Utility of Micronucleus Study on Breast Cytology Smears

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ABSTRACT

Background

Micronuclei (MN) are extranuclear chromatin bodies that arise due to chromosomal breakage and serve as biomarkers of genomic instability. Their presence has been linked to malignant transformation. Breast cytology offers a minimally invasive method for early detection of breast lesions. Evaluating micronuclei in these smears may enhance diagnostic accuracy by providing an additional objective parameter.

Objective

To compare the MN score in benign and malignant epithelial neoplasms of the breast as well as compare it within different grades of breast carcinomas.

Method

A retrospective cross-sectional study was conducted including epithelial breast neoplasms over a period of three years from February, 2021 to February, 2024. May–Grünwald–Giemsa (MGG) and Papanicolaou stained cytology smears of cases whose biopsy sample was also received in department of Pathology were included after ethical approval from institutional review committee. Micronuclei were identified based on established criteria and scored in 1,000 epithelial cells per case. The micronuclei frequency was compared across cytologically diagnosed benign and malignant lesions proven histopathologically. Statistical analysis was performed to evaluate significance.

Result

The mean micronuclei score was significantly higher in malignant smears compared to benign lesions ($p < 0.05$). A progressive increase in micronuclei frequency was observed from grade 1 to grade 3 amongst the malignant category.

Conclusion

Micronuclei scoring on breast cytology smears is a simple, cost-effective and potentially reliable marker of malignancy. It can serve as a useful adjunct in routine cytological evaluation, and also help in predicting the grade of malignancy.

KEY WORDS

Breast, Chromosomal instability, Neoplasm

INTRODUCTION

Breast cancer remains the most common malignancy among women worldwide (46.8% of all cancers in females) and a leading cause of cancer-related mortality in females (12.6%).[1] Early and accurate diagnosis is critical for improving prognosis and guiding appropriate management. Fine-needle aspiration cytology (FNAC) is widely used as a first-line diagnostic tool for evaluating palpable breast lesions due to its simplicity, cost-effectiveness, and minimal invasiveness.[2] However, the cytological differentiation between benign and malignant lesions can occasionally be challenging, particularly in cases with borderline features.

Micronuclei (MN) are small, extranuclear bodies formed from chromosomal fragments or whole chromosomes that fail to incorporate into the daughter nuclei during mitosis. They are formed due to chromosomal aberrations leading to lagging of whole chromosome or acentric chromosome fragments during anaphase in cell division. Their presence reflects underlying genomic instability, a hallmark of cancer.[3] The International Human Micronucleus project (HUMN) was launched in 1997 to study the baseline frequency and ability to predict genomic damage by micronucleus in lymphocytes and exfoliated buccal cells in the human population. The project proved the micronucleus score to be a minimally invasive biomarker of genomic damage and chromosomal instability.[4]

Over the past few decades, the micronucleus assay has been recognized as a reliable indicator of DNA damage and chromosomal aberrations in various tissues. In breast lesions, increased MN frequency has been reported in malignant smears compared to benign and borderline counterparts, suggesting its potential as an ancillary marker in cytological evaluation.[5-8] However, these studies are relatively fewer, performed in limited samples and some have conflicting results.

Despite its promise, micronuclei scoring is not routinely incorporated into breast cytology practice. Establishing its diagnostic utility could provide a valuable, objective parameter to supplement morphological assessment, particularly in resource-limited settings or equivocal cases. This study was undertaken to evaluate the frequency of micronuclei in breast cytology smears and assess its correlation with cytological diagnosis, thereby exploring its role as a supportive diagnostic marker in distinguishing benign from malignant breast neoplasms.This study will also try to assess the role of micronuclei in grading the malignant cases.

METHODS

This was a retrospective cross-sectional analytical study that was conducted in the Department of Pathology of Nobel medical college and teaching hospital from February, 2021 to February, 2024 for duration of three years. Ethical clearance from the Institutional review committee of Nobel medical college and teaching hospital was obtained before the study (Reference no 16/2024). Patients with palpable breast lumps who underwent both cytological as well as histopathological examination of the lump that proved to be neoplastic were included in the study. Breast lesions other than epithelial neoplasms and cases with cytology samples that are inadequate for evaluation due to either less cellularity or staining artefacts were excluded from the study.

The sample size was estimated to be 73 by using the formula, $n=Z^2\ P(1-P)/e^2$, where Z is the confidence level at 95% (1.96); e is margin of error taken as 5% and P is hospital-based prevalence of breast histopathology samples received in the department that turned out to be an epithelial neoplasm which is 95%.One hundred such cases including 50 benign neoplasms and 50 malignant neoplasms were analysed after retrieving the histology slides and corresponding cytology slides stained with Papanicolaou stain. The researcher was blinded about the histopathological diagnosis of each case.

Micronuclei were identified following the criteria by Countryman and Heddle.[9] According to them, a micronucleus must:

1. Not be refractile

2. Be less than 1/3 the diameter of the main nucleus

3. Have similar or lighter color as the nucleus

4. Be clearly separated from the main nucleus and within 3 or 4 nuclear diameters

5. Not be more than 2 associated with a single nucleus

Micronuclei meeting the criteria were searched on oil immersion filed (1000x) and scoring was performed in 1000 continuous non-overlapping, intact and non-apoptotic epithelial tumour cells.

Histological grading of breast carcinoma was done according to the criteria proposed by Elston and Ellis.[10]

The data was analyzed and compiled with the help of tables for descriptive purpose. SPSS (Statistical package for the social sciences) version 16 was used for data analysis. Descriptive analyses were reported as mean and standard deviation of continuous variables. Independent t-test was used to know if there is any significant difference in the mean MN score of benign and malignant groups. ANOVA (Analysis of variance) followed by post hoc analysis by Tukey HSD was used to test the difference in MN score amongst grade 1, grade 2 and grade 3 malignant breast cancers. Value of $p < 0.05$ was taken as statistically significant.

RESULTS

A total of 100 breast cytology smears were analyzed, comprising 50 benign and 50 malignant cases, with their

diagnosis and grade confirmed by histopathological evaluation. The mean mi-cronuclei (MN) score was significantly higher in malignant breast neoplasms compared to benign lesions figure 1.

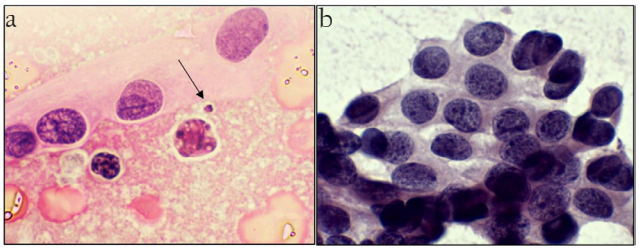

Figure 1. Papanicolaou stained cytology smears of breast lump (a) Invasive breast carcinoma with a micronucleus denoted by arrowhead (1000x) (b) No micronuclei in a case with fibroadenoma (1000x)

This difference in MN scores between the two groups was also statistically significant. A de-tailed comparison is presented in table 1 below:

Table 1. Comparison of micronuclei score amongst benign and malignant breast neoplasms

Lump category	N	Mean MN score	Standard deviation	p-value* (Indepen-dent t-test)
Benign neoplasm	50	1	.948	<0.001
Malignant	50	21.96	9.3	

* p-value < 0.05 considered statistically significant

A total of 50 cytologically diagnosed malignant breast lesions confirmed by histopathology were included in the study and were categorized into Grade 1 (n=14), Grade 2 (n=18), and Grade 3 (n=18) based on corresponding histopathological grading.

A one-way ANOVA test revealed a statistically significant difference in MN scores among the three grades ($p < 0.001$). Post hoc analysis further confirmed that the mean MN score in Grade 3 tumors was significantly higher than in Grade 2, which in turn was significantly higher than in Grade 1 (Table 2).

Table 2. Comparison of micronuclei score within different grades of breast cancer

Grade of malignancy	Mean difference in MN score	Standard error	p-value* (ANOVA with post hoc Tukey HSD)
Grade 1 vs 2	-9.5	1.3	<0.001
Grade 1 vs 3	-21.1	1.3	<0.001
Grade 2 vs 3	-11.6	1.2	<0.001

* p-value < 0.05 considered statistically significant

DISCUSSIONS

In this study, we evaluated the utility of micronuclei (MN) scoring in breast cytology smears as a potential marker for distinguishing benign from malignant breast lesions. Our results demonstrated a statistically significant increase in the mean MN score among malignant breast neoplasms compared to benign neoplasms, suggesting that MN frequency may serve as a reliable indicator of genomic instability and malignancy in breast cytology.

The elevated MN score in malignant cases aligns with the well-established role of micronuclei as biomarkers of chromosomal damage, mitotic dysfunction, and genomic instability all of which are hallmarks of cancer.[8,11] Malignant cells often undergo abnormal mitoses and have impaired DNA repair mechanisms, leading to an increased formation of micronuclei. This biological basis supports our findings and highlights the relevance of MN scoring as a supplementary cytological tool.

Our findings are consistent with previous studies that have reported higher MN frequencies in malignant breast lesions. For example, studies by Sylvia et al. and Samanta et al. also demonstrated significantly elevated MN scores in cytologically or histologically confirmed malignant breast aspirates.[5,12] These studies reinforce the diagnostic value of MN scoring in differentiating between benign and malignant pathology, particularly in cases with ambiguous cytomorphological features.

The major advantage of MN scoring lies in its simplicity, cost-effectiveness, and minimal training requirement.[13] It can be performed on routine FNAC smears which is a simple, inexpensive and non-invasive diagnostic test, without the need for additional staining or equipment.[14]

Our findings demonstrated a progressive increase in MN frequency with higher tumor grade, with Grade 3 carcinomas showing the highest MN scores, followed by Grade 2 and Grade 1 lesions. This gradation was statistically significant and reinforces the role of MN as a marker of genomic instability and tumor aggressiveness. Our results are in agreement with previous studies that have shown a positive correlation between MN frequency and histological grade in breast cancer. For example, Hemlatha et al. and Mangam et al. reported a similar trend, highlighting that MN scoring could reflect not only the presence of malignancy but also its biological behavior and potential aggressiveness.[5,8]

Mahanta et al. also studied MN scoring in cervical dysplastic lesions and found sequential and significant increase in MN score from low to high grade dysplastic lesions. This adds to the growing body of evidence supporting the diagnostic and prognostic value of MN analysis in cytological specimens.[15]

Goel et al. however did not find any statistically significant difference in MN score while comparing grade 2 and grade 3 breast cancers. This discrepant finding could be attributed to a small sample size of only 16 malignant cases in their study.[6]

The ability to estimate tumor grade from cytology smears using a simple and cost-effective method like MN scoring is particularly valuable in settings where histopathological

grading may be delayed or unavailable. It can serve as an adjunct to conventional cytological features. Moreover, in cases with equivocal cytomorphology, a higher MN score may prompt more aggressive investigation or early intervention.[16,17]

However, there are some limitations to consider. Micronucleus identification can be subjective and may vary between observers unless strict morphological criteria are applied.[18] Factors such as poor smear quality, overlapping cells, or degenerative changes may affect accurate scoring. Furthermore, while the MN score can support a diagnosis, it should not replace standard cytological assessment but rather complement it. Additionally, while MN score correlates with tumor grade, it does not capture other important prognostic markers such as hormone receptor status, HER2 expression, or lymph node involvement.[19,20]

CONCLUSION

Our study supports the utility of micronuclei scoring as a useful adjunct in the cytological evaluation of breast lesions. The significantly higher MN frequency in malignant smears underscores its potential as an indicator of malignancy. Incorporating MN scoring into routine cytology may enhance diagnostic accuracy, especially in borderline or equivocal cases. The significant increase in micronuclei frequency with higher tumor grade also highlights its probable role as a surrogate marker for tumor aggressiveness in breast cancer.

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