

A STUDY ON THE ROLE OF MICRO NUCLEI IN ASSESSING THE PROGRESSION OF PRECANCEROUS LESIONS OF CERVIX AND THE DIAGNOSIS OF CARCINOMA OF CERVIX

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ABSTRACT: BACKGROUND: Invasive squamous cell carcinoma of cervix remains the most common malignant neoplasm of the female genital tract in many countries. The Papanicolaou stained cervical smear is an excellent and effective method in the diagnosis of invasive carcinoma and precancerous lesions of the cervix. This study was conducted to assess the value of Feulgen stained cervical smears in elucidating nuclear features helpful in the diagnosis of malignancy over conventional Pap stained smears and also to look for micronucleated cells in suspected cases of carcinoma of cervix. **AIM:** To analyse the distribution of cases of invasive squamous cell carcinoma and intraepithelial neoplasia (squamous intraepithelial lesion) of cervix over a period of 3 years, to elucidate additional nuclear features helpful in diagnosis of carcinoma using Feulgen stained cervical smears and to study the distribution of micronuclei in Feulgen stained smears from suspected cases of Carcinoma cervix. **MATERIALS AND METHODS:** A three year analysis of all cases of dysplasia and invasive carcinoma of cervix was done by reviewing Papanicolaou stained cervical smears from all the diagnosed cases of invasive carcinoma and precancerous lesions of the cervix. Cervical smears from sixty clinically suspected cases of carcinoma of cervix and smears from 10 normal women collected during a period of 12 months were studied in detail using Papanicolaou stained and Feulgen Stained Smears and micronuclei analysis (MN analysis) was done. **RESULTS:** A total of 24343 cervical smears were screened during the 3 year period of study. Out of these 24343 cases there were 267(1.09%) cases of cervical intraepithelial neoplasia and 144(0.592%) cases of invasive squamous cell carcinoma. Micronuclei analysis done using Feulgen Stained Smears demonstrated a consistent increase in micronucleated cells proportional to the increase in severity of the lesion from normal to invasive carcinoma. **CONCLUSION:** This study analysed the distribution of intraepithelial neoplasia and invasive carcinoma of cervix with particular reference to age distribution. This study also demonstrated the superiority of Feulgen stain over conventional Papanicolaou stain in elucidating nuclear features and micronuclei analysis showed a consistent increase in micronucleated cells proportional to the increase in severity of the lesions. So it is suggested that micronuclei analysis may be used as a marker of a greater potential for disease progression.

KEYWORDS: Precancerous lesions of cervix, Invasive carcinoma of cervix, Feulgen stained smears, Micronucleated cells.

INTRODUCTION: Invasive squamous cell carcinoma of cervix remains the most common malignant neoplasm of the female genital tract in many countries and it is the most common malignant neoplasm in women in India. There is sufficient evidence that invasive carcinoma of

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cervix arises from abnormal cancerous surface epithelium.¹ All invasive carcinomas are preceded by an intraepithelial stage.² Preventive measures such as periodic screening have helped to reduce the incidence of invasive carcinoma of cervix in developed countries.^{3,4} But in developing countries it remains a major health hazard.

The papanicolaou stained cervical smear is an excellent and effective method in the diagnosis of invasive squamous cell carcinoma and precancerous lesions of the cervix. However current evidence strongly suggests that a single cervical smear carries with it an unexpectedly high margin of failure in the detection of precancerous lesions and invasive carcinoma of the cervix. There are instances in cytologic practice when a smear pattern is difficult or impossible to interpret accurately and the term atypical smear is frequently used in such instances.

Increased frequency of micronucleated cells in the buccal mucosa of individuals at high risk of oral cancer and oral cancer patients and cervical cancer and precancerous lesions is well documented. Micronuclei are composed of extrusions from the nucleus during cell division of damaged chromosomes or lagging chromosomes. Micronuclei analysis can be used as an effective method in assessing the genotoxic damage to cells.^{5,6,7}

In this study an attempt is made to investigate the value of Feulgen stained cervical smears in elucidating nuclear features helpful in the diagnosis of malignancy over conventional pap stained smears. The study also aims to look for micronucleated cells in suspected cases of carcinoma of cervix. It is hoped that this study will lead to better understanding of the pathology of carcinoma of cervix and in turn lead to better patient care.

MATERIALS AND METHODS: A three year retrospective analysis of all cases of dysplasias and invasive carcinoma of cervix was done. Papanicolaou stained smears from all the diagnosed cases were reviewed and reclassified into squamous intraepithelial lesion low and high grades and invasive squamous cell carcinomas into three major sub types – large cell – nonkeratinising (LCNK), small cell – nonkeratinising (SCNK) and large cell keratinising (LCK) based on the WHO classification. Simultaneously the age distribution was studied with reference to squamous intraepithelial lesion and invasive carcinoma. Cervical smears from sixty clinically suspected cases of carcinoma cervix and smears from 10 normal women collected during a period of 12 months were studied in detail. Additional smears were taken from these cases. One smear was stained with Papanicolaou stain and another smear as per the Feulgen and Rosenbeck method.

After screening the pap smears cytological diagnosis of each case was made. Feulgen stained smears were screened for micronuclei (MN) and nuclear anomalies like karyorrhexis, karyolysis, chromatin clumping, nuclear buds and atypical mitoses. Micronuclei analysis (MN analysis) was performed following criteria described by Sarto et al and Tolbert et al. Structures were considered micronuclei when distinctly separated from the nucleus and with a configuration and staining similar to those observed in the nuclei. All cells with degenerative changes like karyolysis and karyorrhexis and cells with binucleation and nuclear buds were excluded from the analysis. Two hundred cells in each smear were screened for micro nuclei.

RESULTS: A total of 24343 cervical smears were screened during the period of study. Out of these 24343 cases there were 267(1.09%) cases of squamous intraepithelial lesion and

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144(0.592%) cases of invasive squamous cell carcinoma. A total number of 267 SIL cases were obtained during the 3 year period. The distribution of different grades of SIL is given in Table 1.

SIL	Number of cases	Percentage
Low	176	65.9
High	91	34.1
Total	267	100

Table 1: Distribution of squamous intraepithelial lesion (SIL)

The age of the patients ranged from 24 to 78 years in squamous intraepithelial lesion. Mean age of SIL – low was 46.55 and SIL high grade 49.805 (Table 2).

SIL	N	Mean Age	SD	Range (Years)
Low	176	46.55	10.97	24-76
High	91	49.805	11.32	29-78

Table 2: Mean age of squamous intraepithelial lesion (SIL)

More than 50% patients with SIL - low grade were under 50 years of age, whereas more than 50% of patients were above 50 years in SIL – high grade. There were 144 cases of invasive squamous cell carcinoma of cervix during the study period. The distribution of different types of invasive squamous cell carcinoma is given in Table 3.

Type	Number of cases	Percentage
LCNK	85	59.0
SCNK	02	01.4
Keratinising	57	39.6
Total	144	100

Table 3: Distribution of invasive squamous cell carcinoma

LCNK – Large cell non-keratinizing.

SCNK – Small cell non-keratinizing.

The age ranged from 32 to 84 years. Most of the large cell non keratinising carcinoma cases were in the 5th & 6th decades whereas keratinising types showed a predilection for 6th & 7th decades (Table 4).

Age group (in decades)							
	30-39	40-49	50-59	60-69	70-79	80-89	Total
No. of LCNK Cases	10	30	24	18	1	2	85
%	11.8	35.3	28.2	21.2	1.2	2.4	100%
No. of Keratinising	8	8	20	18	2	1	57
%	14.0	14.0	35.1	31.6	3.5	1.8	100%

Table 4: Age distribution of invasive squamous cell carcinoma

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Micronuclei (MN) analysis: MN analysis demonstrated a consistent increase in micronucleated cells proportional to the increase in severity of the lesion from normal to invasive carcinoma (Table 5).

	Total cases	Total cells counted	Micronucleated cells	Rate/1000
Normal	15	3000	1	0.33
SIL – Low Grade	08	1600	1	0.63
SIL – High	07	1400	2	1.43
Invasive carcinoma	40	8000	12	1.50

Table 5: Distribution of Micronucleated cells

DISCUSSION: Carcinoma of uterine cervix and its precursors belong to the best studied forms of human cancer. There is excellent evidence that invasive squamous carcinoma of the cervix develops from abnormal cancerous surface epithelium. The prevalence rates of invasive carcinoma and intraepithelial carcinoma in India were different from the prevalence rates in United States reported by Sadeghi et al who observed a higher incidence of intraepithelial lesions among younger women. The difference in the rates may be attributed to the inadequate screening programmes in our set up. So this reflects the need to strengthen our screening programmes. The low prevalence rate of invasive carcinoma in Western countries may be due to the adequate prevention programmes following periodic screening.

Feulgen staining of smears was done for the detailed cytomorphological study of different lesions. The nuclear anomalies studied were karyorrhexis, karyolysis, chromatin clumping, nuclear buds, atypical mitoses and micronuclei. Feulgen stained smears were found to be superior to Papanicolaou stained smears in elucidating nuclear features.

Micronuclei are formed by chromosomal fragments that fail to be included in the nuclei during the process of cell division, as a result of genotoxic damage. Some studies showed an increase in micronuclei frequency in cells with aging and following cytotoxic drug therapy. Some other studies demonstrated an increased incidence of micronucleated cell in the buccal smears of persons with precancerous lesion of oral cavity and in high cancer risk groups. This study demonstrated an increase in micronucleated cells proportional to the increase in severity of the lesion that is from low grade lesion, to high grade lesions and invasive carcinoma. Eneida et al made a similar observation. The higher number of MNs observed in high grade lesions and invasive carcinoma suggests that progression to malignant transformation involves an increase in the frequency of chromosomal damage. So the presence of a high number of MNs in cervical intraepithelial neoplasia could be considered as a marker of a higher probability for disease progression.

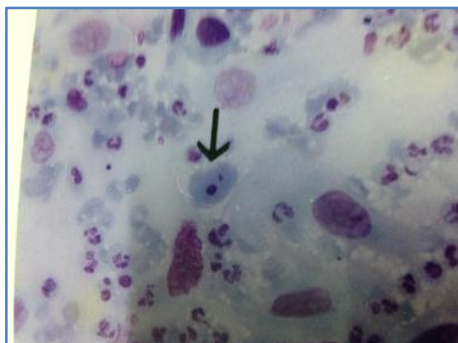
CONCLUSION: Invasive carcinoma of the cervix is the leading cancer in women in our country reflecting the inadequacy of our screening programmes and points to the need to strengthen our screening programmes. This study demonstrated the superiority of Feulgen stain over Papanicolaou stain in elucidating nuclear features and showed a consistent increase in

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micronucleated cells proportional to the increase in severity of the lesions. So MN analysis using Feulgen stained smears could be considered as a marker of a higher probability of disease progression.

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Feulgen stained Cervical smear showing micronucleus

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