Journal of Cardiovascular Disease Research

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 09, 2023

ROLE OF DNA PLOIDY IN PROGNOSIS OF BREAST CARCINOMA

Jyoti Namdev[1], Meena Singrol[2], Nancy Mourya[1], Puja Singh[1*], Sachin Sharma[3], Manish Kaushal[3], Shikha Ghanghoria[3]

1: Bundelkhand Government Medical College, Sagar
2: Atal Bihari Vajpayee Government Medical College, Vidhisha
3: Mahatma Gandhi Medical College, Indore
* Corresponding Author

Abstract

Breast cancer, which accounts for over a quarter (24.2%) of all cancer cases and is the most frequently diagnosed cancer and the leading cause of cancer death in women globally, is expected to see 2.1 million new cases diagnosed in 2022.

For determining the prognosis of a patient with breast cancer, there are numerous new adjunctive modalities available. The patient's prognosis can be learned a great deal from the flow cytometry.

Important prognostic data can be obtained from DNA ploidy analysis, which can also aid in forecasting the behavior and aggressiveness of breast carcinomas. An aberrant DNA content condition called aneuploidy is frequently linked to more aggressive tumour characteristics, a higher likelihood of recurrence, and a worse prognosis in breast cancer patients.

The present study was carried out to evaluate the impact of DNA ploidy on patient outcomes, such as disease-free survival, overall survival, and response to treatment.

The prospective cohort study was conducted over a two-year period with the patient's previous agreement and with institutional ethical approval.

Keywords

Breast carcinoma, DNA Ploidy, Tumour grade, Immunohistochemistry, Flow cytometry

Introduction

A whopping 14% of all cancers in women are breast cancer, which is the most common malignancy among Indian women. 87,000 reported deaths and 1.6 lakh new cases of breast cancer were mentioned in a 2022 study on the subject. [1,2]. It was the cause of 15% of all cancer-related fatalities in women. [3] Although it is believed to be a disease of the affluent world, incidence rates are rising in almost every region worldwide. [4] Increased life expectancy, increased urbanization, the adoption of a Western lifestyle, and changes in reproductive behavior are to blame for the rising incidence of breast carcinoma in the developing world. [5]. When compared to Western women, breast cancer in Indian women is found to be a decade younger, indicating that breast cancer develops in India earlier in the premenopausal period. Cancers tend to be more aggressive in young people. [6]. It can happen at any age, but in India, the incidence rates grow in the early 30s and reach their peak between the ages of 50 and 64. [4-5].

Although breast cancer survival rates vary from country to country, they have improved since the global awareness campaign. This is due to the fact that breast cancer is discovered sooner and treatment regimens have improved over time in nations where individuals have access to quality healthcare. [7]. The five-year survival rate for breast cancers

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 09, 2023

found at an early stage is 80–90% in many developed countries with access to high-quality healthcare; this number drops to 24% for tumors discovered at a later stage.

The Global Surveillance of Trends in Cancer Survival 2000-14 (CONCORD-3) study found that there is still a significant disparity between countries, with numbers as low as 66.1% in India. [7,8].

One of the most prevalent forms of malignant neoplasm is invasive breast cancer, which has a variable prognosis due to the variety of its clinical course and receptivity to treatment.

The different clinic pathological characteristics that are used to evaluate the prognosis and therapeutic outcomes for this condition frequently fall short of capturing the complexity and clinical diversity of breast cancer. The course of breast cancer is influenced by a variety of predictive and prognostic markers. [9]

Prognostic factors are linked to the nature of the disease, while predictive factors determine how well a patient will respond to treatments. [5,7]

The American Joint Committee on Cancer (AJCC)'s published TNM (tumor size and extent, lymph node involvement, and distant metastases) method is used to stage breast cancer. [9,10].

Aims and Objectives

- To evaluate the reliability of DNA ploidy analysis by flow cytometry on cell suspension of Fine Needle Aspirate of Breast lump
- To investigate the relationship between DNA ploidy and prognosis in patients with breast carcinoma.
- To determine whether DNA ploidy can serve as a prognostic biomarker for breast carcinoma.

Methodology

After receiving proper approval from the departmental scientific committee and the institutional ethical committee, the prospective cohort study was conducted. The clinical information and tumour samples were gathered from a group of people who have breast cancer.

Study Design: A prospective cohort study of DNA Ploidy on breast tissue during the COVID peak wave in India.
Sample Size: A total of 50 cases were taken for the study after due consent was obtained from the patient.
Inclusion Criteria: All clinically suspected cases of breast carcinoma
Exclusion Criteria: All benign breast lesions.

Each case included clinical information such as age, sex, parity, site, length of symptoms, size of lump, use of oral contraceptives, use of neoadjuvant chemotherapy status, and radiological information such as USG/CT scan. A fine needle aspirate (FNA) sample from the breast lump was collected prior to surgery in order to perform flow cytometry and determine the DNA ploidy.

The standard procedure for surgical grossing of the specimens was adhered to in every instance. The Beckman Coulter flow cytometer was used for the flow cytometry. Each example of a malignant breast tumour was compared to a benign breast lesion (a fibroadenoma). The investigation of DNA ploidy was conducted. DNA index, diploidy, and aneuploidy were calculated.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 09, 2023

Results

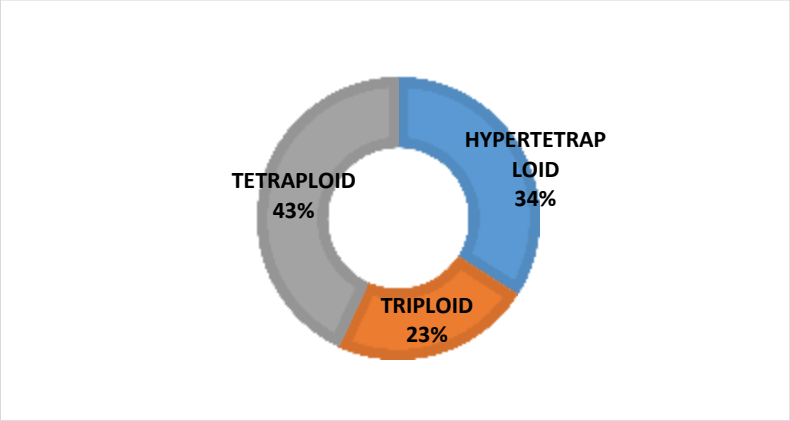
A total of 50 probable breast cancer cases were noted throughout a two-year period. On fine needle aspiration samples, a DNA flow cytometric assay was performed prior to surgery. Each case of a malignant tumour has been compared to a benign breast lesion (fibroadenoma). The DNA ploidy was studied both for diploidy and anaploidy represented in Table 1 and figure 1.

Table 1: Frequency of cases and controls according to ploidy in the study group

	Benign Breast Tumour	Malignant
	Control	Case
Diploid DI (<1.5)	50 (100%)	11 (22%)
Aneuploid (>1.5)	0	39 (78%)

In our study group, the benign breast lesions were taken as controls and the malignant ones were the cases. Among 39 aneuploid cases (DI >1.5) 9 tumors (23.10%) were found to be triploid, 17 (43.50%) were tetraploid and 13 (34.40%) were hyper tetraploid. The same has been depicted in Figure 1.

Figure 1: Frequency of Aneuploid cases in the study group



There was significant correlation between ploidy and tumor size (p-0.01) which showed that larger tumors (T3 >5cm) were aneuploidy as shown in (Table 2)

Table 2: Association of ploidy with multiple factors such as tumour size, and node involvement with prognosis

	Ploidy		Total
	Diploid	Aneuploid	
Size			
T1 (>2 CM)	3 (27.3%)	1 (2.6%)	4
T2 (2-5 CM)	5 (45.4%)	13 (33.3%)	18
T3 (> 5 CM)	3 (27.3%)	25 (64.1%)	28
Total	**11**	**39**	**50**

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 09, 2023

Node status			
Negative (N-0)	6 (54.6%)	9 (23.1%)	15
Positive (N 1-3)	5 (45.4%)	30 (76.9%)	35
Total	**11**	**39**	**50**
Prognosis			
Good	8 (72.7%)	8 (20.5%)	16
Bad	3 (27.3%)	31 (79.5%)	34
Total	**11**	**39**	**50**

The table 2 shows the correlation between the prognostic factors along with tumor size and has been studied and it shows a higher association of T3 along with positive node having a poorer prognosis.

Figure 2-Correlation between DNA Index (DI) and Age

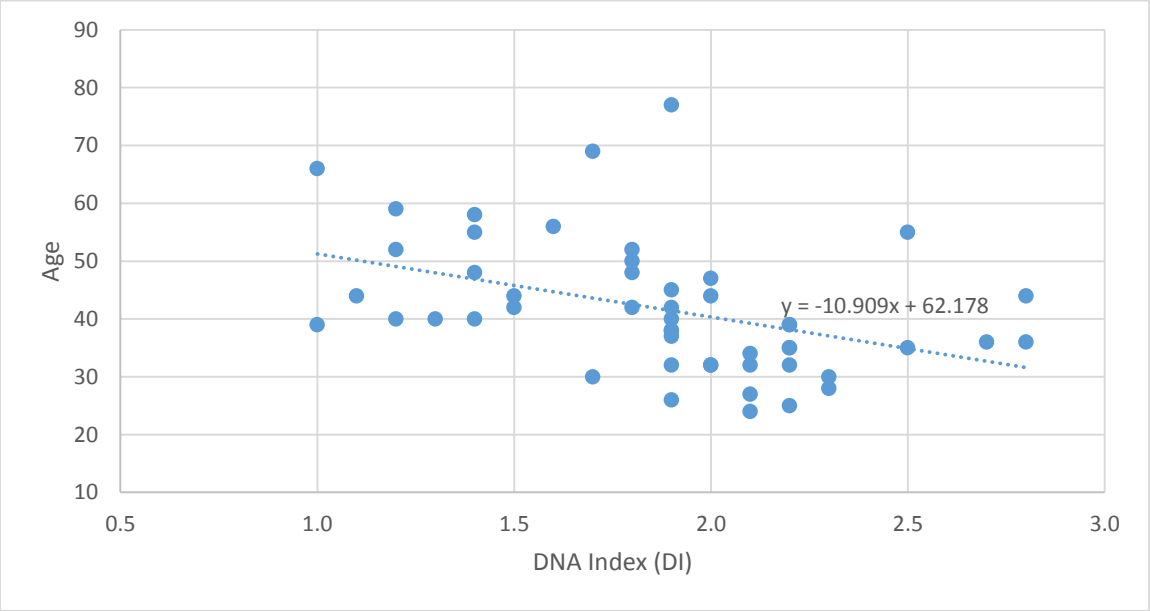


Figure 2 demonstrates a Pearson association coefficient, indicating a strong inverse association between age and DNA index.

Table 3: Histological grading, ER/PR, and HER2 immunohistological stain grading

Histological Grade	Ploidy		Total
	Diploid	Aneuploid	
Grade II	4 (80%)	7 (13.3%)	11
GradeIII	1 (20%)	23 (86.7%)	24
Total	**5**	**30**	**35**
ER/PR status			
Positive	4 (60%)	6 (6.7%)	10
Negative	1 (40%)	24 (93%)	25

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 09, 2023

Total	**5**	**30**	**35**
HER 2nU			
Positive	2 (40%)	7 (23.3%)	9
Negative	3 (60%)	23 (76.7%)	26
Total	**5**	**30**	**35**
Menopause			
Pre menopause	4 (44.40%)	23(86.90%)	27
Post menopause	5 (55.6%)	3 (10.4%)	8
Total	**9**	**29**	**35**

The table makes it clear that aneuploidy is associated with higher histological grading along with the postmenopausal age group being more commonly affected. Her 2 and ER/PR is also more negative in association with aneuploidy.

Figure3: DNA Diploid histogram(DI-1.0)

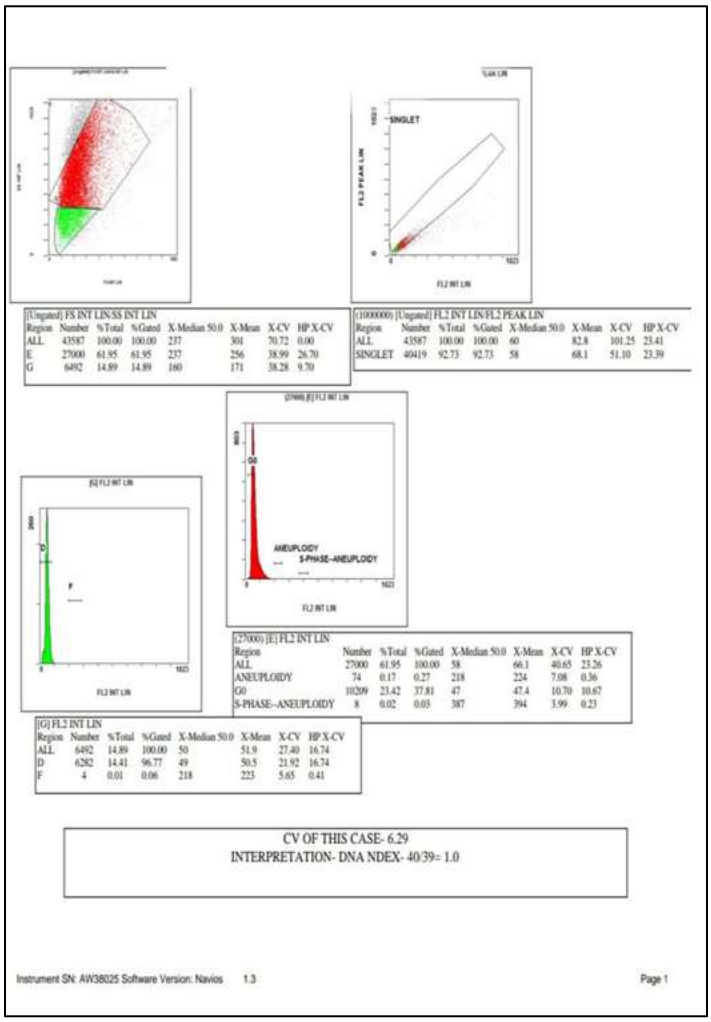


Figure 3 shows the actual histogram of the cases undergoing flow cytometry.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 09, 2023

Discussion

The study was carried out in India during the Covid wave peak over a period of two years. The DNA ploidy of 50 consecutively diagnosed breast cancer patients was assessed using the flow cytometric technique. When the results of the present study were scrutinized against the background of the available literature, many results were concordant with the previous studies.

According to a study by Pinto et al., tumours in younger premenopausal females (under the age of 40) were more likely to be associated with aneuploidy, which is consistent with our data (above the age of 45, p-0.03). [11]

The number of diploid and aneuploid tumors was roughly the same in postmenopausal females, but the number of aneuploid tumours was more than six times higher in premenopausal patients. In premenopausal females, ploidy was an additional, independent predictive factor.

According to Moureau et al. research, larger tumours were discovered to have higher DNA indices and aneuploidy, which is consistent with our study's findings that ploidy and tumour size were correlated ($p = 0.01$) and that 64.1% of aneuploidy tumours were larger than 5 cm. [12]

According to research by Pinto et al., aneuploidy and DNA index were shown to be higher in larger tumours (T3->5cm) (73.9% and p-0.006). Similar findings were made in our investigation, where we discovered that two-thirds (64.1%, $p = 0.01$) of the tumours with a diameter larger than 5 cm were aneuploid. [11]

Ploidy and grading were found to have a substantial correlation in the study by Gazic B, Pizem, et al. According to our study, which indicated that patients with aneuploidy tumours were of grade III (64.1%, p-0.02), patients with aneuploid grade III tumours exhibited poor clinical outcomes. [13]

According to Xuang J. et al., aneuploidy and positive lymph nodes have a positive correlation (pN0 vs. pN1-3: p0.00001), which is consistent with our study's finding that aneuploid tumours are more likely to metastasize to the lymph nodes (p-0.04). [14]

In line with a study by Tsutsui et al., which found that tumours with lower DNA ploidy tend to be ER/PR positive and those with higher DNA ploidy were more likely to be ER/PR negative, our current study suggests that tumours lacking hormone receptor activity (Oestrogen receptors and Progesterone receptors) are more likely to be aneuploid (p-0.019). [15]

Dayal et al. investigated aneuploidtumours and found no significant relationships, which is consistent with our data. They also evaluated tumours with human epidermal growth factor receptor 2 (HER2) status. Ploidy of the DNA was more likely to be ER/PR negative. [16]

According to research by Kawauchi et al., 69.4% of the tumours were aneuploid. Similar findings were made in our investigation, where we found that aneuploid tumours tended to be present in more than two-thirds (78%) of the cases. [17]

Our study suggested that DNA aneuploidy tended to have a high grade of tumor differentiation ($p<0.02$) [11,18] greater tumor size ($p<0.01$) [11,15], lack of hormone (estrogen and progesterone) receptors ($p<0.019$) [9, 11,13] and nodal status ($p<0.04$) [11,18, 19].

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 09, 2023

Our study also found that younger age females are more likely to have higher tumours (p-0.02), a lack of hormone (oestrogen and progesterone) receptors (p-0.01), and a high DNA index (DI>1.9; p-0.01), which suggests that younger females are more likely to present with highly aggressive breast tumours. These findings are almost identical to those of Pinto et al.'s research. [11]

Conclusion

This study suggested that when DNA flow cytometric analysis was performed on fresh tumor samples, DNA aneuploidy tended to be one of the substantial prognostic factors for breast cancer.

DNA ploidy status has been shown to be an independent prognostic indicator in breast carcinoma. Patients with aneuploid tumors, characterized by abnormal DNA content, generally have a poorer prognosis compared to patients with diploid tumors.

The majority of the cases of invasive breast cancer were aneuploid. Aneuploid tumors with a DNA Index of more than 1.5 were related to large tumor size, nodal involvement, high histological grade, and lack of hormone receptors. Tumors with poor histomorphological prognosis were more commonly aneuploid.

Limitation

The small sample size is because the study was carried out between COVID periods so the number of non-covid admissions had reduced in the hospital.
There is a need for studying more of other immunohistochemistry parameters along with DNA ploidy for the outcomes to be measured in carcinoma breast.

Conflict of interest: The authors do not have any conflict of interest.

References

1. Breast Cancer Statistics: India versus The World & Comparison of Global Statistics from Globocan 2022
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68(6): 394-424. doi: 10.3322/caac.21492.
3. WHO.http://www.who.int/cancer/prevention/diagnosis-screening/breast-cancer/en/.
4. Nandakumar A. National Cancer Registry Programme. Consolidated Report of the Population Based Cancer Registries. Incidence and Distribution of Cancer: 1990–96. Indian Council of Medical Research, New Delhi. Cancer. 2019; 125: 3184-97.
5. Das DS, Yengkhom IS, Sekar V, Akoijam S, Adhikarimayum AD, Chongthu JL. Study of evolving trends in the clinicopathological profile of breast cancer patients attending a tertiary cancer center. Journal of Medical Society. 2019; 33(2): 71-75. doi: 10.4103/jms.jms_82_18
6. Karim MO, Khan KA, Khan AJ, Javed A, Fazid S, Aslam MI. Triple Assessment of Breast Lump: Should We Perform Core Biopsy for Every Patient? Cureus. 2020; 12(3): e7479. doi: 10.7759/cureus.7479.
7. Malvia S, Bagadi SA, Dubey US, Saxena S. Epidemiology of breast cancer in Indian women. Asia Pac J Clin Oncol. 2017;13(4):289-295. doi: 10.1111/ajco.12661.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 09, 2023

8. Mahmood H, Faheem M, Mahmood S, Sadiq M, Irfan J. Impact of age, tumor size, lymph node metastasis, stage, receptor status and menopausal status on overall survival of breast cancer patients in Pakistan. Asian Pac J Cancer Prev. 2015; 16(3): 1019-24. doi: 10.7314/apjcp.2015.16.3.1019.
9. da Costa Vieira RA, Biller G, Uemura G, Ruiz CA, Curado MP. Breast cancer screening in developing countries. Clinics (Sao Paulo). 2017; 72(4): 244-253. doi: 10.6061/clinics/2017(04)09.
10. Moutafoff C, Coutant C, Bézu C, Antoine M, Werkoff G, Benbara A, et al. Facteurs prédictifs et pronostiques des cancers du sein multifocaux [Prognostic and predictive factors in multifocal breast carcinoma]. Gynecol Obstet Fertil. 2011; 39(7-8): 425-32. French. doi: 10.1016/j.gyobfe.2011.05.001.
11. Pinto AE, Pereira T, Santos M, Branco M, Dias A, Silva GL, et al. DNA ploidy is an independent predictor of survival in breast invasive ductal carcinoma: a long-term multivariate analysis of 393 patients. Ann Surg Oncol. 2013; 20(5): 1530-7. doi: 10.1245/s10434-012-2804-6.
12. Moureau-Zabotto L, Bouchet C, Cesari D, et al. Combined flow cytometry determination of S-phase fraction and DNA ploidy is an independent prognostic factor in node-negative invasive breast carcinoma: analysis of a series of 271 patients with stage I and II breast cancer. Breast Cancer Res Treat. 2005; 91: 61–71. doi: 10.1007/s10549-004-7047-1
13. Gazic B, Pizem J, Bracko M, Cufer T, Borstnar S, Pohar-Marinsek Z, et al. S-phase fraction determined on fine needle aspirates is an independent prognostic factor in breast cancer - a multivariate study of 770 patients. Cytopathology. 2008; 19(5): 294-302. doi: 10.1111/j.1365-2303.2007.00528.x.
14. Xu J, Huang L, Li J. DNA aneuploidy and breast cancer: a meta-analysis of 141,163 cases. Oncotarget. 2016 Sep 13;7(37):60218-60229. doi: 10.18632/oncotarget.11130.
15. Tsutsui S, Ohno S, Murakami S, et al. Prognostic value of DNA ploidy in 653 Japanese women with node-negative breast cancer. Int J Clin Oncol. 2001; 6: 177–182. doi:10.1007/PL00012103
16. Dayal JH, Sales MJ, Corver WE, Purdie CA, Jordan LB, Quinlan PR, Baker L, ter Haar NT, Pratt NR, Thompson AM. Multiparameter DNA content analysis identifies distinct groups in primary breast cancer. Br J Cancer. 2013; 108(4): 873-80. doi: 10.1038/bjc.2013.42.
17. Kawauchi S, Furuya T, Ikemoto K, Nakao M, Yamamoto S, Oka M, Sasaki K. DNA copy number aberrations associated with aneuploidy and chromosomal instability in breast cancers. Oncol Rep. 2010; 24(4): 875-83. doi: 10.3892/or.2010.875.
18. Pinto AE, André S, Mendonça E, Silva G, Soares J. Overall survival in advanced breast cancer: relevance of progesterone receptor expression and DNA ploidy in fine-needle aspirates of 392 patients. Int J Biol Markers. 2003; 18(1): 7-12. doi: 10.1177/172460080301800102.
19. llemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Nikšić M, et al. CONCORD Working Group. Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet. 2018; 391(10125): 1023-1075. doi: 10.1016/S0140-6736(17)33326-3.