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Original Research Article

MICRONUCLEUS SCORING AS A PROGNOSTIC INDICATOR AND A BIOMARKER OF GENETIC DAMAGE IN CERVICAL LESIONS ON PAP SMEAR

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ABSTRACT

Background: Cervical cancer ranks fourth among women worldwide. The most popular and economical way to screen for cervical cancer is with Pap smears. Micronuclei (MN) are small nuclei formed by chromosomal fragmentation or chromosomal loss. MN frequency has been linked to genomic instability and cancer risk. **Objective**: To evaluate prognostic value of micronucleus, count in cervical lesions found on pap smears.

Materials and Methods: In this retrospective study, we compared the micronucleus score over a two-year period, from August 2022 to August 2024, in seven groups of cervical lesions: normal (526), inflammatory (335), ASC-US (121), ASC-H (2), LSIL (10) and HSIL (6). Under oil immersion magnification, two observers independently counted the number of micronucleated cells per 1000 epithelial cells, which was then expressed as an MN score per 1000 cells.

Results: The number of micronuclei increased progressively from the inflammatory to ASC-US to ASC-H to LSIL and HSIL. The mean MN scores ±SD for cervical lesions in the following cases: normal (2.57±1.38), inflammatory (8.21±1.26), ASC-US (10±2.83), ASC-H (13±2.83), LSIL (15.8±1.44) and HSIL (20.33±1.63). Statistical significance was achieved with a p-value of less than 0.0001.

Conclusion: MN counting is a useful prognostic indicator, which is easy, reliable, simple, objective test performed on routinely stained Pap smear.

Key words: Pap smear, Micronucleus, Cervical lesions.

INTRODUCTION

Micronuclei (MN) are small, extranuclear structures that emerge from chromosomal fragments or entire chromosomes that fail to integrate into the daughter nuclei during cell division. Their presence reflects underlying genotoxic stress or mitotic malfunction, serving as a tangible sign of chromosomal instability—a phenomenon at the core of many pathological conditions, including cancer. Due to their sensitivity and specificity, MN are frequently utilized as biomarkers to assess genetic damage in a wide range of biological and environmental studies.[1] They can be easily observed in exfoliated cells using simple cytological techniques, making them particularly valuable in large-scale screening programs aimed at early detection of genomic alterations.[2]

Cervical cancer is among the most common malignancies affecting women worldwide, particularly in low- and middle-income countries. It is predominantly caused by persistent infection with high-risk types of human papillomavirus (HPV), which induces DNA damage and disrupts the regulation of cell proliferation and apoptosis.[3.4]

The Papanicolaou (PAP) smear has been a cornerstone in the early detection and prevention of cervical cancer for decades, allowing the identification of precancerous changes in cervical epithelial cells before progression to invasive disease. Its value lies in its simplicity, cost-effectiveness, and ability to drastically reduce cervical cancer incidence when used in organized screening programs.[5]

Incorporating micronucleus scoring into PAP smear analysis enhances the diagnostic and prognostic power of conventional cytology. The presence and

frequency of MN in cervical exfoliated cells correlate with the severity of epithelial abnormalities, ranging from inflammation and low-grade dysplasia to high-grade lesions and carcinoma.[6] By quantifying MN, clinicians can gain additional insights into the extent of genomic instability within the cervix, which may not be fully captured by cytological morphology alone. This is especially helpful in ambiguous or borderline cases where traditional PAP results may be inconclusive[7,8]

Micronucleus analysis represents a valuable adjunct to cervical cytology, offering both diagnostic precision and prognostic insight. Its integration into PAP smear interpretation provides a more comprehensive picture of cervical cellular health, particularly in settings where access to advanced molecular diagnostics may be limited.[9] As we move toward more personalized and predictive models of care, leveraging micronucleus (MN) scoring in cervical screening much like its demonstrated utility in distinguishing malignant from benign effusions based on chromosomal instability markers—may not only improve early detection but also guide tailored monitoring and intervention strategies for women at risk.[10] Objectives of our study include assessing the prognostic significance of micronucleus (MN) counts in cervical epithelial lesions identified through Pap smear cytology.

MATERIALS AND METHODS

A retrospective study was conducted in the Cytopathology department of Karwar Institute of Medical Sciences in Karwar involving of 1000 Pap smear samples, including Normal (526), Inflammatory (335), ASC-US (121), ASC-H (2), LSIL (10) and HSIL (6). from August 2022 to August 2024. The slides with cell material were treated with Papanicolaou stain. The findings were evaluated based on the Micronuclei Scoring method for every 1,000 Exfoliated Squamous cells examined under a light microscope.

MN scoring: Two observer separately counted the number of micro nucleated cells on pap stain per 1000 epithelial cells in oil immersion magnification in a zig-zag method and were expressed as MN score per 1000 cells.

Criteria used for identification of micronucleus were

a) Regular rounded structure with perimeter suggestive of a membrane located within inner half of the cytoplasm.
b) Diameter variable from 1/16 to 1/3 the diameter of the main nucleus without any overlap or a bridge to the nucleus.
c) Staining intensity and texture similar to that of the nucleus and in same focal plane as nucleus.

Inclusion Criteria

- Squamous cells lying singly will be preferred for counting of MN.
- Squamous cells not obscured by blood, inflammatory cells, debris and organisms.

Exclusion Criteria

- Clumps of cells with obscured nuclear or cytoplasmic boundaries and overlapping cells were avoided.
- Degenerated cells, cell covered with debris, mucus, WBC, bacteria and RBC were exempted from counting and scoring.
- Anucleate squames, endocervical and endometrial cells were avoided.

Inadequate and faded slides were excluded

Statistical Analysis: Data was entered in excel sheets of both observers. Average score was taken for both observers and analysed using. further One-way analysis of variance (ANOVA) was employed to assess the statistical significance of differences in micronucleus (MN) scores across seven histopathologically defined categories of cervical lesions.

RESULTS

In this retrospective analysis of 1000 Pap smear samples spanning August 2022 to August 2024, micronucleus (MN) scoring was utilized to assess genomic damage across a spectrum of cervical lesions. The cases were stratified into six diagnostic categories: normal, inflammatory, ASC-US (Atypical Squamous Cells of Undetermined Significance), ASC-H (Atypical Squamous Cells—cannot exclude HSIL), LSIL (Low-grade Squamous Intraepithelial Lesion), and HSIL (High-grade Squamous Intraepithelial Lesion).

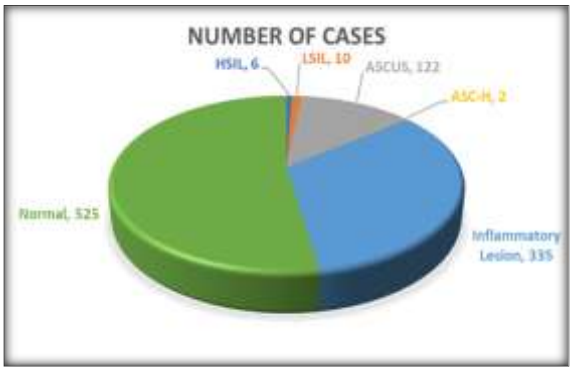

Figure 1: Distribution of cervical lesion

The MN scores showed a clear, statistically significant upward trend correlating with lesion severity. Specifically, the mean MN scores were lowest in normal cytology (2.57 ± 1.38) and progressively increased through inflammatory (8.21 ± 1.26), ASC-US (10 ± 2.83), ASC-H (13 ± 2.83), LSIL (15.8 ± 1.44), reaching the highest values in HSIL cases (20.33 ± 1.63).

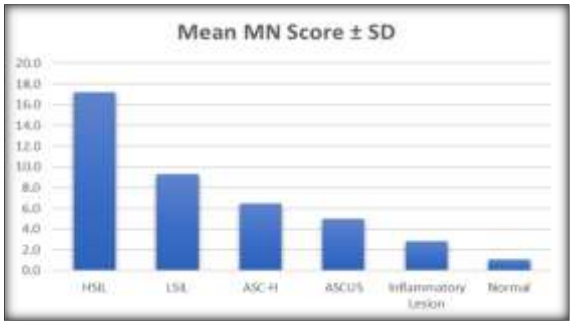

Figure 2: Relation between MN score and cervical lesions

One-way ANOVA revealed a highly significant difference between these groups ($F=155.603$, $p<0.001$).

Further analysis using the Games-Howell post hoc test confirmed significant pairwise differences between nearly all groups, particularly between HSIL and the rest, underscoring the strong association between MN frequency and epithelial atypia. These findings validate micronucleus scoring as a quantitative and reproducible marker of genomic instability, enhancing the diagnostic depth of routine Pap smear evaluation and providing potential prognostic utility in cervical cytology.

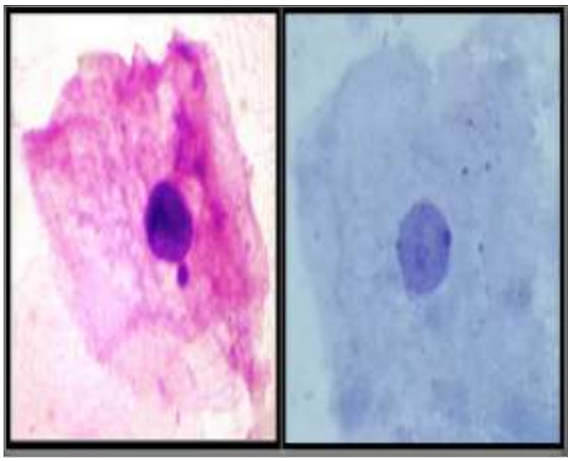

Figure 3: Micronuclei in squamous cells 100x oil, Pap stain

Table 1: Comparison between groups

ANOVA					
MN Score	**Sum of Squares**	**df**	**Mean Square**	**F**	**p-value**
Between Groups	643.488	2	321.744	155.603	< 0.001*
Within Groups	76.506	37	2.068		
Total	719.994	39			

Significant difference

ANOVA with p< 0.001* indicated that, there was significant difference between the groups and hence, Games-Howell post-hoc was carried out for making pairwise comparisons.

Table 2: Games-Howell post hoc test for multiple comparisons

Multiple Comparisons						
Category		**Mean Difference**	**Std. Error**	**P-value**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
HSIL	LSIL	7.9	1.3	0.008*	2.6	13.2
HSIL	ASCUS	12.2	1.3	0.001*	6.8	17.6
HSIL	ASC-H	10.7	1.4	0.002*	5.2	16.1
HSIL	Inflammatory Lesion	14.4	1.3	0.001*	8.9	19.8
HSIL	Normal	16.1	1.3	0.0001*	10.6	21.5
LSIL	ASCUS	4.3	0.4	< 0.001*	2.8	5.8
LSIL	ASC-H	2.8	0.7	0.116	-1.1	6.7
LSIL	Inflammatory Lesion	6.5	0.4	< 0.001*	5	8
LSIL	Normal	8.2	0.4	< 0.001*	6.7	9.7
ASCUS	Inflammatory Lesion	2.2	0.1	< 0.001*	1.9	2.4
ASCUS	Normal	3.9	0.1	< 0.001*	3.6	4.1
ASC-H	Normal	5.4	0.5	0.131	-8.8	19.7
Inflammatory Lesion	Normal	1.7	0	< 0.001*	1.6	1.8
*The mean difference is significant at the 0.05 level.						

DISCUSSION

MN has generally been used as a biomarker of chromosomal damage, genome Instability and cancer risk.[11] The micronucleus test on exfoliated cells has been successfully used to screen population groups at risk for cancers of oral cavity, urinary bladder, cervix and oesophagus. Micronucleus assay in buccal cells serves as a sensitive, non-invasive biomarker for monitoring genomic damage linked to environmental exposures, lifestyle factors, and cancer risk.[12] The analysis of data from the present study has revealed an elevated MN score in cervix smears of NILM to Inflammatory smear to ASC-U to ASC-H to LSIL to HSIL.(FIGURE 2)

The mean MN scores in our study (normal: 2.57±1.38; inflammatory: 8.21±1.26; ASC-US: 10±2.83; ASC-H: 13±2.83; LSIL: 15.8±1.44; HSIL: 20.33±1.63) were consistent with previously

published studies. Navya BN et al. reported a similar increasing trend, with MN counts rising proportionately with the severity of cervical epithelial abnormalities, reinforcing MN's role as a reliable marker of chromosomal instability. Our statistical analysis using one-way ANOVA and subsequent Games-Howell post hoc tests further emphasized the significance of these differences ($p < 0.001$), especially between HSIL and all other diagnostic categories.

Navya BN et al. study showed a clear, progressive elevation in micronucleus (MN) scores corresponding to the severity of cervical epithelial lesions. The MN count ranged from 2.57 ± 1.38 in normal cytology to 8.21 ± 1.26 in inflammatory smears, and continued rising through ASC-US (10 ± 2.83), ASC-H (13 ± 2.83), LSIL (15.8 ± 1.44), HSIL (20.33 ± 1.63), culminating in the highest count seen in invasive carcinoma (23 ± 2.83). These findings highlight MN scoring as a reliable and quantifiable marker of chromosomal instability that correlates strongly with cytological atypia. The study validates the integration of MN analysis in routine Pap smear interpretation as a supplemental diagnostic tool.[6]

Bhat et al.'s research examined both MN scoring and apoptotic cell frequency in cervical smears. Their findings reinforced the premise that genomic instability, as evidenced by increased MN count, is paralleled by elevated rates of apoptosis in dysplastic lesions. Statistically significant correlations were observed between lesion severity and both parameters, suggesting that combining MN and apoptosis analyses offers enhanced sensitivity in detecting early cellular transformation. The dual-parameter approach may be particularly beneficial in ambiguous or borderline cases where morphological assessment alone is insufficient.[13]

Gandhi G et al. this study further expanded the scope of cytogenetic screening by simultaneously evaluating MN formation and apoptotic cell counts in exfoliated cervical cells. Gandhi G et al. reported a concurrent rise in both indicators as lesions progressed from benign to malignant stages. Their research emphasized the diagnostic synergy of MN and apoptosis, proposing that their combined assessment yields more robust insights into the extent of genotoxic damage and disease progression. Particularly in low-resource settings, this method provides a feasible and cost-effective alternative to molecular diagnostics.[14]

Sujatha Kanetkar et al. study conducted at the Krishna Institute of Medical Sciences in Karad, Maharashtra, this study evaluated the utility of micronucleus (MN) scoring in cervical Pap smears over the course of one year. Analysing 406 samples, the researchers observed a statistically significant variation in MN scores across different grades of cervical lesions ($p < 0.05$). The results revealed a gradual and consistent increase in MN frequency from normal cytology through inflammatory changes to dysplastic lesions. This reinforced the hypothesis that MN formation parallels chromosomal damage and cellular atypia, supporting its role as a reliable cytogenetic biomarker for early detection and progression monitoring in cervical pathology.[15]

Ambroise MM et al. In a four-year study conducted at Pondicherry Institute of Medical Sciences (PIMS), Puducherry, Ambroise MM et al. explored the predictive value of MN and binucleated cell counts in cervical intraepithelial neoplasia and carcinoma. Analysing 132 samples, they performed Receiver Operating Characteristic (ROC) curve analysis and found that MN counts exhibited high sensitivity and specificity for identifying high-grade squamous intraepithelial lesions (HSIL) and invasive carcinoma. Additionally, the study demonstrated a positive correlation between MN score and apoptosis, indicating that both biomarkers reflect underlying genomic instability. The inclusion of binucleated cell count was shown to enhance the predictive value of MN assessment, making it a powerful and practical biomarker for routine screening in cervical cancer diagnostics.[16]

In relation to earlier studies, my study can demonstrates a clear correlation between increasing MN frequency and the severity of cervical lesions across a wide diagnostic spectrum, our findings support the integration of MN assessment into routine Pap smear analysis. This is particularly important in low-resource settings, where it can serve as a valuable adjunct to the Bethesda system, aiding early detection, patient stratification, and follow-up without the need for advanced molecular testing.

CONCLUSION

Micronucleus scoring provides a reliable, and simple method for assessing genetic damage in cervical lesions. Micronucleus score increases from NILM to invasive carcinoma. We can conclude that a standard scoring system for micronuclei can 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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