Section: Pathology

Original Research Article

FREQUENCY OF MICRONUCLEI IN EXFOLIATED SQUAMOUS EPITHELIAL CELLS IN SPECTRUM OF CERVICAL LESION: A CROSS-SECTIONAL STUDY IN A TERTIARY CARE CENTRE

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Received : 10/11/2023
Received in revised form : 03/01/2024
Accepted : 20/01/2024

Keywords:
Micronuclei, Cervical smear, Bethesda categories, MN score.

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DOI: 10.47009/jamp.2024.6.1.320

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2024; 6 (1); 1614-1617

Abstract

Background: Micronuclei are round or oval aberrant cytoplasmic chromatin masses adjacent to the nucleus that can be observed under a light microscope. Micronuclei are formed during cell division from lagging chromosomes or chromosome fragments caused by mitotic errors or DNA damage. The identification of MN, an early marker of genetic damage, is a valuable tool. This study aimed to assess the frequency of micronuclei in squamous epithelial cells from cervical smears in various cytology categories of the current Bethesda classification system in the study population. **Materials and Methods:** A cross-sectional study evaluating 200 cervical smears stained with Papanicolaou staining was reviewed and categorised based on the Bethesda system. The frequency of micronuclei in each smear category was observed, and the mean micronuclei scores were calculated in various categories. Analysis of variance was performed among various categories to determine the significance of differences in micronuclei scores. **Result:** Mean of micronuclei score in NILM - 0.943±0.647, Inflammatory - 1.589±0.745, ASC–US - 2.062±0.742, ASC-H - 3.856±1.502, LSIL-4.27± 1.670, HSIL- 7.56±1.564 and invasive carcinoma-10.032 ±2.288. The ANOVA test showed a significant increase in the MN score between the SIL and IC groups. Among LSIL and HSIL, a significant increase in Mn score was observed. **Conclusion:** Our study showed significant variation in MN scores among the study population's inflammatory, preneoplastic, and neoplastic categories. Hence, the MN score can be used as a sensitive indicator for early detection of genomic damage.

INTRODUCTION

Cervical carcinoma is the fourth most frequent malignancy in women worldwide, and the most common histologic type is squamous cell carcinoma (80-90%), of which 76% occurs in countries with no screening programmes.[1] In India, the frequency of cervical carcinoma is 6-29%, and it is the third most common malignancy, with a mean age of 50.[2] The incidence has decreased in countries with effective screening programs. Dysplasia-carcinoma sequence progression has occurred over the past several years. Human papillomavirus is strongly associated with the progression of dysplasia to invasive carcinoma. HPV is associated with 90% of squamous cell carcinoma cases. Early screening and detection of premalignant lesions will help in early intervention and reduce mortality and morbidity. The Bethesda reporting system is currently recommended and used in reporting cervical smear cytology for both conventional and liquid-based cytology. The Bethesda system comprises three major categories: glandular cell abnormalities and a two-tier system for reporting intraepithelial lesions.

Micronuclei are extranuclear cytoplasmic bodies of damaged chromosomes and fragments not incorporated into the nucleus during cell division.[3] Earlier micronuclei were identified in red blood cells. Micronuclei screening is used as a measure of genotoxic substances.[4] Various Micronuclei screening and scoring studies have been conducted on exfoliated cells of the oral cavity mucosa, urothelial cells, cervix, and oesophageal mucosa. A linear relationship was observed between the dose and micronucleus induction in irradiated in vitro lymphocytes. Micronucleus was established as a

ISSN (O): 2687-5365; ISSN (P): 2753-6556

reliable method for assessing chromosomal damages caused by cytotoxic agents in vivo.[5] Doubling of frequency of MN was noted in cultured fibroblast when exposed to tobacco-specific nitrosamine (NNK), suggesting that smoking could induce MN in repair-deficient cells show clear evidence of rising MNI from no tobacco users to PMD (pre-cancer) to cancer in various stages.[6,7]

The identification of MN, an early marker for genetic damage, can be used as a valuable tool for tracking people or communities exposed to mutagenic and teratogenic agents.[8] Several papers have demonstrated the utility of MN screening of buccal mucosa in oral cancers and pre-cancers.[9] Micronuclei can be assessed using commercial stains like Papanicoloau, May Grunwald Giemsa, orcein, and feulgen. Papanicolaou staining is routinely used to screen cervical smears. This is a cost-effective and easy-to-use cytological stain.

Aim

This study aimed to assess the frequency of micronuclei in squamous epithelial cells of cervical smears in the study population's Bethesda classification system cytology categories. Micronuclei score indicates early genomic damage and is a supportive tool in detecting premalignant and malignant cervical neoplasm. To study the use of micronuclei, a score was used to differentiate neoplastic from inflammatory and NILM categories.

MATERIALS AND METHODS

Retrospectively, cervical smear slides of 200 cases were studied. Cervical smears obtained using the conventional method and stained using Papaniculou stain were studied. According to the Bethesda system for cervical smears 10, the slides were classified as NILM, inflammatory, ASCUS, ASH, LSIL, HSIL, or invasive squamous cell carcinoma.

Inclusion Criteria

All cervical smears of patients above 30 years with adequacy based on the recent Bethesda classification criteria were included.

Exclusion Criteria

Unsatisfactory slides with inadequate squamous cell counts were excluded.

In each slide, 1000 squamous epithelial cells were examined under 40x and 100x oil immersion. Micronucleated squamous cells were counted after 100x oil immersion (Figure 1). A score of one was assigned for squamous cells with either single or multiple micronuclei. In slides with micronuclei scores < 5, approximately 2000 squamous cells were counted.11 Micronuclei scoring in each slide was expressed as per 1000 squamous cells.

MN Criteria.[12]

The following criteria were used to evaluate micronuclei. The diameter of the micronucleus (MN) ranges from 1/16 to 1/3 of the mean diameter of the main nuclei, corresponding to 1/256 and 1/9 of the area of one of the main nuclei in a BN cell. MN are round, oval, and non-refractile, making identifying artefacts such as staining particles easy. The MN may touch but not overlap with the main nuclei, and the micronuclear boundary should be identifiable from the nuclear boundary. MN normally have the same intensity as the main nuclei; however, staining may be more intense on rare occasions. Keratohyaline bodies, nuclear karyorrhectic debris, and bacteria carefully examined interference.

Statistical Analysis

The number of micronucleated cells frequency per 1000 squamous epithelial cells was counted. Statistical analysis was performed, and statistical significance was determined by one-way analysis of variance (ANOVA) using SPSS version 27.

RESULTS

The age range in our study was 32–72 years, and the age range for SIL and invasive carcinoma was 50.5-58.5.

The macronuclei score of squamous cell carcinoma was significantly higher than those of normal and inflammatory cytology ($p<0.05$). Micronuclei score of SIL is higher significantly than normal and inflammatory ($p<0.05$). Squamous cell carcinoma and HSIL showed significantly higher rates than ASCUS and ASC-H. LSIL showed a significantly higher Micronuclei score than ASCUS ($p<0.05$). The ASCUS and ASC-H groups showed significantly higher inflammatory cytology than the normal group ($p<0.05$).

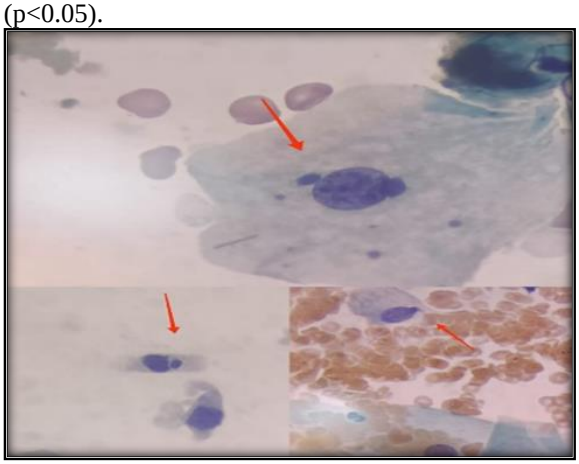

Figure 1: Micronuclei in squamous cells 100x oil Pap stain

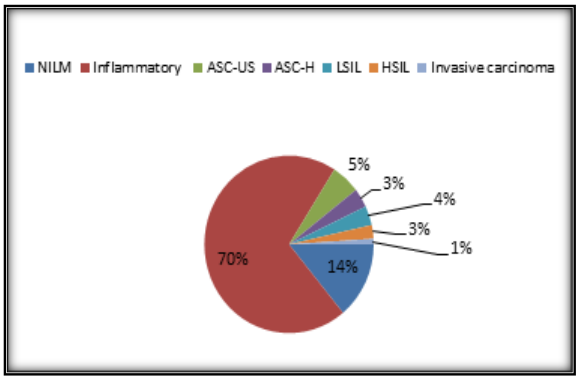

Figure 2: Distribution of carcinoma

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Table 1: Distribution of carcinoma according to the age and mean micronuclei score

	Age range	Number of cases	Micronuclei score mean ±SD
NILM	32- 71	28	0.943 ±0.647
Inflammatory	32- 68	140	1.589± 0.745
ASC-US	38- 65	11	2.062 ±0.742
ASC-H	35- 72	7	3.856 ±1.502
LSIL	46-68	7	4.27± 1.670
HSIL	42-58	5	7.56± 1.564
Invasive carcinoma	55-62	2	10.032 ±2.288

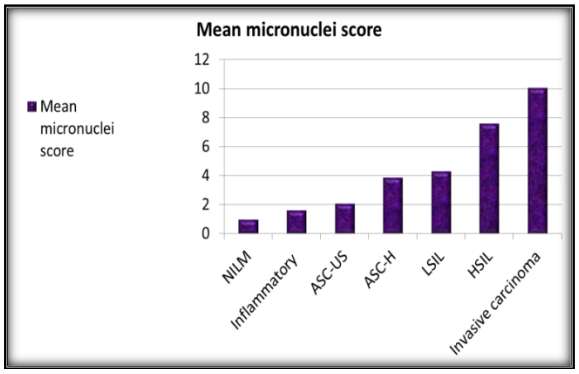

Figure 3: Mean micronuclei score in squamous epithelial cells of cervical smears for Bethesda categories

DISCUSSION

Micronuclei, a known marker for chromosomal damage, were significantly increased in the HSIL and invasive carcinoma groups. Previous studies have shown that micronuclei scores vary widely between the NILM and invasive carcinoma groups. In our study, the MN score of HSIL and invasive carcinoma was significantly higher than the NILM group, on par with a study by Aires et al. showing higher frequency of apoptosis and micronucleus observed frequency was $p<0.005$ in HSIL compared to inflammatory cervical smears.[13] Mahanta et al. have noted a significant difference between the MN score of NILM and ASCUS (1.9607) and HSIL (9.3931) categories, similar to our study result.[14]

Our study showed the highest MN score in invasive carcinoma, in contrast to the study by LSIL. HSIL has a significant difference in our study as contrary to Guzman et al.[15] Gayathriet al., a statistically significant difference was observed in MN score of LSIL (4.062) and ASC- H HSIL (8.032) as in our study.[16] Mahanta et al. have established a significant sequential increase in MN score from NILM to SIL and squamous cell carcinoma.[14] A study by Samanta et al. has shown a gradual uptrend of MN score from NILM (1.02) and inflammatory (2.87) smears to neoplastic smears and invasive carcinoma (18.50), as in our study.[17]

Bueno et al. proved that the MN score was significantly($p<0.0001$) higher in the precancerous and cancer group than the control group.[18] A micronuclei study by Sylvia et al. established a statistically significant increase in the frequency of micronuclei in benign and malignant breast neoplasms. Increased MN score is a biomarker for increased cancer risk.[19] Similar to our study, Micronuclei occurred with increased frequency in cases of dysplasia and malignancies as in a study by Haddad et al.[20] Similar results were noted in our study between NILM and neoplastic groups.

Tiwana et al. showed micronuclei scoring gradually an increasing frequency micronucleus with a comparatively lesser mean MN score in invasive carcinoma than our study results (10.032) mean MN score in invasive carcinoma.[21] Moya et al. have demonstrated an increased frequency of Micronuclei in cervical cytology and peripheral blood lymphocytes associated with HPV using comet assay studies.[22] Chromosomal instability can be easily monitored in the micronucleus test.[23] Micronucleus count possesses a high degree of sensitivity and specificity for identifying HSIL and invasive carcinoma.[24]

Further studies, including many cases with histopathological correlations, can establish micronuclei scores as an effective and early screening tool.

CONCLUSION

The early detection of chromosomal aberrations and genetic damage by assessing micronucleus scores can be helpful in early intervention, reducing mortality and morbidity. Micronucleus score increases from NILM to invasive carcinoma. We conclude that a standard scoring system for micronuclei should be established, and the micronuclei score can be used as an additional criterion along with The Bethesda system for screening cervical smears, aiding in periodic follow-up and treatment of patients.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556

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ISSN (O): 2687-5365; ISSN (P): 2753-6556