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Original Research Article

The Role of Micronucleus Scoring in Cervical Papanicolaou Smears

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Abstract

Aims and Objectives: 1) To evaluate micronucleus scoring in all major diagnostic categories as defined by "The Bethesda System for Reporting Cervical Cytology" 2014, including negative for intraepithelial lesions and malignancy (NILM), inflammatory, abnormal squamous cells of undetermined significance (ASC-US), abnormal squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL) and invasive carcinoma (IC) in cervical Pap smears. 2) To study the frequency and pattern of MN from NILM to invasive carcinoma (IC) categories in cervical Pap smears.

Materials and Methods: Pathologists independently assessed 1000 conventional cervical smears stained with Papanicolaou (Pap) stain, which included unsatisfactory for evaluation (93), NILM (154), inflammatory (673), ASC-US (25), ASC-H (19), LSIL (15), HSIL (14) and IC (7). The MN score per 1000 cells was determined by counting the number of MN cells in high-power (×400) and oil immersion (×1000).

Results: The mean MN score ± standard deviation was found to be 0.99 ± 0.744 in NILM cases, 0.67 ± 0.782 in inflammatory cases, 1.57 ± 0.507 in ASC-US cases, 1.63 ± 0.50 in ASC-H cases, 1.56 ± 0.511 in LSIL cases, 2.47 ± 0.516 in HSIL cases and 3.0 ± 0.00 in IC cases. A step-wise increase was observed in MN score from inflammatory to IC categories.

Conclusions: MN score is a reliable and easy test that can be used in conjunction with routine cervical PAP to assess the risk of malignant transformation in the uterine cervix as a biomarker for predicting the risk of carcinoma.

Keywords: Cervical Smear, Micronucleus, Screening.
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Introduction

According to the WHO Global Cancer Observatory, cervical cancer is the fourth most common malignancy in women with 604,127 new cases and 341,831 deaths reported worldwide in 2020. India alone accounts for 123,907 of these cases and nearly one-third of global cervical cancer–related deaths with a cumulative incidence risk of 2.01% and a cumulative mortality risk of 1.30%.[1,2,3] Cervical cancer has an excellent prognosis when identified early through efficient screening and diagnostic methods.[4]

Micronuclei (MNs) were initially described as Howell–Jolly bodies in erythroid precursors and later observed in lymphocytes, exfoliated buccal cells and cervicovaginal epithelial cells.[5,6] Micronuclei (MNs) are small, nuclear protrusion, extra nuclear bodies which are formed during mitosis, chromosomal fragments or whole chromosomes are not incorporated into the main daughter nuclei. [16] MN analysis in exfoliated cells has been applied as a screening tool for cancers of the oral cavity, urinary bladder, cervix, and esophagus. The frequency of MN is significantly higher in IC than NILM.

The present study assesses MN frequency across the full range of diagnostic categories in cervical Papanicolaou (PAP) smears based on the 2014 Bethesda System for Reporting Cervical Cytology. [7]

Materials and Methods

The present study included 1,000 conventional cervical smears obtained as part of routine screening from the Department of Obstetrics and Gynecology. All smears were stained using the Papanicolaou (PAP) method and examined

microscopically. Each smear was categorized into one of the following diagnostic groups: unsatisfactory for evaluation (93), negative for intraepithelial lesion or malignancy (NILM) (154), inflammatory (673), atypical squamous cells of undetermined significance (ASC-US) (25), atypical squamous cells—cannot exclude HSIL (ASC-H) (19), low-grade squamous intraepithelial lesion (LSIL) (15), high-grade squamous intraepithelial lesion (HSIL) (14) and invasive carcinoma (IC) (7).

Micronucleus (MN) scoring was performed independently by pathologists. For each case, MNs were counted in 1,000 epithelial cells observed under high-power magnification (×400), and their presence was confirmed using oil immersion (×1000). All smears were systematically screened using the zig-zag technique.

Inclusion and exclusion criteria

Inclusion Criteria

1. Patients who have been diagnosed/ histopathological confirmed cases.
2. Patients who have not undergo chemotherapy or radiotherapy prior to PAP smear formation.
3. Clumps of cells with obscured nuclear, cytoplasmic boundaries and overlapping of cells will be separated and cells lying singly will be preferred for counting of MN.

Exclusion Criteria

1. Pregnant women or women in the immediate postpartum period, due to hormonal and cellular alterations. 2. Patients with known exposure to genotoxic agents or occupational hazards. 3. Degenerated cells, apoptotic cells and cytoplasmic fragments will be exempted from counting and scoring.

Criteria for identifying micronucleus [17]

• MN diameter less than one-third of the main nucleus diameter. • The shape, colour and texture of MN similar to those of nucleus. • Staining intensity similar to or slightly weaker than that of the nucleus. • MN round to oval in shape having close proximity but no actual contact with the nucleus. • Plane of focus same as that of the main nuclei. • Cells with double or multiple MNs were given a score of 1 and the number of MN cells in each case was expressed per 1000 cells (MN score).

The results obtained were statistically evaluated using the IBM SPSS 27 Statistics software.

Results

The mean age of the patients in unsatisfactory for evaluation, NILM, inflammatory, ASC-US, ASC-H, LSIL, HSIL and IC categories is shown in Table 1.

Table 1: Age distribution of cases taken for micronucleus scoring

Groups	Number of cases	Age range (years)	Mean age (years)
Unsatisfactory	93	36-81	42
NILM	154	23-34	27.4
Inflammatory	673	19-43	28.5
ASC-US	25	25-48	31.7
ASC-H	19	25-38	30.5
LSIL	15	22-41	27.2
HSIL	14	20-48	31.3
IC	7	30-64	44.4
Total	1000		

NILM=Negative for intraepithelial lesions and malignancy, LSIL=Low-grade squamous intraepithelial lesion, IC=Invasive carcinoma, ASC-US=Abnormal squamous cells of undetermined significance, ASC-H=Abnormal squamous cells cannot exclude HSIL and HSIL=High-grade squamous intraepithelial lesion.

The mean age was more in the IC category as compared to other categories accept unsatisfactory for evaluation category. Biopsy follow-up obtained in various cases is shown in Table 2.

Table 2: Biopsy outcome

Unsatisfactory	Nil	Nil
NILM	Nil	Nil
Inflammatory	Nil	Nil
ASC-US	Nil	Nil
ASC-H	10	6 (moderate-to-severe dysplasia), 4 (chronic cervicitis), 5 (moderate-to-severe dysplasia)
LSIL	12	5 (chronic cervicitis), 7 (moderate-to-severe dysplasia)
HSIL	16	CINI II/CIN III
IC	4	Squamous cell carcinoma

NILM=Negative for intraepithelial lesions and malignancy, LSIL=Low-grade squamous intraepithelial lesion, IC=Invasive carcinoma, CIN=Cervical intraepithelial neoplasia, ASC-US=Abnormal squamous cells of undetermined significance, ASC-H=Abnormal squamous cells cannot exclude HSIL and HSIL=High-grade squamous intraepithelial lesion.

We received biopsy specimens for 10 cases of ASC-H, 12 cases of LSIL and all the cases of HSIL and IC for histopathological correlation. The biopsy was not available in unsatisfactory, NILM, inflammatory and ASC-US categories. Of the ten available biopsies of ASC-H cases - six showed moderate-to-severe dysplasia and four showed chronic cervicitis ; out of the twelve LSIL biopsies - five showed chronic cervicitis and seven showed moderate-to-severe dysplasia ; while out of 20 HSIL and IC biopsies - all showed either a cervical intraepithelial neoplasia (CIN) II/III and invasive squamous cell carcinoma . Thus a complete concordance was found between cytological and histological findings in the HSIL and IC cases. The mean MN score in various cervical lesions is shown in Table 3.

Table 3: Mean micronucleus score in cervical lesions

Group	MN score±SD
Unsatisfactory	0.00±0.00
NILM	0.98±0.746
Inflammatory	0.63±0.780
ASC-US	1.59±0.509
ASC-H	1.60±0.48
LSIL	1.58±0.509
HSIL	2.50±0.513
IC	2.99±0.00

NILM=Negative for intraepithelial lesions and malignancy, LSIL=Low-grade squamous intraepithelial lesion, MN=Micronucleus, SD=Standard deviation, IC=Invasive carcinoma, ASC-US=Abnormal squamous cells of undetermined significance, ASC-H=Abnormal squamous cells cannot exclude HSIL and HSIL=High- grade squamous intraepithelial lesion.

The number of MN cells per 1000 epithelial cells was counted under oil immersion magnification by observer independently. The mean MN score ± standard deviation was found to be 0.98±0.746 in NILM cases, 0.63±0.780 in inflammatory cases ,1.59±0.509 in ASC-US cases , 1.60±0.48 in ASC-H cases , 1.58±0.509 in LSIL cases, 2.50±0.513 in HSIL cases and 2.99±0.00 in IC cases . A step-wise increase was observed in MN score from inflammatory to IC categories.

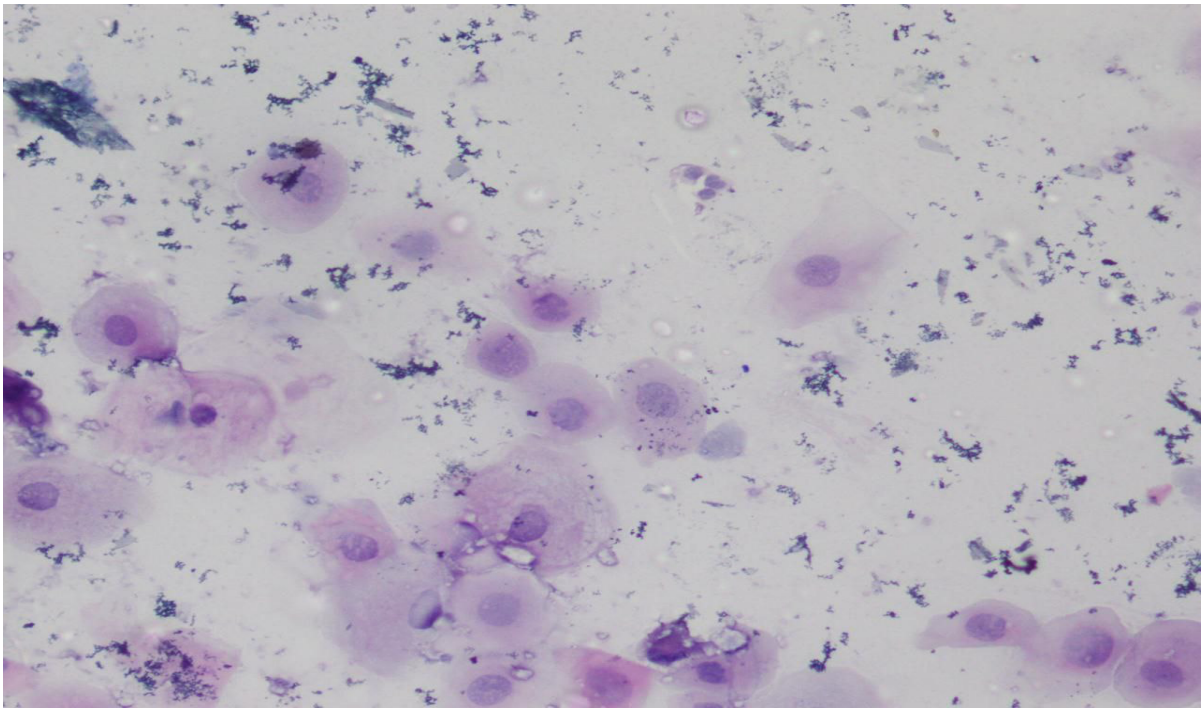

Figure 1: Photomicrographs showing ASCUS.

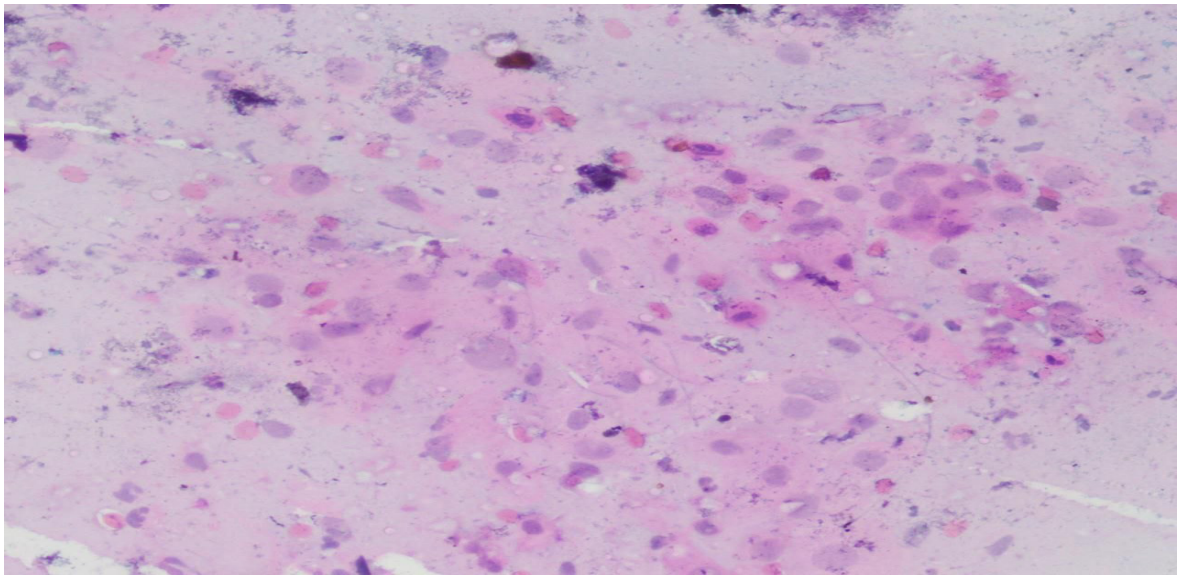

Figure 2: Photomicrographs showing Candida.

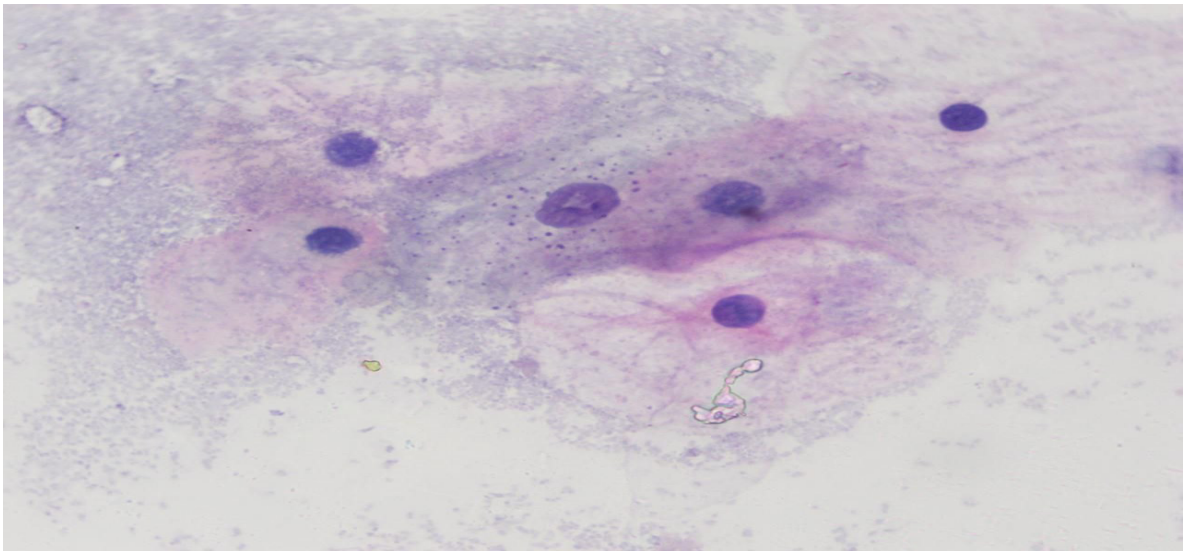

Figure 3: Photomicrographs showing Bacterial vaginosis.

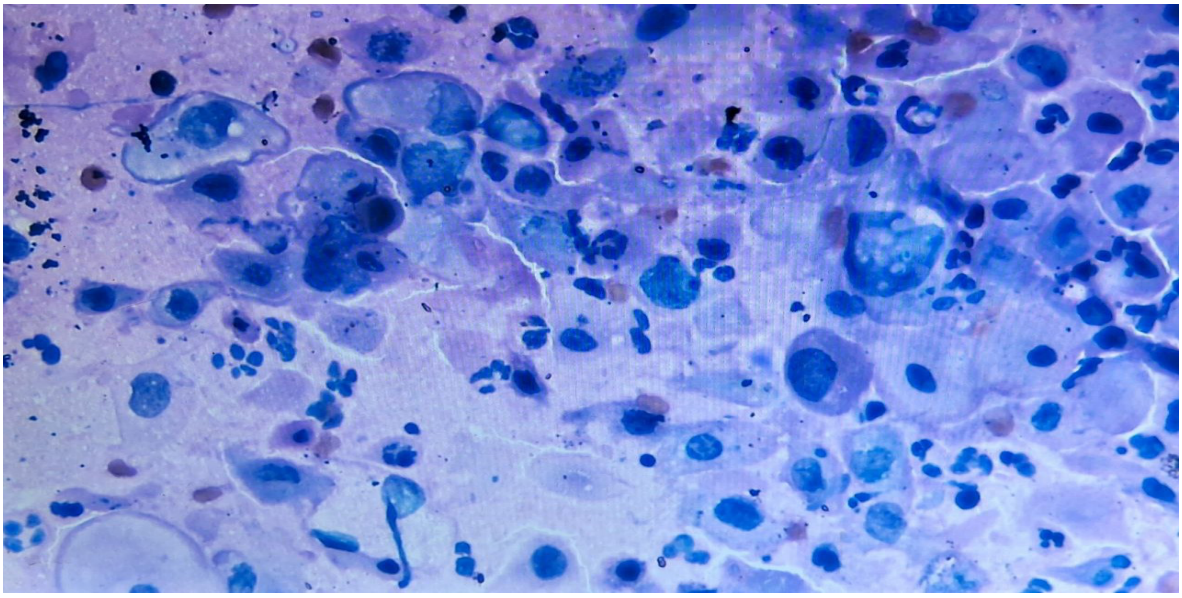

Figure 4: ASC-H.

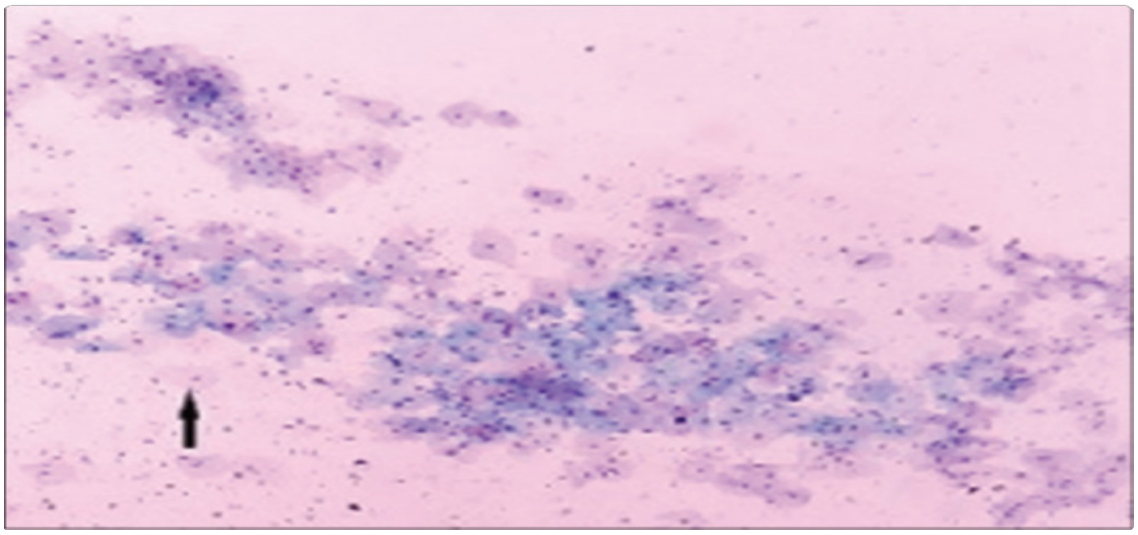

Figure 5: Photomicrographs showing micronucleus (arrow) in negative for intraepithelial lesions and malignancy (Papanicolaou stain, × 100).

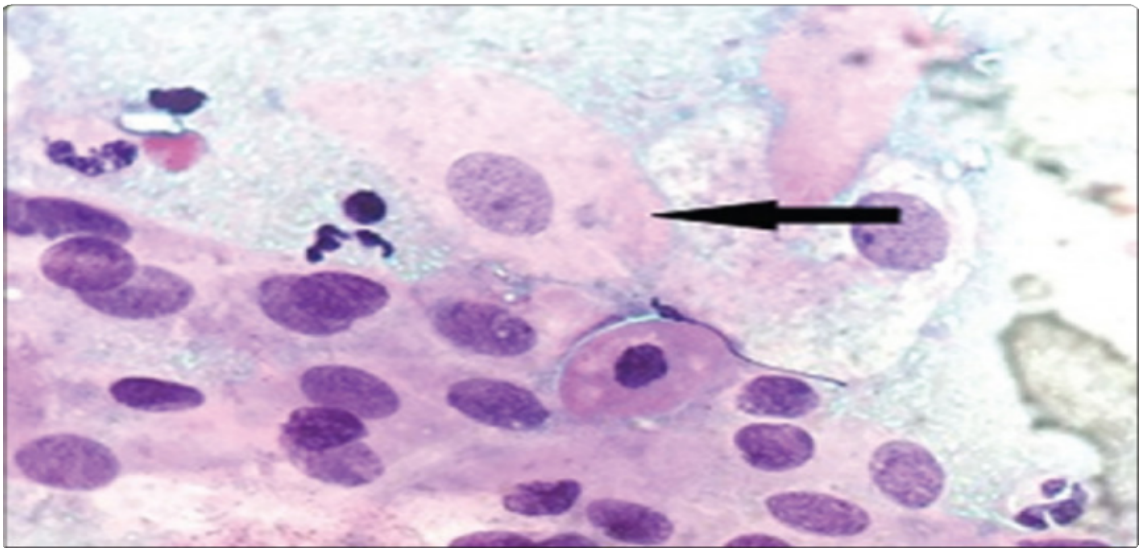

Figure 6: Photomicrographs showing micronucleus (arrow) in abnormal squamous cells cannot exclude high-grade squamous intraepithelial lesion (Papanicolaou stain, ×400).

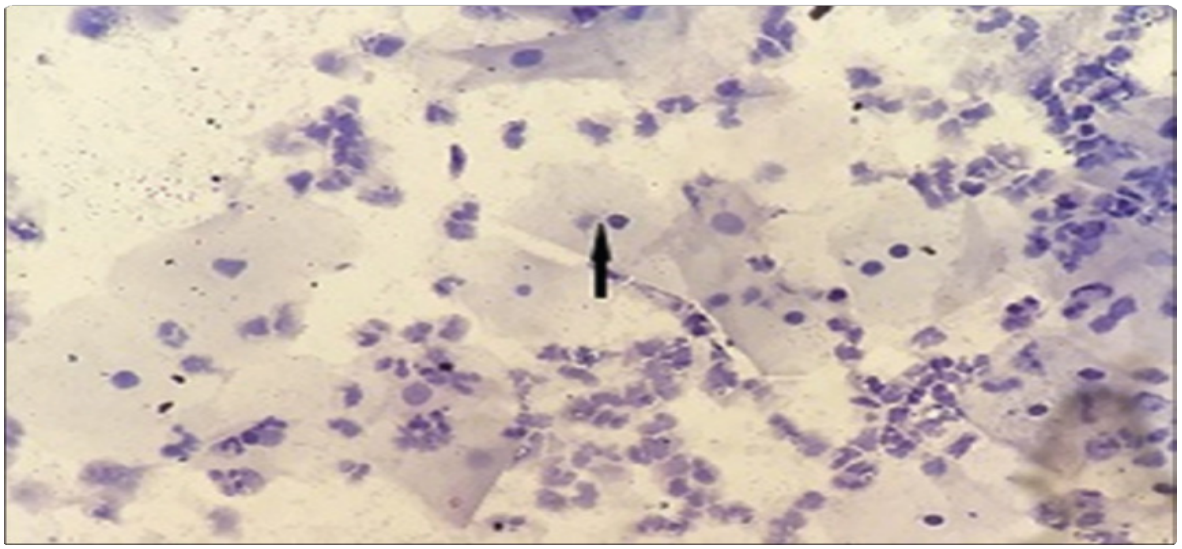

Figure 7: Photomicrographs showing micronucleus (arrow) in candidiasis (Papanicolaou stain, ×400).

One-way analysis of variance (ANOVA) was applied to analyze the significance of variance in mean MN scores among different groups, as shown in Table 4.

Table 4: Result of analysis of variance (P value) (post hoc test)

	Unsatisfactory	NILM	Inflammatory	ASC-US	ASC-H	LSIL
Unsatisfactory		0.000	0.000	0.000	0.000	0.000
NILM	0.000		0.000	0.013	0.025	0.039
Inflammatory	0.000	0.000		0.000	0.000	0.000
ASC-US	0.000	0.013	0.000		1.000	1.000
ASC-H	0.000	0.020	0.000	1.000		1.000
LSIL	0.000	0.039	0.000	1.000	1.000	
HSIL	0.000	0.000	0.000	0.007	0.025	0.006
IC	0.000	0.000	0.000	0.000	0.004	0.001

P value is significant if ≥0.05. NILM=Negative for intraepithelial lesions and malignancy, LSIL=Low-grade squamous intraepithelial lesion, IC=Invasive carcinoma, ASC-US=Abnormal squamous cells of undetermined significance, ASC- H=Abnormal squamous cells cannot exclude HSIL and HSIL=High-grade squamous intraepithelial lesion. Analysis of MN score obtained by ANOVA in various categories [Table 4] revealed as follows [Figure 8]:

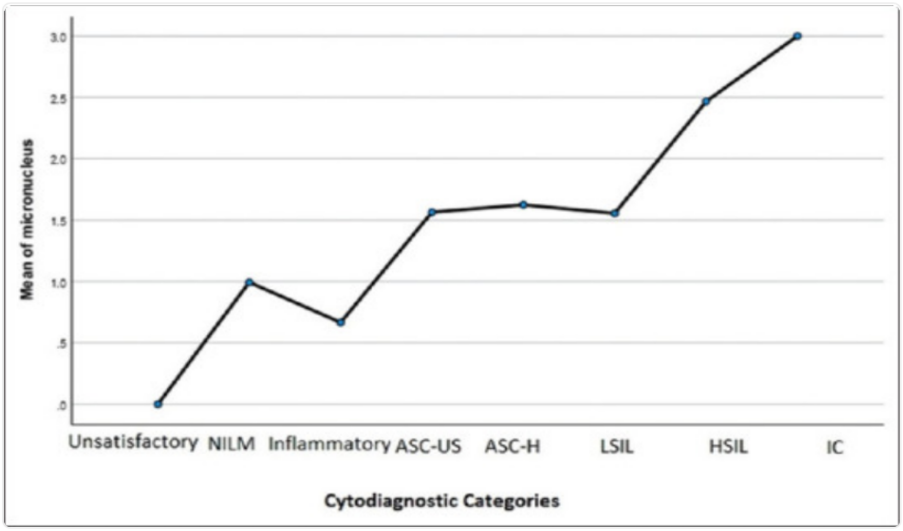

Figure 8:

Trend of micronucleus score in various cytodiagnostic categories. NILM = Negative for intraepithelial lesions and malignancy, LSIL = Low-grade squamous intraepithelial lesion, IC = Invasive carcinoma, ASC-US = Abnormal squamous cells of undetermined significance, ASC-H = Abnormal squamous cells cannot exclude HSIL and HSIL = High-grade squamous intraepithelial lesion.

1. The MN score was significantly higher in IC compared to the other categories (P = 0.000), except HSIL (P = 0.794).
2. HSIL showed significant difference with the other categories (P = 0.000), except IC (P = 0.794).
3. The MN score of LSIL was significantly different from other categories (P = 0.000) except ASC-US and ASC-H (P = 1.000).
4. The MN score of ASC-H was significantly different from other categories (P = 0.000) except ASC-US and LSIL (P = 1.000).
5. The difference in MN score between ASC-US and other categories was significant (P = 0.000) except ASC-H and LSIL (P = 1.000).
6. The difference of MN scores between that of NILM and inflammatory was significant (P = 0.000).

Discussion

Cervical cancer is a leading cause of death among women—particularly in developing nations—the number of cases continues to grow despite existing screening options.

To improve early detection, researchers are focusing on micronuclei (MNs) as critical biological indicators.

MNs are tiny fragments of genetic material that sit outside the main nucleus of a cell. They form when

chromosomes or fragments fail to integrate properly during cell division. Because these structures directly reflect genetic damage and chromosomal instability, an increase in MN frequency often appears long before clinical symptoms of cancer. Consequently, monitoring these biomarkers provides a powerful way to identify cancer risks and detect the disease in its earliest stages.

In the current research, micronucleus (MN) scoring was applied across the full spectrum of the 2014 Bethesda System for cervical cytology. The results indicated that MN scores were significantly higher in cases of high-grade squamous intraepithelial lesions (HSIL) and invasive carcinoma (IC) than in other diagnostic groups. Furthermore, a steady, incremental rise in MN frequency was observed as lesions progressed from NILM to IC, reinforcing the concept that MNs are key indicators of cervical epithelial disease progression.

Aires et al. (2011) used Chi-square testing on 59 smears to demonstrate that HSIL exhibited significantly higher MN counts than LSIL or inflammatory samples.[13] The present study reached comparable conclusions using ANOVA.

Samanta et al. (2011) observed a rise in MN scores from NILM to HSIL but a minor drop in IC cases, the current research showed a continuous upward trajectory through to IC.[14] This consistent increase mirrors more recent work by Mahanta et al. (2020), whose study of 106 cases also confirmed a sequential, significant rise in MN scores across the Bethesda categories.[15]

Gayathri et al. (2012) also reported a gradual elevation in MN scores from NILM to IC, specifically noting a highly significant gap between LSIL and HSIL ($P = 0.000$), a finding corroborated by the current study's own results ($P = 0.008$).[11] Bueno et al. (2014) quantified this trend, showing mean MN counts increasing significantly alongside the severity of CIN lesions and cancer ($P < 0.001$), which matches the progressive rise observed in this research.[12]

Tiwana et al. (2022), in a one-year study of 1000 conventional cervical Pap smears, demonstrated a progressive and statistically significant increase in mean MN scores from inflammatory and NILM categories through ASC-US, ASC-H, LSIL and HSIL, with the highest values observed in invasive carcinoma (IC).[18] Their findings support the role of micronucleus scoring as a simple and reliable adjunct to routine cervical cytology for assessing the risk of malignant transformation. Despite the clear diagnostic benefits, MN scoring is hindered by practical obstacles such as the presence of nuclear debris, bacterial colonies and staining artifacts that can interfere with accuracy. The manual process is also labor-intensive and slow. Nevertheless, implementing modern solutions—such as automated image analysis, liquid-based cytology, and DNA-specific staining—could mitigate these issues, making MN scoring a more reliable and economical biomarker for assessing cervical cancer risk.

Conclusions

In cervical Pap smears, the current study shows a distinct, progressive increase in micronucleus (MN) frequency from NILM to invasive carcinoma (IC) categories. MN assessment is a straightforward, reliable method that might be a useful supplement to normal Pap smear screening for determining cervical cancer risk, despite the fact that it is rather labor-intensive and time-consuming.

MN are small, nuclear protrusion, extranuclear bodies which are formed during mitosis, chromosomal fragments or whole chromosomes are not incorporated into the main daughter nuclei. [16]

They developed biomarkers of chromosomal instability and genotoxic damage that represent catastrophic mitosis, chromosomal breakage, cell deaths during mitosis, and pandemonium in the genome.[10]

MN assessment is a useful method for early cancer screening and risk prediction since an increase in MN frequency has been seen in tissues exposed to carcinogens long before clinical manifestations appear.

Ethical Consideration

Ethical clearance will be obtained from the institutional ethics committee before undertaking the study. Written informed consent will be taken from all patients included in the study.

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