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Pathology

COMPARATIVE STUDY USING PAPANICOLAOU STAIN AND AGNOR STAIN IN CERVICAL SMEARS IN A TERTIARY CARE HOSPITAL

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ABSTRACT In India Cervical cancer is the second most common cancer among women. The AgNOR dots are quite useful in tumour detection.

AIM: A comparative study using Papanicolaou and AgNOR stain in cervical smears.

MATERIAL AND METHODS : present study was prospective analytical study,carried out in pathology department in tertiary care hospital. 100 patients from Gynaec OPD were selected .Two cervical smears were prepared . One stain with conventional Papanicolaou stain and other with AgNOR stain . Cytopathological findings were compared with number of AgNOR dots of corresponding slide.

OBSERVATIONS: The AgNOR dots increases (NILM1.62 , Chronic cervicitis -2.01, LSIL-2.75and HSIL-4.06)severity of lesion increases.

CONCLUSION: AgNOR dots tend to increase as the severity of lesion of cervix increase and also indicates AgNOR counts in cervical dysplasia has potential diagnostic importance.

KEYWORDS : Agnor , Cervical Smear ,cytopathology

INTRODUCTION

In India Cervical cancer is the second most common cancer among women between 15-44 years of age and is considered to arise from cervical intraepithelial neoplasia (CIN) . In India, over the past 5 decades, there has been a definitive shift in the trend of the cancer development amongst females, thus making cervical carcinoma slip down to the second position, being due to lack of basic understanding or awareness of the importance of the routine screening of cervix has been a major contributor to the higher incidence of cancer of the cervix amongst the women in the developing countries second only to breast cancer.

Screening using cervical smears has long been established as a mainstay for detecting early cytological abnormalities and has contributed to a significant fall in death rate from cervical carcinoma in many countries.[1]

In contrast to developed countries ,cervical cancer is a public health problem in developing countries like India, so much so that India alone accounting for one- quarter of the world wide [2]

It is one of the leading cause of cancer mortality , accounting for 17% of all cancer deaths among women aged between 30-69 years. It is estimated that cervical cancer will occur in approximately 1 in 53 Indian women during their life time compared with 1 in 100 women in more developed regions of world. In India Cervical cancer contributes to approximately 6-29% of cancer in women. [3]

Cervical Intraepithelial Neoplasia or cervical dysplasia is the stage that can precedes to carcinoma cervix in near future and this stage can be considered as a vital stage for the diagnosis because early diagnosis at this stage may have better prognosis.

'AgNOR' represents Nucleolar Organiser Regions (NORs) stained with colloidal silver. In rapidly dividing cells like neoplastic cells, nucleolar dysaggregation occurs and resulting in dispersion of individual AgNOR. Recent studies show that AgNOR are significantly higher in number in malignant cells [4]

The normal transformation zone present on the cervix represent the zone of of physiologic metaplastic epithtelium that has replaced the columnar epithelium.This site for obtaining the cervical smear is of greatest interest as it has the potential for developing neoplasia of cervix. CIN lesions with low AgNOR counts are more likely to regress in comparison to CIN lesions with high AgNOR counts.[5]

The reliability of this method in the evaluation of malignancy has been frequently demonstrated even by a simple visual assessment. 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.

MATERIAL AND METHODS:-

MATERIAL

STUDY DESIGN AND PLACE:

Comparative Prospective analytical study was carried out in Department of pathology attached to a Tertiary care hospital over a period of 2 years.

SAMPLE COLLECTION

Hundred (100) Samples were randomly collected for this study, who were routinely attended Obstetrics & Gynecology Department (OPD and ward) with the help of endocervical brush on the glass slide.

After taken the detailed history of patients and obtaining the informed consent and institutional ethical clearance , Pap-smear were prepared, two slides were obtained, one for routine Pap staining and other for staining with colloidal silver stain. Pap smear was prepared by routine conventional method, then slides will be examine under the microscopy and interpretate the result.

AgNOR count was done and correlated with severity of the lesion Stastical analysis of the data was done by using ' F' test [6]

OBSERVATION & RESULTS:

A total of 100 samples were collected, out of which, 14% were 21-30 years, 35% were 31-40 years, 36% were 41-50years and 15% women were 50yrs of age and above.

Out of 100 cases studied 31% women had complain of p/v bleeding, 32% women had white discharge and rest of the 37% women had no complain.

Among the 100 women studied, 15% had <5 years of marital life, 30% have 5-10 years of marital life, another 40% had 11-20 years of marital life and 15% women had more than 20 years of marital life.

Among 100 women , only one women had (1%) H/O sexual contact .

P/V BLEEDING & NUMBER OF CASES:

Out of 100 cases studied 31% women had a complaint of bleeding p/v , out of 31 cases 15% had history of irregular bleeding ,10% had H/O

post coital bleeding and 6% women had c/o post menopausal bleeding.

CYTOPATHOLOGICAL FINDINGS & NUMBER OF CASES :
Out of 100 cases cytopathologically studied 56% were Negative for Intra Epithelial Malignancy(NILM),33% of Chronic cervicitis(CC), 8% of High grade Squamous Intraepithelial lesion(HSIL) and 3% of Low grade Squamous Intraepithelial Lesion (LSIL)was found.

There was no correlation found between parity and number of AgNOR dots. ('P'value>.05)

AGNOR SCORE AND MULTIPLE SEXUAL PARTNERS:
In this study women having history of multiple sex partners had higher AgNOR score than the women having single sex partner.
AgNOR score and history of multiple sex partners was significantly correlated ('p ' value<.003).

PRESENTING COMPLAINTS AND AGNOR DOTS WISE DISTRIBUTION (TABLE 1):
The women were having complaints of white discharge and bleeding p/v had high Ag NOR count, the correlation between these two complaints and number of AgNOR count was highly significant . (p value .0001)

PER SPECULUM FINDINGS AND NUMBER OF AGNOR DOTS WISE DISTRIBUTION (TABLE 2)
Out of 100 case, 48 cases were positive,Of 48 positive cases, 39 cases were positive for cervical erosion and 9 cases were positive for cervical hypertrophy. The women who had cervical erosions on per speculum examination ,AgNOR count was higher as compared to normal cervix and the correlation was statistically significant.

CYTOPATHOLOGICAL FINDINGS AND AGNOR SCORE (TABLE 3)
The mean AgNOR score for NILM was 1.62, for Chronic cervicitis 2.11, for LSIL 2.75 and for HSIL 4.06.

The various cytopathological findings exhibited statistically significant difference in AgNOR values.(p value <.0001)

Negative for Intra Epithelial Lesion(NILM) and Chronic cervicitis had low score and other cases LSIL and HSIL had higher AgNOR score.

DISCUSSION
Cervical cancer is the most common cause of death among women in India next to breast cancer. The cervix is easily accessible site to obtain Pap smear ,so it is easy to detect squamous cell abnormality in cervix at very early stage which may further progress to Squamous Cell Carcinoma.

Many times Pap smear has certain limitations because of subjective error, the pathologist may have different views for the same lesion ,so to avoid the conflicts smear can also stain with AgNOR stain.

On the basis of number of intranuclear dots we can correlate our findings and strengthen our diagnosis, So by counting number of AgNOR dots can be extremely helpful for pathologist in diagnosis as well as in prognosis of cancer and also Can play important role in differentiating between different spectrum of cervical lesions.[7]

In our study there was a statistically significant correlation('p' value <.00001) found between nucleolar organiser regions(NORs) and various cervical lesion . Highest number of dots was reported in High Grade Squamous Intra Epithelial Lesion(4.06)and lowest score in normal cervical smear(mean score (1.62).The significant difference in score was also observed between HSIL , LSIL and Chronic cervicitis.
Similar study done by Basanta S et al[8] , 2016 they find out of 60 cases, 25% were benign. 26.6% were CIN and 48.4 % were diagnosed as carcinoma cervix. AgNOR count for benign lesion was1.5 ,for CIN 3.7 and for malignancy was 5.6. and AgNOR score was significantly higher in malignant lesion as compared to benign lesion .

Another study conducted by KC Shiv Raj *et al*[9], they selected 53 cases of cervical lesion and subjected to cytology and biopsy with AgNOR stain.The mean AgNOR score of LSIL in cervical smear was 2.9 and 1.9 in cervical biopsy and in HSIL 3.45 in cervical smear , 3.00 in cervical biopsy respectively. Similar to our study this study also concluded that as severity of cervical inepithelial neoplasia increases mean AgNOR count also increases significantly.

CONCLUSION
Finding of our study shows, AgNOR dots present within the nucleus of squamous cells have a definitive proliferative activity with severity of cervical pathology.

The AgNOR dots or granules tend to increase as the as the severity of lesion of cervix increases progressively.

Furthermore the studies also reported that dysplasia with low AgNOR counts are more likely to regress as compared to those with high AgNOR counts .Which on other hand are likely to progress to invasive carcinoma showing prognostic significance. Although more studies are necessary , our preliminary study indicates AgNOR counts in cervical dysplasia has potential diagnostic as well as prognostic importance.

Table 1 : Presenting complaints and AGNOR dots wise distr ib ution

Variable	Presenting complaints	Cases	Mean±SD	F- test	p-value
AgNOR dots	BPV	31	2.43±0.91	8.280	0.0001 Significant
	NC	37	1.77±0.42		
	WD	32	1.94±0.65		
	Total	100	2.03±0.72		

Table 2:- Per speculum findings and number of AgNOR dots wise distribution

Variable	Per speculum findings	No.of cases	Mean±SD	F- test	p-value
AGNOR dots	CE	39	2.46±0.89	15.895	0.0001 (highly significant)
	CH	9	2.04±0.47		
	GN	52	1.70±0.34		
	Total	100	2.02±0.72		

Table-3: Cytopathological findings and AgNOR score

Variable	Cytopathological findings	Cases	Mean±SD	F tests	p-value
AGNOR dots	NILM	56	1.62+-0.32	167.31	<0.0001
	CC	33	2.11+-0.29		
	LSIL	3	2.75±0.14		
	HSIL	8	4.06+-0.12		

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