Original Article

DOI: 10.7860/JCDR/2022/49784.16810

Pathology Section

Micronucleus Scoring in Fine Needle Aspiration Cytology of Breast Lesions- A Retrospective Analytical Study

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ABSTRACT

Introduction: Micronuclei and other nuclear abnormalities are biological indicators of genotoxicity and chromosomal instabilities. Breast lesions are frequently encountered in routine clinical practice and Fine Needle Aspiration Cytology (FNAC) is used as a routine diagnostic modality.

Aim: To identify the utility of micronucleus scoring in classifying and diagnosing palpable breast lesions with FNAC.

Materials and Methods: The present study was a two year retrospective analysis in the Department of Pathology of Guntur Medical College and Government General Hospital, Guntur, Andhra Pradesh. Case records and FNAC smears of breast lesions in the period from March 2018 to February 2020 were retrieved from the departmental archives. Data was analysed in the month of September 2020. A total of 108 cases were included in the study. Micronucleus scoring was done on the FNAC smears independently by two institutional pathologists who were blinded to clinical data and final diagnosis and mean micronuclear scores were obtained. Statistical analysis was done using Chi-square test on Statistical Package of the Social Sciences (SPSS) software (version 14) to determine the significance of micronuclear score in differentiating benign and malignant lesions, and in grading the malignant tumours.

Results: All the cases were classified into four categories: benign, atypical favouring benign, suspicious of malignancy, invasive breast carcinoma on cytology. In the present study it was found that micronucleus scoring was effective in differentiating various benign and malignant breast lesions (p-value=0.0001) and also in grading of malignant tumours (p-value=0.05). The results obtained showed that there exists a significant level of correlation with other well established standard grading systems (Pearson correlation coefficient=0.94).

Conclusion: The present study revealed that micronucleus scoring is indeed a useful and reliable method for diagnosing breast lesions and can be used as an adjunct in classifying difficult and borderline cases on cytology.

Keywords: Biomarkers, Chromosomal instability, Diagnostic method, Genotoxicity

INTRODUCTION

Micronuclei and other nuclear abnormalities are biological indicators of genotoxicity and chromosomal instabilities [1]. One of the first acknowledged micronuclei are Howell Jolly bodies that are seen in erythrocytes of patients suffering from nutritional deficiencies of folate and cyanocobalamin [1]. The causes of micronucleus formation can vary from being spontaneous, infective, chronic inflammatory, metabolic, genotoxic chemical and radiation exposure, neoplastic and genetic as described in various studies [1,2]. Micronuclei are derived most commonly from acentric chromosomal fragments or from a lagging whole chromosome during the anaphase of mitotic cell division [3,4,5]. There are various theories postulating the origin of micronucleus like dysfunctional mitotic spindle apparatus formation of dicentric chromatids, ring chromosomes and united sister chromatids [6,7]. These omitted chromosomes ultimately get enclosed by a nuclear membrane and remain within the cytoplasm of the parent cell. Thus, they remain morphologically analogous to normal nuclei, except for the smaller size (ranging between 1/3rd and 1/16th of the nucleus), when stained by conventional smears [8].

The International Human Micronucleus Project launched in 1997 has proved behind doubt that micronuclear assays are minimally invasive and simple indicators of genomic instability [9]. Hence, Micronucleus scoring (MN score) studies done in various preneoplastic and neoplastic lesions of oral, cervical, hepatic and urothelial regions have shown that there exists a clear cut correlation between degree of malignancy and MN score [5,10-12].

In most micronucleus studies of patients with breast lesions, spontaneous micronuclei of lymphocytes from peripheral blood or exfoliated squamous cells from buccal smears were studied [13,14]. There are only a few studies about occurrence of micronuclei in breast aspirates and its correlation with the type of lesion and grading of tumours [15,16,17]. Hence, the objective of the present study was to establish if there exists a correlation between micronucleus scoring and epithelial breast lesions (benign and malignant) and to find if this scoring can be used as an adjunct to classify challenging borderline cases. Additionally, correlation between micronuclear score with Robinson's cytological scoring system grades of malignant lesions was also done [18].

MATERIALS AND METHODS

The present retrospective analytical study was conducted in the Department of Pathology of a tertiary care centre in the month of September 2020 by retrieving data of 24 months period (March 2018 to February 2020). Ethical clearance was taken as per the Institutional protocol (Approval No. Faculty/525/20, Dr. PSIMS and RF- IEC). All fine needle aspiration smears of breast pathology, relevant clinical details and histopathologic reports were retrieved from the departmental archives.

Inclusion criteria: Cases diagnosed as epithelial lesion of the breast with good quality staining, histopathological correlation and relevant clinical details were included in the study.

Exclusion criteria: Fibrocystic disease, abscesses, non epithelial malignancies, cases with no histopathological correlation and smears with poor staining, obscuring elements or degenerated cells were excluded from the study.

Study Procedure

Giemsa stained cytological smears prepared from breast lesion aspirations were studied using binocular microscope (Olympus CX21i). Micronucleus counting was done for all cases using oil immersion

objective (100X magnification) by two individual qualified pathologists, following the standard criteria as described by Patino-Garcia B et al., [19]. A minimum of 1000 epithelial cells were counted using zigzag method and the scores were given as number per 1000 cells. Areas with overlapping nuclei were avoided to evade false positives. Both the pathologists were blinded to the final diagnosis, in order to avoid confirmation bias. The interobserver reliability was excellent (Cohen's kappa value: 0.8). Mean of the two blinded observers was considered as the final value of micronucleus score.

Cases were classified into four categories (benign, atypical favouring benign, suspicious of malignancy, invasive breast carcinoma) on cytology after confirmation with their final histopathological diagnosis [20]. Mean micronucleus scores for each category were calculated separately. Invasive breast carcinomas (category 4) were categorised into three grades based on Robinson's cytological scoring and grading system [18]. This system uses six cytological parameters (cell dissociation, cell size, cell uniformity, nucleolus, nuclear margin and nuclear chromatin) for grading the invasive breast carcinomas. Each parameter is given a score between 1-3, and the scores are added up to determine the grade of the tumor. Tumours with sum of 6-11 are graded as grade I, sum between 12-14 are graded as grade II tumours and sum between 15-18 are graded as grade III tumours. The degree of correlation between micronucleus scores and Robinson's grades was also established.

STATISTICAL ANALYSIS

Data was charted using Microsoft Excel (2015 version) and analysed using SPSS software (version 14). Chi-square test was used for calculating value of significance. p-value of less than or equal to 0.05 was taken as significant. Cohen's kappa value was used to calculate the interobserver reliability. Pearson's correlation test was used to compare micronuclear scores with Robinson's cytological grading system.

RESULTS

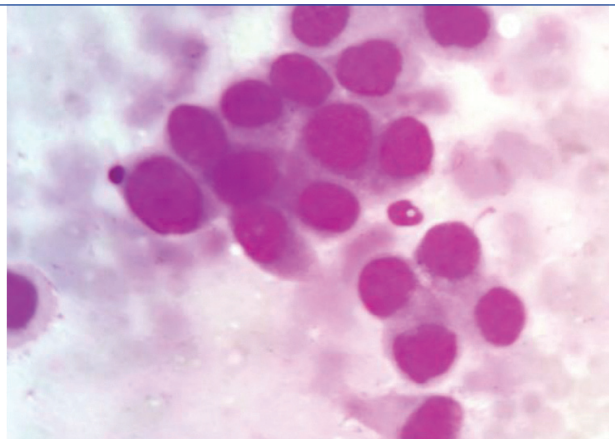
A total of 108 cases which fit into the inclusion and exclusion criteria were taken into the study. All the cases were classified into four categories: benign, atypical favouring benign, suspicious of malignancy, invasive breast carcinoma on cytology. The case distribution, final histopathological diagnosis, mean age distribution, mean micronucleus scores are depicted in the [Table/Fig-1].

Round to oval, small (diameter size 1/3rd to 1/16th of nucleus), non refractile, intracytoplasmic bodies with smooth contour, colour and texture similar to that of the nucleus were counted as micronuclei [Table/Fig-2].

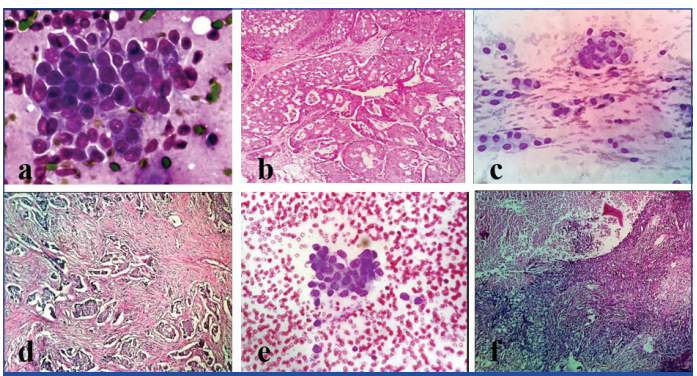
All invasive breast carcinomas were sub categorised using Robinsons grading system and further correlated with subsequent histological grades as depicted in the [Table/Fig-3]. With the application of Chi-square test, it was found that micronucleus scoring can be used effectively in differentiating various benign and malignant breast lesions (p-value=0.001) as depicted in [18] [Table/Fig-4]. However, it was found that micronucleus scoring may not be as effective in distinguishing category 1 and category 2 (p-value=0.22) and category 3 and invasive breast carcinoma grade I (p-value=0.12), as depicted in the [Table/Fig-4]. As depicted in a significant p-value was obtained on comparing micronucleus scores with Robinsons cytological scoring in the invasive breast carcinoma category (p-value=0.05) [Table/Fig-5]. The level of correlation between micronucleus score and Robinsons score was established using Pearson's correlation coefficient, which showed that there was significant level of correlation between them (0.94).

S. No.	Cytological category	Final histopathological diagnosis (number of cases)	Number of cases	Mean age (years)	Mean micronucleus score (number per 1000 cells)
1.	Benign	Fibroadenoma (48)	53	28.5±6.5	0.56±0.5
		Phyllodes tumour (5)			
2.	Atypical favouring benign	Fibroadenoma withadenosis (3)	5	33.8±6.4	2.8±0.8
		Tubular adenoma (1)			
		Lactating adenoma(1)			
3.	Suspicious of malignancy	Usual ductal hyperplasia (3)	11	42±5.3	6.7±2.4
		Atypical ductal hyperplasia (8)			
4.	Invasive breast carcinoma	Invasive carcinoma NST (35)	39	51.5±10.6	22±6.4
		Medullary carcinoma (2)			
		Mucinous carcinoma (2)			

[Table/Fig-1]: Table depicting diagnostic categories, number of cases, age distribution and mean micronucleus scores.
NST: No special type



[Table/Fig-2]: May Grunwald-Giemsa (MGG) stain, oil immersion (100X): Microphotograph of a cytosmear of a breast lesion which was diagnosed as invasive ductal carcinoma on cytology and confirmed as breast carcinoma NST (No special type) on histopathology showing a micronucleus in one of the cells.



[Table/Fig-3]: Microphotographs of cytological and corresponding histological sections of all three grades of invasive breast carcinoma (a) MGG stained cytological smear, 100X, invasive breast carcinoma, grade I (b) Haematoxylin and Eosin (H&E) stained corresponding histological section, 10X, invasive breast carcinoma, grade I (c) MGG stained cytological smear, 40X, invasive breast carcinoma, grade II (d) H&E stained corresponding histological section, 10X, invasive breast carcinoma, grade II (e) MGG stained cytological smear, 40X, invasive breast carcinoma, grade III (f) H&E stained corresponding histological section, 10X, invasive breast carcinoma, grade III.

S. No.	Cytological category	Number of cases	Mean age	Mean micronucleus score	p-value (Chi-square test)
1	Benign	53	28.5±6.5	0.56±0.5	0.001
	Malignant	39	51.5±10.6	22±6.4	
2	Benign	53	28.5±6.5	0.56±0.5	0.22
	Atypical favouring benign	5	33.8±6.4	2.8±0.8	
3.	Benign	53	28.5±6.5	0.56±0.5	0.02
	Suspicious for malignancy	11	42±5.3	6.7±2.4	
4.	Suspicious for malignancy	11	42±5.3	6.7±2.4	0.12
	Invasive breast carcinoma grade I	11	55.8±6.7	13.72±1.73	

[Table/Fig-4]: Comparative analysis of micronucleus scores between various categories of breast lesions [18].

Invasive breast carcinoma (Robinsons cytological scoring system)	Number of cases	Mean age	Mean micronucleus score	p-value (Chi-square test)	Pearson's coefficient of agreement
Grade III	12	48.2±12.6	29.75±1.71	0.05	0.94
Grade II	16	50.8±10.6	23.31±2.21		
Grade I	11	55.8±6.7	13.72±1.73		
Total	39	51.5±10.6	22±6.4		

[Table/Fig-5]: Comparative analysis of micronucleus scores between different grades of malignant lesions.

DISCUSSION

With the evolution of tumour genetics, various genes have been implicated in breast carcinogenesis. Of note are Breast cancer gene 1 (BRCA1) and Breast cancer gene 2 (BRCA2) gene products which play an important role in initiation and/or progression of inherited as well as sporadic cases of breast carcinogenesis [21]. It has been shown in various studies that these two gene products along with RAD51 (DNA repair protein), BRCA1-Associated Ring Domain protein 1 (BARD1), p53 and Retinoblastoma (RB) proteins play an important role in centrosome formation [22] and control of chromosome segregation [23-25]. Thus, defective centrosomes that are formed as result of decreased BRCA1 or BRCA2 gene expression result in loss of integrity of chromosome segregation and non dysjunctional chromosomal loss or gain and spontaneous micronuclei formation [26]. Thus, micronucleus scoring in breast carcinoma can be used as a simple morphological biomarker for chromosomal breakage and genomic instability and may be used as a screening test [15].

In the present study, it was found that micronucleus scores are significantly different in various benign and malignant lesions and also in various grades of malignant lesions of the breast [Table/Fig-5]. The findings of the present study are in concordance with various other studies [15-17]. Thus use of micronucleus scoring in routine practice can be helpful in guiding some difficult diagnoses. As depicted in [Table/Fig-5] there is a gradual increase in the mean micronucleus scores in various grades of invasive breast carcinoma (category 4) with a significant correlation between the micronucleus scores and Robinson's cytological scoring system. This observation points to the fact that micronucleus scores can be used as an additional criteria in sub categorising difficult malignant lesions of the breast. These results are concordant with those obtained by Samantha S et al., [27].

In the study by Meel M et al., mean micronucleus score >6 predicted the presence of invasive breast carcinoma with a high sensitivity and specificity [28]. However, in the present study, it was concluded that differentiation of borderline cases (category 3) from grade I invasive carcinomas by just using micronucleus score may not be reliable. The mean micronucleus scores in the present study were similar to the results of Samantha S et al., study and are much lesser than that obtained in the study of Hemalatha A et al., and are higher than that obtained in Meel M et al., study [15,27,28]. This variation could be due to varying micronucleus number in baseline cases (benign category).

In the present study micronucleus evaluation was done using routine Giemsa stained slides. Several authors acclaimed that use of Deoxyribonucleic acid (DNA) specific feulgen stain, fluorescent stains like auramine rhodamine stain, propium iodide stain yield better results as false positivity can be evaded [29,30]. However, DNA specific stains like MGG are much cost effective, less cumbersome procedures and are routinely used in normal cytology and hence are preferred [15].

Micronucleus scoring is relatively easy, reliable and reproducible test. However, phagocytosed platelets, karyorrhectic debris of apoptotic cells, smearing and staining artifacts can give rise to increased number of false positives. Use of liquid based cytology, which provides excellent single cell thickness smears and automatic micronuclear counters, may provide more reliable data [1].

Limitation(s)

In the present study, baseline levels of micronucleus scores, its variation with age, sex, exposure to chemicals and other factors were not considered.

CONCLUSION(S)

The rising use of adjuvant and neoadjuvant therapy in the treatment of invasive breast carcinomas has resulted in increased demand for exploration of more cost effective and simple prognostic biomarkers that can be performed in less sophisticated laboratories. Micronucleus scoring could be a pace towards it.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Apr 09, 2022
- Manual Googling: Jun 06, 2022
- iThenticate Software: Jul 05, 2022 (7%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Apr 06, 2021**
Date of Peer Review: **Jun 14, 2021**
Date of Acceptance: **Jun 28, 2022**
Date of Publishing: **Aug 01, 2022**