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ROLE OF MICRONUCLEI (MN) SCORING AS POTENTIAL MARKER OF GENOTOXICITY IN VARIOUS EPITHELIAL LESIONS-SPECIAL EMPHASIS ON BREAST LESIONS: A DESCRIPTIVE OBSERVATIONAL STUDY

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ABSTRACT

Background and Objectives: Cancer is genomic disease associated with genetic damage accumulation. Majority of solid tumors show large number of chromosomal aberrations. Carcinoma of breast is on rise in our country. Like other cancers even breast carcinomas are known to have chromosomal instabilities that play an important role in cancer development and progression. **Aims:** 1. To score the spontaneously occurring micronuclei in epithelial lesions of breast, cervix and liver 2. To score and compare micronuclei frequency in benign tumours and various grades of infiltrating ductal carcinomas of breast. **Materials and Methods:** Cross-sectional Retrospective analysis of breast cytology smears stained with May Grunwald Giemsa stain, received over a period of 1 year formed the basis of the study. Micronucleus scoring was done by counting the number of micronuclei in 1000 epithelial cells under oil immersion and score was calculated in various epithelial lesions and specifically compared these in the fibroadenoma, usual/atypical ductal hyperplasia, and the three grades of infiltrating ductal carcinomas of breast. Statistical Analysis: Descriptive analyses and one-way analysis of variance was used for statistical analysis. **Results:** Out Of the 478 cases, statistically significant mean micronucleus score of fibroadenoma (152), usual ductal hyperplasia (16), atypical ductal hyperplasia(9), and ductal carcinoma grade I, II, III (83) were 0.36,2.18,5.22,8.83,11.51 and 15.357 respectively. MN score for high grade intra epithelial lesion of cervix(9) was 8.22 and for metastatic liver carcinoma (209) was 5.42. **Conclusion:** MN scoring on the epithelial cells of breast, cervix and liver could serves as an additional biomarker for the cancer screening, diagnosis and grading of breast lesions. This is an easy, simple, reliable, reproducible & objective test which can be performed on routinely stained May Grunwald Giemsa stain and pap smears.

KEYWORDS: Micronuclei (MN), Genetic, Breast cytology aspirates.

INTRODUCTION

Accumulation of genetic damage over the time leads to genomic disease such as carcinoma. Most of solid tumors show large number of chromosomal aberrations, thus screening for chromosomal instabilities is very important. The screening for chromosomal instabilities can be done by using micronucleus scoring.

Micronucleus (MN) is formed by chromosomes or chromosome segments that fail to be incorporated in cell nuclei during cell division. MN formation represents a measure of both chromosome breakage as well as chromosome loss and is a sensitive indicator of chromosomal damage.[1] These micronuclei are round to oval in shape with a diameter range from 1/3 to 1/16th of main nucleus. Their intensity and texture is similar to that of the main nucleus & these must be located within the cytoplasm of the cell.[2] The first study of micronucleus was done by Evans et al. on the effect of neutrons on plant root tips.[3] Later, micronucleus scoring was used to demonstrate genomic damage secondary to radiation and chemicals.[4] The term micronucleus test was suggested for the first time by Boller and Schmidt and Heddle who showed that this assay provided a simple method to detect the genotoxic potential of mutagens after in vivo exposure of animals using bone marrow erythrocytes. The International Human Micronucleus project (HUMN)- 1997, proved the micronucleus score to be a minimally invasive biomarker of genomic damage. Micronuclei scoring in routinely stained smears has been applied to study the different epithelial preneoplastic and neoplastic conditions of the

head and neck, cervical intraepithelial lesions, cervical carcinomas and also in liver carcinogenesis.[5] Chromosomal instabilities in breast cancer occur in form of p53 mutations, BRCA1 & BRCA2 mutations, CHEK mutations. cervical carcinoma have chromosomal instabilities in form of MDM2 gene mutation, RB gene mutation. Fine needle aspiration cytology (FNAC) is applied as the primary tool for diagnosis in breast and liver masses because of its ease, rapidity.

AIMS AND OBJECTIVES

1.To score the spontaneously occurring micronuclei in epithelial lesions of breast, cervix and liver. 2. To score and Compare micronuclei frequency in benign tumours and various grades of infiltrating ductal carcinomas of breast. 3. To establish the importance of micronucleus scoring in cytology smear.

MATERIALS AND METHODS

The present study was a retrospective study of micronuclei scoring on fine needle aspiration cytology (FNAC) smears of various epithelial lesions special emphasis on breast lesions conducted in the department of pathology, SMS Medical College, Jaipur over a period of 1 year from June 2019 to June 2020. The study consisted of 478 cases in which breast lesions, cervical lesions and liver lesions were included. The clinical details and histopathology reports were retrieved from the archives of department of Pathology. Benign proliferative breast diseases, fibrocystic disease, abscess, nonepithelial malignancies, cases without histopathological confirmation, degenerated, poorly stained, and smears obscured by hemorrhage or necrotic material, Infiltrating ductal carcinomas occurring in males, carcinomas with neoadjuvant chemotherapy & radiotherapy were excluded from the study. The morphologic mimics of micronuclei - apoptotic cells, stain deposits, inflammatory cells, and nuclear fragments were excluded. Cytological Examination of Breast Lesions: Smears were stained with H & E stain & MGG stain; examined for Micronucleus scoring of various epithelial lesions and specifically compared these in the fibroadenoma, usual/atypical ductal hyperplasia, and the three grades of infiltrating ductal carcinomas of breast graded according to Robinson's cytograding method.

Cytological Examination of Cervical Smears: Prepared slides of cervical smears which were received from gynecology department, stained with H& E stain; were examined for micronuclei.

Cytological Examination of Liver lesions: H & E and MGG stained smears of liver lesions in which metastatic carcinoma was diagnosed, were used for MN score.

Micronucleus scoring was done by counting the number of micronuclei in 1000 epithelial cells under oil immersion.

MN Scoring Criteria

1. Cytoplasm should be intact and relatively flat cell position on the slide
2. No/little overlap with adjacent cells
3. No/little debris
4. Nucleus intact with smooth and distinct nuclear membrane
5. Texture and staining intensity of micronucleus should be similar to that of nucleus
6. Should be as same focal plane as nucleus

Statistical Analysis

The data were entered in Microsoft Excel programme and analyzed. The categorical data were analysed and presented as frequencies and percentages. Age and total score were presented as mean and standard deviation (SD). One-way analysis of variance test (ANOVA) was used for significant difference occurring between benign and malignant and also among all grades of IDC and the value of $P < 0.05$ was taken as statistically significant.

RESULTS

Out Of the 478 cases, 260 cases were of breast lesions, 209 cases were of liver in which metastasis were diagnosed, and 9 cases were of cervix diagnosed as high grade squamous intraepithelial lesion (HSIL).[Table 1][figure 1] Breast lesions includes Fibroadenoma(152), usual ductal hyperplasia(16), atypical ductal hyperplasia(9), and ductal carcinoma (83).[Table 2]. Out of 260 cases of breast lesions in Fibroadenoma group MN score of <2 had a high sensitivity (99.3%) and specificity (98.2%) for confirming benign cases in our study. In UDH group(16 cases) had an average micronucleus score higher than the benign group and statistically significant ($P < 0.001$). MN score of 2-5 had a sensitivity of 93% and specificity of 98%. ADH cases (9 cases) showed a high margin of statistically significant difference from the benign, and UDH groups. The difference in the micronucleus score was statistically significant ($P < 0.001$). Micronucleus score of ≥5 and <8 had a moderate sensitivity (75%) but a high specificity of 99% in detecting cases of ADH[Table 3].

The malignant group (total 83 cases) had a high margin of difference from the rest of the categories and the difference in the mean micronucleus score between the four groups and three grades of tumor were found statistically significant ($P < 0.001$). Further, micronucleus score of ≥8 had a high sensitivity (91%) and specificity (99%) of detecting malignancy [Table 4].

209 cases were of metastatic liver carcinoma incidence of which is more in female patients (53.58%). The mean MN Score of liver FNAC cases was 5.42 with a average age of 56.14 years [Table 5]. In cervical lesions 9 cases were of HSIL with a mean age of 57.4 years and micronucleus score value of 8.22[Table 6].

Table 1: Site distribution of cases (n= 478).

Site of collection	No. of cases
Breast	260
Liver	209
Cervix	9

Table 2: Breast lesions (n= 260).

Breast lesions	No. of cases
Fibroadenoma	152
Usual ductal hyperplasia	9
Atypical ductal hyperplasia	16
IDC I	32
IDC II	37
IDC III	14

Table 3: Distribution of breast lesions in four categories (n=260) according to diagnosis, age & Micronuclei score.

Type of Lesion	Number of cases	Age range	Mean age	Micronuclei range (1000 cells)	Mean micronuclei
Fibroadenoma	152	17-50	26.76	0-2	0.36
Usual ductal hyperplasia	16	20-53	32.4	1-3	2.18
Atypica ductal hyperplasia	9	30-50	39	3-8	5.22
Infiltrating ductal carcinoma	83	23-77	48.13	6-18	11.158

Table 4: Distribution of Micronuclei in different grades of ductal carcinoma breast.

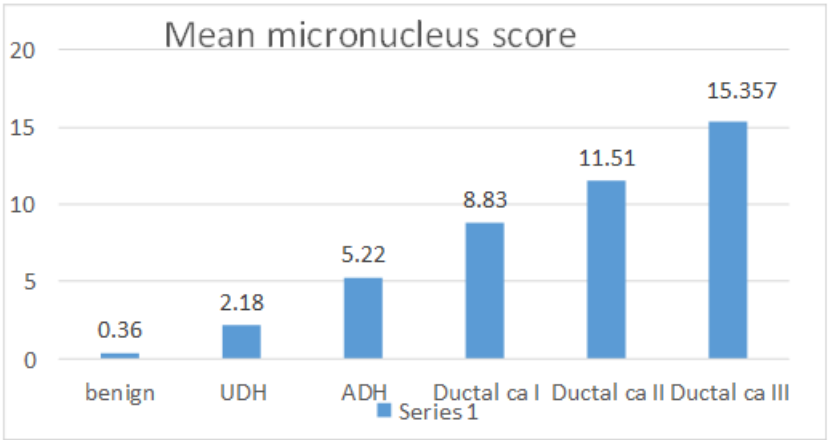
Robinsons grading of infiltrative duct carcinoma (83)	Number of cases	Age range	Mean age	Micronuclei range (1000 cells)	Mean micronuclei
Grade I (score 6-11)	32	35-75	45.2	6-12	8.83
Grade II (score 12-14)	37	23-73	48.13	7-15	11.51
Grade III (score 15-18)	14	35-77	57.5	12-18	15.357

Table 5: Occurrence of metastatic liver carcinoma according to age & sex and mean MN score.

	Total cases	Age range	Age mean	MN score range	MN score mean
Male	97	22-98	55.58	1-13	5.75
Female	112	24-81	56.74	2-14	5.07
Total	209	22-98	56.144	1-14	5.420

Table 6: MN score in cervix lesions (HSIL)

	Total cases	Age range	Age mean	MN score range	MN score mean
HSIL	9	45-85	57.44	6-12	8.22



Graph 1: Shows increasing mean MN Score from benign to malignant cases in breast lesions.

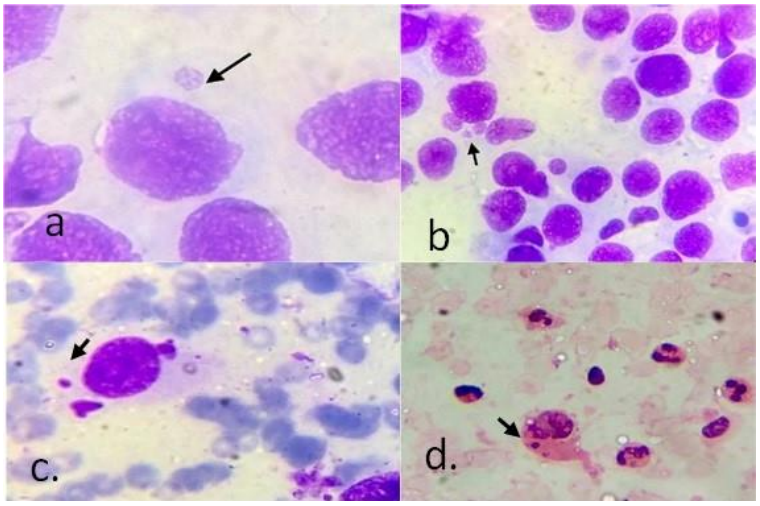


Figure 1: Arrow point to micronuclei in cytoplasm of infiltrative ductal carcinoma of breast., MGG stain, & HE stain at 400x, and 1000x in a, b, c: in cytoplasm of metastatic liver neoplasm, MGG stain at 1000x d: in HSIL cervix, H&E 1000x.

DISCUSSION

Cancer is a genomic disease associated with genetic damage accumulation and have chromosomal instabilities that play an important role in cancer development and progression. Chromosomal instabilities in breast occur in the form of P53 mutations, BRCA 1, BRCA 2 mutations, CHEK 2 mutations which occur both in familial and sporadic carcinomas. Thus screening for chromosomal instabilities is very important. This can be done by using micro nuclei scoring, and by looking into the aneuploidy status as they are very sensitive indicators of chromosomal instabilities.[6,7] As Micronuclei considered as an upcoming marker for genotoxic damage, the present study was conducted to observe the potential role of micronucleus score in identifying a susceptibility to cancer related to familiar or genetic factors in fine needle aspiration cytology smears of various epithelial lesions.

In our study we noticed a significant difference in occurrence of micronuclei between benign and malignant cases as well as between various grades of breast malignancy [Graph 1]. Similar findings has been described by Samantha S and Goel et al.[8,9] In our study mean micronucleus scoring for fibroadenoma was 0.36 ± 0.9 (Range 0–2). This is comparable to the baseline micronucleus scoring of normal individuals of 1.08–1.23, proving there is no genomic damage in the benign group.[10] When the micronucleus score is ≥2 in the benign appearing cytology smear, it gives us a clue of proliferative activity and warrants further evaluation with biopsy. There is only 1 study in this category. UDH group (16 cases) had an average micronucleus score of 2.18 ± 1.26 (Range 1–3) which is higher than the benign group and statistically significant ($P < 0.001$). MN score of 2-5 had a sensitivity of 93% and specificity of 98%. ADH cases had an average micronucleus score of 5.22 ± 2.792 (Range 3–8) showing high margin of statistically significant difference from the benign, and UDH groups. The mean MN score of our study for malignant group 11.158 ± 5.56 (Range 6–18), is similar to studies done by Goel et al. and Samanta et al. who showed an average score of 9.3 and 13.6, respectively.[9,8] They also did not find much variation according to the grade in contrast to our study. The difference in the average micronucleus score between the four groups and three grades of tumor were statistically significant ($P < 0.001$). Further, comparing the grade of the tumor with the micronucleus score there was a steady increase in score from grade one to three tumors (8.83 ± 2.594, 11.51 ± 3.794, and 15.357 ± 2.98 respectively). Further, micronucleus score of ≥8 had a high sensitivity (91%) and specificity (99%) of detecting malignancy. In present study only cases of HSIL were included, in 9 cases of HSIL mean MN scoring is 8.22 which is compatible with the study done by Gayathri et al (2012) with a mean MN score of 8.03±1.64 but less than study done by Samanta et al (2011) with a mean MN score of 21.30±17.18.[11,8] In present study 209 cases of metastasis to liver from epithelial origin are taken. In which mean micronuclei scoring was 5.42 which is less than study done by sheikh SS et al (2019).[12]

CONCLUSION

To summarize MN scores were low in the benign and there is a gradual increase in scores from the benign to the malignant category. Hence, it proves the gradual increase of genomic damage from benign to malignant cases and also serves as an additional tool in the classification of breast lesions on cytology, especially the borderline grey zone categories of ductal hyperplasia. MN scoring on the cytology smear is a tedious process but it is cheap & readily be done in any laboratory in routinely processed & stained smears. MN scoring on the epithelial cells of cervix and liver could be used as biomarker in cancer screening and manifestation of genetic damage or chromosomal breakage. It is a simple, reliable, reproducible test which can be performed with routine staining.

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