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Original Research Article

Diagnostic significance of micronucleus evaluation in breast fine needle aspiration cytology

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ABSTRACT

Background: Breast cancer is one of the most common malignancies among females in India. Micronucleus (MN) scoring, a marker of chromosomal instability, has shown potential in evaluating breast lesions cytologically. The International Human Micronucleus Project confirmed that MN assays are simple and minimally invasive indicators of genomic instability, with higher scores correlating with malignancy. To compare MN scores across benign, borderline and malignant breast lesions, correlate MN scores with Robinson's cytological grading in malignant cases and evaluate the diagnostic utility of MN scoring in fine needle aspiration cytology (FNAC).
Methods: A cross-sectional study was conducted in the Department of Pathology of Melmaruvathur Adhiparasakthi Institute of Medical Sciences and Research from December 2023 to February 2025 on 100 breast FNAC samples. Smears were alcohol-fixed, H&E-stained and classified per the Yokohama System. Robinson's cytological grading was applied to malignant cases and MN scoring was performed on 1000 cells per case. Statistical analysis was done using SPSS version 20, with $p<0.05$ considered significant.
Results: Among 100 cases (age 19–62 years), there were 50 benign, 10 atypical, 10 suspicious and 30 malignant lesions. Mean MN scores were 0.53±0.45, 2.8±0.85, 6.4±2.1 and 21.9±5.9, respectively ($p=0.000$). Significant differences were found between most groups, except benign versus atypical ($p=0.15$). MN scores increased with Robinson's grades I–III, showing significant correlation ($p=0.04$).
Conclusions: Micronucleus scoring, reflecting genomic instability, serves as a simple, cost-effective adjunct cytological marker enhancing diagnostic accuracy and prognostication in breast malignancy.

Keywords: Breast cytology, Fibroadenoma, Invasive ductal carcinoma, Micronucleus score

INTRODUCTION

When cells undergo division, small fragments of the nucleus, known as micronuclei, may be left behind due to chromosomal abnormalities. These abnormalities arise from acentric chromosome fragments or the lagging of whole chromosomes during mitosis. Micronuclei can be visualized in the cytoplasm of cells under oil immersion microscopy as round to spherical structures, sharing the same texture and staining characteristics as the nucleus, typically measuring between one-third to one-sixteenth the size of the main nucleus.[1] The formation of micronuclei has been attributed to a wide range of etiological factors, including spontaneous genetic events, infectious agents, chronic inflammation, metabolic disturbances, exposure to genotoxic chemicals, radiation, neoplastic processes and hereditary conditions.[2] Owing to their association with chromosomal instability, micronuclei serve as valuable molecular marker and biomarker for assessing genotoxicity and chromosomal damage.[3] Breast cancer remains a significant public health concern in India, with an estimated 1,45,000 new cases reported annually having an age-standardized incidence rate of 25.8 per 100,000 women, making it one of the most frequently diagnosed

malignancies among Indian females.[4] Fine Needle Aspiration Cytology (FNAC) is widely employed as a primary diagnostic tool for evaluating breast masses, favoured for its rapidity, cost-effectiveness and minimally invasive nature. However, distinguishing borderline or atypical lesions on FNAC can present diagnostic challenges. In such instances, the evaluation of micronuclei offers a valuable adjunctive method, providing objective and reproducible diagnostic insights.[5]

The present study aimed to compare micronucleus scores in breast cytology aspirates among benign, borderline and malignant lesions and to correlate MN scores with Robinson's cytological grading system in malignant cases and also to assess the utility of MN scoring in enhancing diagnostic accuracy and contributing to the refinement of cytological assessment protocols.

METHODS

The present cross-sectional study was conducted in the Department of Pathology of Melmaruvathur Adhiparasakthi Institute of Medical Sciences and Research from December 2023 to February 2025. Ethical clearance was taken as per the institutional protocol. Females above 18 years old with breast lump were included in the study. Females with less than 18 years of age, male breast lesions, smears with poor staining, obscuring elements or degenerated cells were excluded from the study. After obtaining informed consent, FNAC was done for all breast lesions and smears were fixed in 85% isopropyl alcohol for 15 minutes and stained with Haematoxylin and eosin.

Smears were screened and routine Breast cytology diagnosis were made based on Yokohama System for reporting breast fine needle aspiration cytology. Cases were cytologically classified into four categories benign, atypical, suspicious of malignancy and invasive breast carcinoma. Invasive breast carcinomas were further subcategorized into three grades according to Robinson's cytological scoring system, which incorporates six morphological parameters: cell dissociation, cell size, cell uniformity, nucleoli, nuclear margin and chromatin pattern.[6] Each parameter was scored from 1 to 3, with the cumulative score determining tumor grade. Scores of 6–11 corresponded to grade I, 12–14 to grade II and 15–18 to grade III carcinomas. Then Micronuclei scoring was done for 1000 cells on haematoxylin and eosin-stained smears under oil immersion (×1,000).

Criteria for micronuclei

Diameter of micronuclei should be 1/16 to 1/3rd of diameter of the main nucleus. The shape, colour and texture of Micronuclei should be similar to the nucleus. Staining intensity should be similar to or slightly weaker than that of the nucleus. Micronuclei should be round to oval having close proximity but no actual contact with the nucleus. Plane of focus should be same as that of the main nucleus.[7] Mean MN scores were calculated separately for each category and for the three grades of malignancy. Finally, the degree of correlation between micronucleus scores among all four categories of breast lesions and Robinson's grades were also established.

Statistical analysis

Statistical analyses were conducted using SPSS version 20. Group comparisons were evaluated with the Chi-square. P value<0.05 was considered as statistically significant.

RESULTS

The present study included 100 cases of breast aspirate smears collected over a period of 1 year from the department of pathology. The age of the patients in this study ranged from 19 to 62 with a mean age of 38.3 years.

In this study, 50 (50%) cases were categorised as benign, 10 (10%) cases as atypical, 10 (10%) cases as suspicious and 30 (30%) cases as Invasive carcinoma. In the present study, 50 benign cases had a mean age of 29.2±6.8 years. The 10 cases categorized as atypical showed a slightly higher mean age of 35.1±5.9 years. The 10 cases of suspicious of malignancy had a mean age of 44.2±6.3 years, while the 30 cases of invasive carcinoma demonstrated the highest mean age of 52.6±9.8 years. Thus, the mean age of patients showed a progressive increase from benign lesions (29.2±6.8 years) to invasive carcinoma (52.6±9.8 years).

Benign lesions had the lowest MN score (0.53±0.45), whereas invasive carcinoma showed the highest (21.9±5.9). Intermediate categories also reflected this trend, with atypical cases having a mean MN score of 2.8±0.85 and suspicious cases showing 6.4±2.1. This highlighted a clear stepwise increase in MN frequency from benign to malignant lesions, supporting its potential role as a marker of malignancy (Table 1).

In this study, Comparative analysis of micronucleus scores between various categories of breast lesions revealed that the difference in MN scores between benign and malignant cases was highly significant ($p<0.001$), confirming the strong discriminatory value of MN scoring between these categories. The comparison between benign and suspicious cases also reached statistical significance ($p=0.02$), reflecting the marked rise in MN frequency in the latter.

Atypical versus suspicious and suspicious versus malignant cases likewise demonstrated a highly significant difference ($p<0.001$), corresponding to the sharp increase in MN scores. However, the comparison between benign and atypical breast lesions was not statistically significant ($p=0.15$), suggesting that MN scores in atypical lesions partially overlap with benign cases, limiting their reliability as an independent discriminator in this borderline group (Table 2).

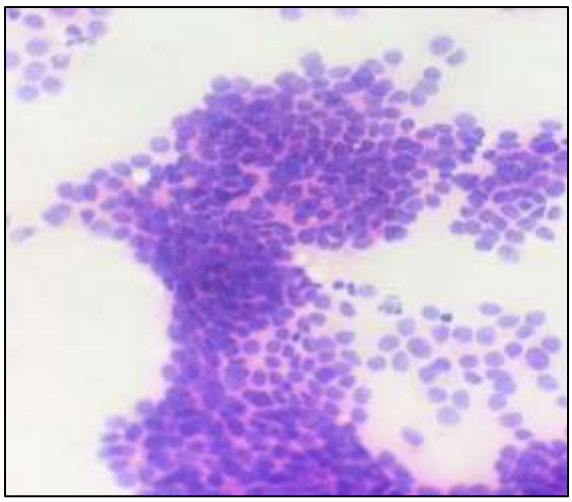

Figure 1: Micronuclei in fibroadenoma (H&E stain, 1000X).

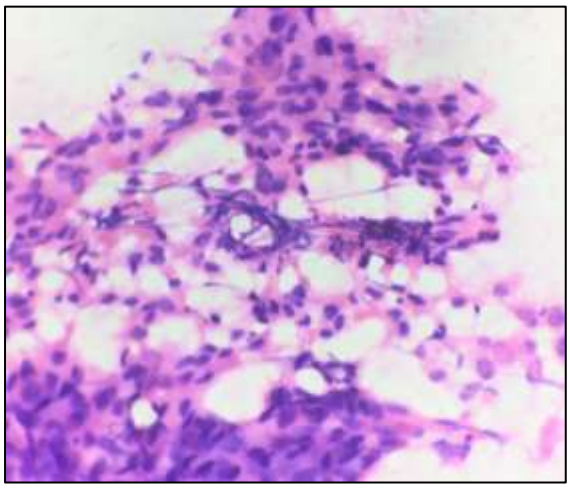

Figure 2: Micronuclei in atypical breast lesion (H&E stain, 1000X).

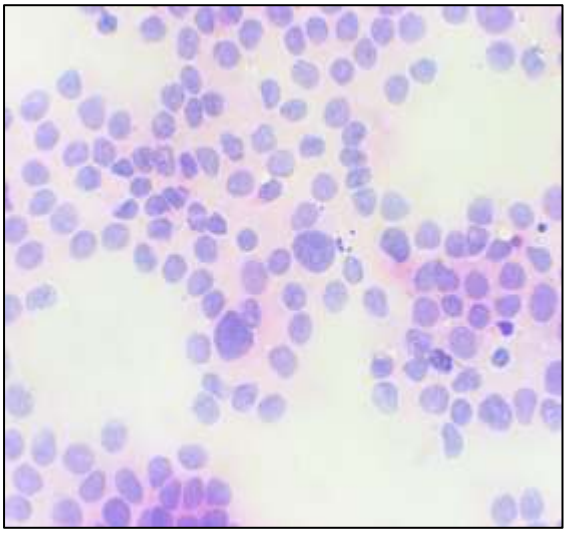

Figure 3: Micronuclei in a case suspicious of malignancy (H&E stain, 1000X).

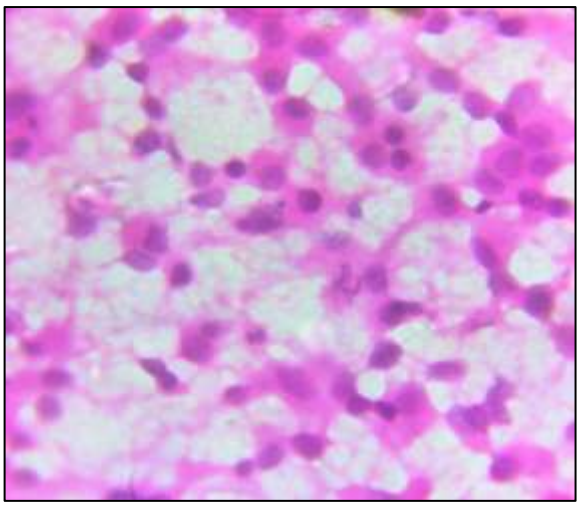

Figure 4: Micronuclei in Invasive carcinoma – grade1 (H&E stain, 1000X).

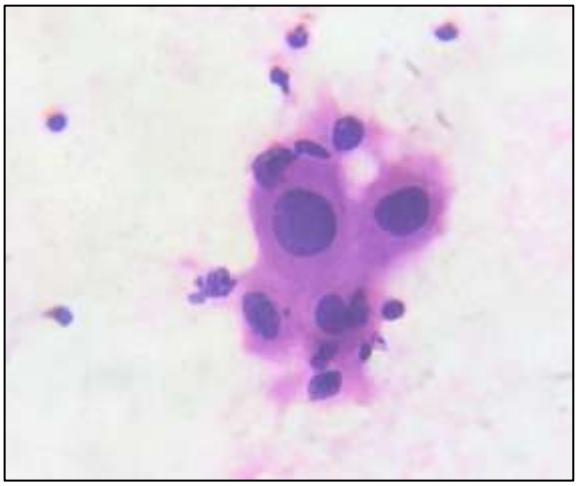

Figure 5: Micronuclei in Invasive carcinoma–grade 2 (H&E stain, 1000X).

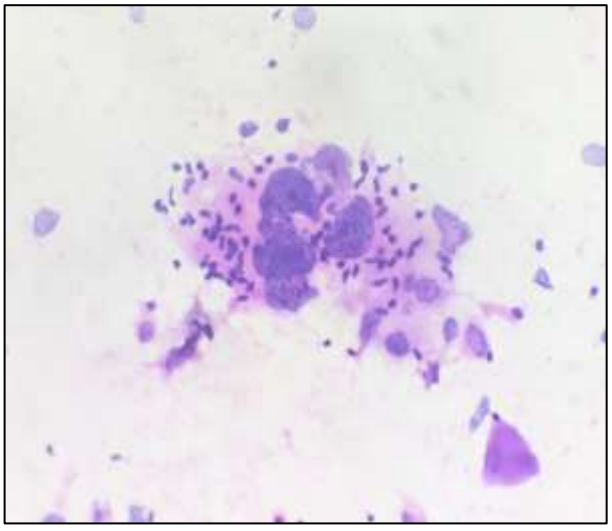

Figure 6: Micronuclei in grade 3 (H&E stain, 1000X).

In this study, Invasive carcinoma cases were further stratified according to Robinson cytological grade (Grade I, II and III) and the distribution of mean age, mean micronucleus (MN) score per 1000 cells and statistical correlations were summarized. A total of 30 malignant cases were included, comprising 10 (33.3%) cases in Grade I, 12 (40%) cases in Grade II and eight (26.7%) cases in Grade III carcinomas. The mean age of patients among different grades of breast malignancy revealed that patients with Grade I carcinoma had a mean age of 49.5±11.3, Grade II cases had a mean age of 51.8±10.2 years and Grade III cases had a mean age of 56.4±7.1 years. This showed that higher malignancy grades are associated with advancing age and tend to occur in relatively older patients compared with lower-grade tumors. Grade I carcinomas exhibited the lowest MN score of 13.5±1.8 per 1000 cells, while Grade II carcinomas showed a significant rise to 23.1±2.1 per 1000 cells. Grade III carcinomas demonstrated the highest MN score at 30.0±1.5 per 1000 cells. The mean MN scores showed a progressive and marked increase across the grades, reflecting the association between nuclear instability and tumor aggressiveness. Statistical analysis revealed a significant difference in MN scores among the carcinoma grades (p=0.04), supporting the association between increasing MN frequency and higher cytological grade (Table 3). Taken together, these findings indicated that micronucleus scoring not only differentiates benign from malignant lesions but also demonstrates a clear correlation with tumor grades within malignant lesions. Higher MN frequencies were consistently associated with higher grades of carcinoma, reflecting greater chromosomal instability in more aggressive tumors.

Table 1: Table depicting comparison of mean age and micronucleus score between various categories of breast lesions.

Cytological category	Number of cases	Mean age (years)	Mean micronucleus score (per 1000 cells)
Benign	50	29.2±6.8	0.53±0.45
Atypical	10	35.1±5.9	2.8±0.85
Suspicious of malignancy	10	44.2±6.3	6.4±2.1
Invasive carcinoma	30	52.6±9.8	21.9±5.9

Table 2: Comparative analysis of micronucleus scores between various categories of breast lesions.

S. no.	Cytological category comparison	Mean micronucleus score (per 1000 cells)	P value (Chi-square test)
1	Benign	0.53±0.45	<0.001
	Malignant	21.9±5.9	
2	Benign	0.53±0.45	0.15
	Atypical	2.8±0.85	
3	Benign	0.53±0.45	0.02
	Suspicious of malignancy	6.4±2.1	
4	Atypical	2.8±0.85	<0.001
	Suspicious of malignancy	6.4±2.1	
5	Suspicious of malignancy	6.4±2.1	<0.001
	Malignant	21.9±5.9	

Table 3: Micronucleus scores across Robinson's grades of invasive carcinoma.

Grade	Number of cases (30)	Mean age (years)	Mean micronucleus score	P value
I	10	49.5±11.3	13.5±1.8	0.04
II	12	51.8±10.2	23.1±2.1	
III	8	56.4±7.1	30.0±1.5	

DISCUSSION

FNAC is widely acknowledged for its simplicity, cost-effectiveness and minimally invasive approach in the initial evaluation of breast lesions. Nonetheless, the technique presents diagnostic challenges in distinguishing certain borderline or atypical cases from clearly benign or malignant lesions. In the study, there was a progressive increase in mean age with advancing cytological category where patients with benign lesions had a mean age of 29.2±6.8 years, whereas those with atypical lesions were slightly older, with a mean age of 35.1±5.9 years. This increased further in the suspicious category (44.2±6.3 years) and the highest mean age was observed in malignant cases (52.6±9.8 years). Thus, there was a consistent age gradient, with an overall difference of more than two

decades between benign and malignant lesions. This age gradient is consistent with the epidemiological profile noted by kata et al and Swathi et al, reinforcing the pattern that breast cancer presents at a relatively older age compared to benign conditions.[8,9] Further higher-grade carcinomas tend to occur in relatively older patients (mean age-56.4±7.1) compared with lower-grade tumors (mean age-49.5±11.3) which was consistent with the findings of Ramya kata et al.[8]

In our study, the mean micronucleus score, calculated per 1000 cells, demonstrated a clear stepwise increase from benign to malignant breast lesions. Benign lesions exhibited the lowest score whereas malignant lesions demonstrated the highest MN score. Incremental differences were evident at each step: from benign to atypical there was an increase of +2.27, from atypical to suspicious a further increase of +3.6 and from suspicious to malignant a substantial rise of +15.5. In total, malignant lesions showed an absolute increase of 21.37 MN per 1000 cells when compared with benign lesions. This finding closely mirrors the results by Swathi et al, Meel et al and Sylvia et al, where progression from benign to malignant categories showed progressively increasing MN scores.[9-11]

The present study also confirmed that differences in MN scores between benign and malignant, as well as between atypical, suspicious and malignant categories, were statistically significant ($p<0.001$) which was consistent with the findings of Ramya kata et al where difference in MN score between benign versus malignant and suspicious lesions were statistically significant with p value of 0.001 and 0.02 respectively, whereas difference in MN scores between suspicious and malignant lesions was not significant (p value-0.12) which was contradictory to our study.[8] This highlighted the robust discriminatory value of MN scoring, especially in distinguishing clear malignant and suspicious lesions from benign ones. However, the difference between benign and atypical categories was not statistically significant ($p=0.15$) in the study, suggesting some overlap in micronucleus scores that may limit the utility of MN scores as a sole criterion in atypical lesions which was consistent with the findings of Katta et al, where difference in MN score between benign versus atypical categories were not significant (p value=0.22).[8]

Among the tumour grades, the mean MN scores increased with higher tumor grades: Grade I (13.5±1.8), Grade II (23.1±2.1) and Grade III (30.0±1.5). From Grade I to Grade II, the score rose by 9.6, while a further increment of 6.9 was noted from Grade II to Grade III. Overall, the MN score showed a substantial increase of 16.5 from Grade I to Grade III, clearly reflecting a stepwise rise in nuclear abnormalities with advancing tumor grade. The difference across these grades were statistically significant ($p=0.04$) and suggested that MN scoring not only reflects presence of malignancy but also parallels tumor aggressiveness and increasing genomic instability. This trend of rising MN score with escalating tumor grades corroborates the findings by Kata et al, Swathi et al, where a stepwise increase was also observed across channel grades.[8,9] For Grade I tumors, the mean score was 12.5±6.3 in the study by Swathi et al and 13.2±5.7 in the study by Sylvia et al. The increment from Grade I to Grade II was 6.98 in the former and 7.15 in the latter.

Correspondingly, the mean score for Grade II tumors was 19.48±8.2 and 20.35±8.5, respectively. The progression from Grade II to Grade III revealed increments of 7.32 and 7.15 in the two studies. Finally, for Grade III tumors, the mean scores were 26.8±5.27 and 27.5±4.18 in the respective reports. These findings highlighted a parallel trend in both studies, with near-identical increments between successive grades, reinforcing the reproducibility and reliability of micronucleus scoring as a grading parameter.

The progressive and statistically significant escalation in MN scores with higher cytological grades and more severe lesion categories supports the role of MN scoring as a minimally invasive marker for genotoxicity and chromosomal instability in breast pathology. These findings reinforce that MN scores can serve as a valuable adjunctive tool in distinguishing benign from malignant breast lesions and aid in the grading of invasive carcinomas.

However, it is crucial to acknowledge the overlap in MN scores between benign and some atypical categories, which limits the MN score's utility as a standalone diagnostic marker, especially for borderline lesions.

Limitations

The present study, though demonstrating the diagnostic and prognostic potential of micronucleus (MN) scoring in breast cytology, has certain limitations that warrant consideration.

Firstly, the study was conducted on a relatively small sample size of 100 cases within a single tertiary care institution. Hence, the findings may not fully represent the broader population or reflect regional variations in breast lesion profiles. A larger, multicentric study would enhance the generalizability of the results. Secondly, being a cross-sectional study, it was limited to cytological evaluation without longitudinal follow-up. Therefore, the relationship between micronucleus frequency and long-term clinical outcomes such as recurrence, metastasis or survival could not be assessed. Thirdly, although FNAC is a reliable diagnostic technique, histopathological confirmation was not available for all cases, restricting definitive correlation between cytological MN scores and histological diagnosis or grade.

Another limitation is the potential subjectivity inherent in identifying micronuclei on H&E-stained smears. The use of DNA-specific stains such as Feulgen or Giemsa, along

with interobserver reproducibility analysis, could further strengthen the reliability of MN scoring.

CONCLUSION

The study concluded that micronucleus scores progressively increased from benign to atypical, suspicious and malignant breast lesions, with further elevation correlating with higher tumor grades. This pattern underscores the value of micronucleus scoring as a simple, cost-effective adjunct molecular marker in the diagnosis and prognostication of breast lesions. So, incorporating micronucleus scoring into routine cytological evaluation may enhance diagnostic accuracy and offer valuable insights into tumor aggressiveness.

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