeling index, Majority cases (38/100) show KLI>50%. Correlation of Ki67 score with various histological types of SCC of cervix gave Chi square as 19.788 with a significant p value i.e. 0.019 ($p<0.05$=significant).

Correlation of KLI of cases with various histological types of SCC cervix cases gave Chi square value as 16.98 with significant p value i.e. 0.04903. Correlation of m-AgNOR count with histological types of SCC cervix cases gave Chi square value as 24.902 with significant p value i.e. 0.003. Correlation of AgNOR size score with histological types of SCC cervix cases gave Chi square value as 21.088 with significant p value i.e. 0.0122**.** Correlation of AgNOR distribution score with histological types of SCC cervix cases in present study showed Chi square value as calculated is 19.783 gave significant p value i.e. 0.02. Correlation of mean Ki67% with clinical stage of tumor gave a non-significant p value of 0.2 ($p>0.05$=non- significant). Correlation of mean AgNOR count with clinical stage of tumor gave a non- significant p value of 0.2. Correlation between KLI and m-AgNOR count in various histological types of SCC cervix cases gave Chi square value as 40.339 with significant p value i.e. 0.0061 ($p<0.05$=significant).

DISCUSSION:
Table-1: Comparison of frequency of histolological subtypes of Squamous Cell Carcinoma with other studies

STUDY	WELL DIFFERENTIATED	MODERATELY DIFFERENTIATED	POORLY DIFFERENTIATED
Ancuta et. al. (2009) 104	24.6%	49.2%	9.8%
Sharma et. al. (2016)	16.7%	44.4%	38.9%
Choudhary et. al. (2020)	15.0%	62.5%	22.5%
Present study	11.36%	78.40%	10.22%

Results of our study are comparable to a study conducted by **Ancuta et al.** (2009) [104] where moderate differentiated cases were 49.2%, well-differentiated -24.6%, and undifferentiated 9.8%.

Rekha W (2010)[105] observed in her study that majority of cases (58.6%) were moderately differentiated carcinoma which is similar to present study.

Sharma et al. (2016)[106] conducted a study where maximum number of cases belonged to moderately differentiated group (44.4%), followed closely by poorly differentiated (38.9%), well differentiated carcinoma were only (16.7%).

Another study by **Choudhary et al.** (2020)[107] revealed results similar to **Sharma et al**. Out of all Sqaumous Cell Carcinoma included in the study, 62.5% were moderately differentiated, 22.5% were Poorly differentiated and 15.0% were well differentiated. This discrepancy between these two studies and our study can be attributed to difference in sample size. These studies included 18 and 40 cases of squamous cell carcinoma respectively whereas we included 100 cases of SCC.

We observed that most patients presented with complaint of abnormal uterine bleeding (AUB) including post menopausal bleeding (39%), menorrhagia (16%), post coital bleeding (13%), irregular menstrual bleeding (6%). The second common complaint was foul smelling white discharge observed in 23% of total patients.

Table-2: Comparison of presenting complaints of patients with carcinoma cervix with other studies

STUDY	MOST COMMON COMPLAINT	2nd MOST COMMON COMPLAINT
Schalkwyk S et al (2008)108	Abnormal Uterine Bleeding	Fatigue
Eze J et al (2013)109	Abnormal Uterine Bleeding (88.5%)	Offensive vaginal discharge (42.6%)
Sharma et al (2016)106	Vaginal bleeding	Discharge per vaginum
Present study	Abnormal Uterine Bleeding (74%)	Foul white discharge per vaginum (23%)

Sharma et al. (2016)[106] also discussed results alike to our study stating most common symptom was vaginal bleeding, followed by discharge per vaginum.

In a study by **Eze J et al.** (2013)[109] on 61 cases of cervical cancer, it was observed that 54 out of 61 cases (88.5%) presented with complaint of abnormal uterine bleeding followed by offensive vaginal discharge in 26 out of 61 cases (42.6%) which is similar to present study where 74% patients presented with AUB followed by foul smelling white discharge in 23% of the total patients.

Schalkwyk S et al. (2008)[108] observed that most common presenting complaint in females diagnosed with cervical cancer was abnormal uterine bleeding followed by fatigue and offensive vaginal discharge.

In our study 50% of the patients were diagnosed with clinical stage II cervical cancer (IIA- 19% and IIB-31%) followed by 40% patients who were diagnosed with clinical stage III (IIIA-13%, IIIB-27%) at the time of presentation.

Table-3: comparison of clinical stage at time of presentation with other studies

STUDY	MOST COMMON STAGE	2ND MOST COMMON STAGE
Schalkwyk S et al. (2008)108	Stage II (53.3%)	Stage III (33.3%)
Eze J et al. (2013)109	Stage III (36.6%)	Stage II (34.1%)
Choudhary et al. (2020)107	Stage II (71.4%)	Stage III (4.0%)
Present study	Stage II (50.0%)	Stage III (40.0%)

Our finding is analogous to observation by **Schalkwyk S et al.** (2008)[108] where 53.3% cases were in stage II and 33.3% cases in stage

III and **Choudhary et al.** (2020)[107] who also observed stage II (71.1%) to be the most common at the time of presentation **Eze J et al.** (2013)[109] observed 34.1% patients presented with clinical stage II and 36.6% with clinical stage III.

Ki 67 EXPRESSION

35% of total (100) cases in our study showed >50% (high) Ki67 score , 32% showed 30-50% (moderate) score and 29% cases were with 10-30% (low) score.

Table-4: Comparison of Ki-67 score of cases of carcinoma cervix with other studies

STUDY	LOW	MODERATE	HIGH
Brustmann H et. al.111	32.3%	31.5%	33.6%
Ancuta et al. (2009)104	26.2%	42.6%	31.1%
Present study	29%	32%	35%

Results similar to our study were demonstrated by **Brustmann H et al.**[111] They divided Ki- 67 positivity into tertiles: low (<20%), moderate (21-30%), high (>30%) and found that 33.6% cases showed high positivity and 31.5% cases showed moderate positivity and 32.3% cases revealed low positivity.

In a study by **Brown et al.** (1988)[112] on 25 biopsies diagnosed as SCC of cervix maximum cases showed 20-30% positivity staining with Ki67 followed by 40-50% positivity.

Ancuta et al. (2009)[104] in their study on 61 cases of cervical cancer observed results similar to Brown et al. They found 42.6% cases displayed moderate proliferation values as defined by Ki-67 and 31.1% high-Ki-67 proliferation, while only 26.2% were defined by low-tumor proliferation. This difference in results can be attributed to large time gap between our study and study by Brown et al and difference in demography of patients between our study and that by Brown et al and Ancuta et al.

In this study, we were able to establish a significant correlation (p=0.019) between histopathological grade of tumor and Ki-67 positivity. Most of well differentiated cases showed score 1 (mild positivity), while most of the poorly differentiated and non keratinising cases showed score 3 (high positivity).

Table-5: Comparison of Ki-67 scores in various histological types of SCC cervix in present study

Ki-67 SCORE	WELL DIFFERENTIATED (total case=10)	MODERATELY DIFFERENTAITED (total case=69)	POORLY DIFFERENTIATED (total case=09)	NON-KERATINISING (total case=12)
0	-	5.80%	-	-
1	60.00%	28.98%	-	25.00%
2	40.00%	34.78%	22.23%	16.67%
3	-	30.44%	77.77%	58.33%

Brown et al. stated that the distribution of nuclear labelling by monoclonal antibody Ki-67 and hence proliferative activity varied within individual cases with central areas showing cell maturation and keratinization displaying little or no staining compared with the more actively proliferating peripheral portions[112]. This suggests that Ki-67 positivity is more in less differentiated parts of the tumor. It provides a rationale for our findings where 60% of well differentiated cases show mild positivity and 77.7% of poorly differentiated cases show high positivity.

Ancuta et al. established a direct statistical significant moderate correlation between Ki-67 and tumor grading (r=0.386, p<0.005)[104], suggesting higher tumor proliferation rate is associated with loss of tumor differentiated. This again justifies our observation of increased Ki67 positivity in higher grades (less differentiated) tumor cases.

Similar results were stated in a study by **Jian-Qin Yu et al.** (2015)[113] in which 27 out of 28 poorly differentiated cases included in the study were strongly positive and only 1 case was weakly positive. On the other hand out of 29 cases with moderate and high differentiation 15 were weakly positive and 14 were strongly positive.

Chung et al.[114] were also able to establish a significant correlation (p<0.05) between tumor grade and KLI in their study inclusive of 25 biopsies of SCC of cervix.

Following are some studies on lesions other than SCC cervix biopsies which suggested results alike to present study:

Brustmann H et al. found similar results in their study on vulval neoplasm stating that in SCCs, immunoreactive nuclei were accentuated at the marginal regions ofinfiltrating tumor aggregates showing keratinisation and in nonkeratinizing areas of SCCs positive nuclei were distributed diffusely[111].

Sahebali S et al. (2015)[115] performed Ki-67 immunostaining on liquid based cytology cervical smears and found that when comparing the number of Ki-67 immunoreactive cells/1000 cells between the different cytological groups, the HSIL group showed a significantly higher count (p < 0.001) than did the other groups (LSIL, ASCUS).

Marwah et al. (2020)[116] in their study on breast neoplasm found that Grade I tumors had the highest number of Ki-67 negative cases (65.2%). High positivity of Ki-67 was maximally represented in Grade III tumors (69.2%) followed by Grade II tumors (56.4%). The association of Ki-67 with grade was statistically highly significant (P < 0.001).

Goyal et al. (2015)[117] found strongly significant correlation between KLI and tumor grade in urothelial carcinoma. (p=0.000)

Ashraf et al. (2010)[118] in their study of squamous cell lesions of head and neck observed that the Ki67 percentage is significantly increased from normal squamous to SCC group, and the reactivity of staining were related to histological differentiation

Table-6: Summary of correlation of Ki-67 positivity with tumor grade/type in various studies

STUDY	TISSUE /LESION	CORRELATION OF Ki-67 POSITIVITY WITH TUMOR GRADE
Ancuta et al.104	Cervical cancer	Significant (p<0.05)
Chung et al. 114	Squamous cell carcinoma of cervix	Significant (p<0.05)
Sahebali S et al. (2015)115	Liquid based cytology of cervical smears	Significant (p<0.001)
Marwah et al. (2020)116	Breast neoplasms	Significant (p<0.001)
Goyal et al. (2015)117	Urothelial bladder carcinoma	Strongly Significant (p=0.000)
Present study	Squamous cell carcinoma of cervix	Significant (p=0.019)

We did not find any significant correlation between clinical stage of tumor and ki-67 positivity in present study (p=0.2). This result is in concordance with studies by Chung et al.[114], Ancuta et al.[104], Jian-Qin Yu et al. (p=0.354)[113].

The fact that there was no correlation with the clinical stage is not surprising, as staging in this cancer is most dependent on invasion of adjacent tissue.Present study was aimed at finding the role of AgNOR staining as a proliferation marker in SCC of cervix. *We were able to establish a significant correlation between histological grade and mAgNOR count (p=0.003).*

Table-7: Comparison of m-AgNOR count in histological types of SCC of cervix in present study

m-AgNOR COUNT	WELL DIFFERENTIATED (total cases=10)	MODERATELY DIFFERENTIATED (total cases=69)	POORLY DIFFERENTIATED (total cases=09)	NON KERATINISING (total cases=12)
3-5	30.00%	7.25%	11.11%	8.34%
5.1-7	70.00%	31.88%	-	41.66%
7.1-9	-	52.17%	55.55%	50.00%
9.1-11	-	8.70%	33.34%	-

Studies on squamous cell carcinoma of cervix which showed results in concordance with present study:

Kafil Akhtar et al. (2005)[119] compared AgNOR counts in the different grades of squamous cell carcinoma of the cervix and observed a progressive significant increase in the counts with increasing grades of carcinoma

Pahuja S et al. (2003)[120] found significant correlation between histological grade of SCC of cervix and AgNOR count.

Chung et al. found significant correlation between histological grade and mean AgNOR count ($p<0.05$)[114]Studies on lesions other than biopsies of squamous cell carcinoma of cervix which showed results in concordance with present study:

Shukla S et al. (2013)[121] conducted a study on cervical smears found a graded increase m-AgNOR count from normal to inflammatory to premalignant and maximum in malignant smears.

Madhavi et al. (2016)[122] in their study on cytological smears on breast lesions concluded that mean AgNOR count in breast carcinoma is highest (4.76±0.17) followed by benign Phyllodes tumor (2.8±0.75), fibrocystic disease (2.47±0.20). The mean AgNOR counts in fibroadenoma are 2.45±0.05.

Harshi Dhingra et al. (2016)[123] observed that mean number of AgNORs in endometrial adenocarcinoma (5.62 ± 0.37) was significantly higher than that of complex hyperplasia with atypia (4.68 ± 0.23, $p < 0.0001$), complex hyperplasia without atypia (3.63 ± 0.23, $p<0.0001$). Also, mean AgNOR counts in complex hyperplasia with atypia (4.68 ± 0.23) was significantly greater than that of complex hyperplasia without atypia (3.63 ± 0.23, $p<0.0001$).

Devran et al. observed mean AgNOR counts of 6.8 or greater were seen in 98.5% of the malignant breast tumors, whereas all benign breast lesions had mean AgNOR counts less than 6.8[70].

Kakeji Y et al.[124] concluded the AgNOR counts in the primary gastric tumor were significantly higher than in the normal epithelium ($P < 0.01$).

Goyal et al. (2015) found significant correlation between mean AgNOR count and tumor grade in urothelial carcinoma. ($p=0.006$)[117]

Ashraf et al. in their study of squamous cell lesions of head and neck observed that the mAgNOR counts were high in all 44 cases of primary and metastatic SCC and low in normal squamous tissue and increased in dysplastic lesions[118]. The mAgNOR count in SCC increased from well differentiated to poorly differentiated.Studies which revealed results dissimilar to present study in relation to tumor grade and mean AgNOR count:

Pich et al. in their study on renal cell carcinoma stated that even though the mean number of AgNORs per cell was higher in less histologically differentiated and more advanced stage carcinomas, the one-way analysis of variance (ANOVA) showed no significant correlation between AgNOR number and histological grade ($p = 0.132$)[85] or pathologic stage ($p = 0.78$)[85].

Bryan et al.(1990)[125] also failed to find a significant difference between AgNOR number in renal adenomas and carcinomas, supporting the idea that there is a continuous spectrum of kidney tumours. This disparity can be explained by enormous time gap between these two studies and present study. Over the time the staining technique has been modified several times. Also, the tissues under question are different in these studies and present study. Hence it would not be just to draw conclusions out of these comparisons.

Table-8: Summary of correlation of m-AgNOR count with histological tumor grade/types in various studies

STUDY	TISSUE/LESION	CORRELATION OF m-AgNOR COUNT with TUMOR GRADE/TYPE
Chung et. al.114	Squamous cell carcinoma of cervix	Significant ($p<0.05$)
Pahuja S et al 120	Squamous cell carcinoma of cervix	Significant
Kafil Akhtar et al119	Squamous cell carcinoma of cervix	Significant
Harshi Dhingra et al 123	Endometrial lesions	Significant ($p<0.0001$)
Kakeji Y et al124	Primary gastric tumors	Significant ($p<0.001$)
Goyal et al117	Urothelial carcinoma	Significant ($p=0.006$)
Pich et al85	Renal cell carcinoma	Non Significant ($p=0.0132$)
Bryan et. al.125	Renal adenoma and carcinoma	Non significant
Present study	Squamous cell carcinoma of cervix	Significant ($p=0.03$)

We also found in our study, a significant correlation between size of the AgNOR dots and their dispersion with tumour grade ($p=0.0122$).This implies that pleomorphism among AgNOR dots increases with increasing grade of tumor. Srivastav et al. (2013)[126] conducted a study on cervical smears which revealed a positive and significant correlation of cell counts of both pleomorphic ($r = 0.94$; $p < 0.01$) and single dots ($r = 0.95$; $p < 0.01$) with disease severity. Their study indicated the diagnostic potential of pleomorphic dots in the process of cervical carcinogenesis.

But Ashraf et al.[117] found confounding results with present study. They stated there was no statistical difference between the variation of AgNOR dots size and dispersion between various groups of squamous lesions of head and neck included in their study ($P > 0.05$). This can be explained by inter-observer variability in counting NORs and technical factors.[118]

Similar to the correlation between KLI and clinical stage of tumor, the correction between of AgNOR and stage of tumor was also found to be non significant (p=0.2). Similar results were confirmed in studies by Chung et al.[114], Goyal et al.[117], Pich et al. ($p=0.78$)[85]

Correlation Between Ki67 Labelling Index And Mean Agnor

In present study we established a significant correlation between KLI and mean AgNOR count in squamous cell carcinoma of cervix. (p=0.0061).

There have been many studies in the past on different types of lesions aiming at establishing a correlation between KLI and mean AgNOR count. Results of these studies have been variable. Some found no significant correlation between the two variables in question while others found slightly significant to highly significant correlation.

Table-9: following table compares different studies and their results regarding correlation of KLI and mAgNOR count.

Study	Year	Organ system/ lesion	Sample size	Correlation between KLI and mAgNOR
P.A. Hall et al.127	1987	Non Hodgkin's Lymphoma	80	Significant (<0.001)
Devran et al.70	1988	Breast lesions	97	Highly significant (p=0.02)
Hara et al.84	1990	Glioma	14	Linear relation (r=0.91)
Pich et al.85	1991	Renal cell carcinoma	21	Slightly significant
Kakeji et al.124	1991	Gastric carcinoma	91	Significant
Mourad et al.128	1993	Fine needle aspirates of breast	20	Not significant (p=0.13)
Chung et al.114	1994	SCC of cervix	25	Not significant
Pahuja S et al.120	2003	SCC of cervix	18	Not significant
Ashraf et al.118	2010	Squamous lesions of head and neck	64	Significant in SCC group
Goyal et al. 117	2014	Urothelial carcinoma	54	Significant
Present study	2020-2021	SCC of cervix	100	Significant (p=0.0061)

Mourad et al. proposed an explanation for this inconsistent correlation between mAgNOR and KLI. Whenever there is a good correlation this is associated with neoplasms that have a high KLI. The lack of correlation was seen in tumors with low KLI. This might be explained by the fact that those tumors with high proliferative activity measured by KLI are most likely aneuploid with a high mAgNOR count[128].

This explanation is applicable on present study as well because most of the cases in this study showed moderate to high KLI.Goyal et al. defended their finding of significant correlation between KLI and mAgNOR count by explaining how aggregates of AgNORs lead to erroneous counting[117]. Also, technical factors related to staining and fixation limits the practical utility of AgNOR in evaluation of individual cases.

This holds true for present study as well. We faced difficulty in counting NORs due to their aggregation. Technical errors in the staining procedures also could be eliminated completely.

CONCLUSION:

Carcinoma cervix is one of the leading cause of deaths in women all over the world and India carries 25% of total global burden of this disease.

Histopathology is the mainstay for diagnosis of carcinoma cervix and to determine its type. Squamous cell carcinoma is the most common histological type of carcinoma cervix.

Over the years many bio markers and cytokinetic markers have been studied to understand etiology, growth potential and recurrence of tumor. All these have proven useful in planning treatment and determining prognosis of patients.

In this study we have made an attempt to compare Ki67 immuno-staining and AgNOR staining in 100 cervix biopsies diagnosed as squamous cell carcinoma of cervix. Studies have proven both these techniques are markers of proliferation.

We established a significant correlation between the two methods. In order to be able to compare the two methods we must have understanding of limitations of both techniques. Ki67 and AgNOR independently showed significant correlation with tumor grade but not with clinical stage of tumor

The limitations of Ki-67 IHC staining are as follows:

- IHC stains are not standardised worldwide. A number of Ki67 antibodies are commercially available, and their prognostic and diagnostic value in individual tumors can vary significantly.
- Even with the same antibody, results can differ either because of different staining protocols or because of the variables.
- The equipments needed to perform IHC are costly.
- Quantifying results is difficult.
- IHC is subject to human error. Well-trained personnel are must to perform the procedure and interpret results.

The limitations of AgNOR staining are as follows:

- Counting is tedious and time consuming.
- Variation in section thickness and methods of fixation may influence counts.
- Inter-observer variability is a potential problem.
- Technical factors encountered during tissue processing and staining vary in different settings[70].

Observations and conclusions made by researchers in the past from comparing Ki67 and AgNOR stain on various tissues have not been unanimous. Some reported significant correation while others stated there was no significant correlation between these two markers. Possible explanation for these confounding results can be:

- Significant correlation has been found in studies where most cases showed high KLI. Tumors with high proliferative activity measured by KLI are most likely aneuploid with a high mAgNOR count.
- Although AgNOR count is a repeatable, easy, efficient, and low-cost approach, counting of AgNOR can be very subjective, tedious and in cases with greater aggregates of black dots can lead to falsely increased mean AgNOR count and result in significant correlation with KLI.
- Present study was conducted on small sample size without any follow up of cases thus it lacks a statistical power. We can conclude that AgNOR can be employed as a supplement to regular screening and diagnostic methods for determining progression, reccurance and prognosis of squamous cell carcinoma of cervix. But we cannot say this with confidence that AgNOR counts can replace Ki-67 scores in evaluating the proliferative activity of squamous cell carcinoma of cervix.

Therefore further studies with larger cohorts, preferably multicentric are required along with proper follow up to determine whether Ki67 and AgNOR can be used independently as markers in defining prognosis of patients with squamous cell carcinoma of cervix.

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