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Original Research Article

Survivin and Caspase-3 as Biomarkers in Breast Cancer: Correlation with Clinical and Pathological Parameters and the Nottingham Prognostic Index

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Abstract

Introduction: Apoptosis maintains cellular balance by eliminating damaged cells, and its dysregulation is key in cancer progression. Survivin and Caspases regulate apoptosis via the extrinsic and intrinsic pathways. Survivin aids cancer cell survival by blocking apoptosis, while high caspase-3 levels link to poorer breast cancer survival, highlighting its potential prognostic significance as a biomarker.

Aims: To assess the expression of survivin and capase-3 in paraffin-embedded breast cancers samples and examine its association with the clinicopathological characteristics of the patients and the Nottingham prognostic index.

Materials & Methods: The present study was a Cross sectional observational study. This Study was conducted from 18 Months at Department of General Surgery, IHC lab and Department of Pathology IPGMER and SSKM hospital, Kolkata. Total 219 patients were included in this study.

Result: According to statistical analysis, Pearson Correlation Coefficient (r) value was higher in Survivin as compared to Caspase-3. Prognostic significance was more specific in Survivin as compared to Caspase-3 which was statistically significant.

Conclusion: We also conclude that inhibition Survivin may decrease tumour progression in patients and may block breast carcinogenesis, reducing the incidence of breast carcinoma in patients at high risk. To evaluate this fact further study with large sample size is to be contemplated. Further research is warranted to explore the clinical implications of our findings and to translate them into improved outcomes for breast cancer patients.

Keywords: Survivin, Breast Cancer, caspase-3 and Microenvironment.

Introduction

Apoptosis, or programmed cell death, is essential for maintaining homeostasis by removing senescent or damaged cells.[1] Impaired regulation of apoptosis is a defining characteristic of human cancers. Carcinogenesis is linked with an imbalance between apoptosis and cell proliferation. Different genes are either pro-apoptotic or antiapoptotic and they regulate apoptotic signaling pathways. [2]

Survivin and Caspases are proteins maintaining the pathways of apoptosis is initiated through two primary pathways: the extrinsic (death receptor) pathway, which is triggered when ligands bind to death receptors, leading to caspase-8 and then caspase-3 activation; and the intrinsic (mitochondrial) pathway, activated by mitochondrial cytochrome c release. This release forms a complex with Apaf-1 and cytochrome c, aided by ATP, and subsequently activates caspase-9, which in turn activates caspase-3. [3]

Survivin is a protein within the inhibitor of apoptosis (IAP) family. [4] Survivin is involved in multiple biological pathways, with a key role in evading apoptosis. It directly interferes with and suppresses the apoptotic cascade, helping cells avoid programmed cell death. [4] High caspase-3 expression is significantly associated with adverse breast cancer-specific survival. Breast cancer,

which adversely affects women's physical and psychological health, has the highest incidence of female malignant tumors. It is reported that approximately 1.7 million newly diagnosed cases and 521,900 deaths occurred on a global scale in 2012 [5]. Breast cancer is usually divided into ductal or lobular carcinoma according to location, and invasive ductal carcinoma (IDC) is the most common type. Although the diagnosis and treatment techniques have improved greatly, patient mortality remains high because of chemotherapy resistance and distant metastases. Therefore, it is necessary to identify a more valuable and convenient biomarker that can be tested in early breast cancer, then used to reduce the disease mortality.

Several studies have reported that breast cancers with a high apoptosis index have a better prognosis than those with lower or absent levels of apoptosis [6–7]. Some studies also showed that apoptosis factors were over-expressed in advanced breast cancer [8, 9]. Caspase-3, the central member of the cysteine-aspartic acid protease (caspase) family, was found to play a dominant role in the apoptotic signaling pathway and to regulate cellular apoptosis. Poly (ADP-ribose) polymerase 1 (PARP-1) is responsible for DNA repair and programmed cell death and is the most important substrate of caspase-3. During the early stages of apoptosis, caspase-3 is cleaved into 29- and 85-kDa fragments [10] by PARP-1. Furthermore, cleavage of caspase-3 was shown to mediate tumor repopulation in apoptotic tumor cells [11]. The change of caspase-3 expression is related to the carcinogenesis and progression of many tumors, such as colon cancer [12], cervical adenocarcinoma [13], and glioma [14], indicating that caspase-3 level may be a useful biomarker for these tumors.

This study aimed to assess the expression of survivin and capase-3 in paraffin-embedded breast cancer samples and examine its association with the clinical characteristics of the patients and the Nottingham prognostic index.

Material and Methods

Study design- Cross sectional observational study

Study place- Department of General Surgery, IHC lab and Department of Pathology IPGMER and SSKM hospital, Kolkata.

Period of study- 18 Months

Study population-All patients undergoing surgery with HPE proven breast cancer

Sample size- 219

Control-Not needed

Inclusion criteria- All patients with HPE proven breast cancer undergoing surgery

Exclusion criteria

1. Metastatic breast cancer
2. Patient not giving consent for the surgery

Data collection and interpretation

Data from 219 patients undergoing surgery for proven breast cancer was collected during the study period from breast cancer patients coming to SSKM Hospital Surgery OPD and Breast clinic. The following data was taken:-

1. Demographic data such as Age, Sex, Address, Occupation, Socioeconomic status to be noted
2. Relevant history, important clinical findings, TNM Staging, radiological evaluation and metastatic workup to be done as per standardized protocol.
3. Procedure done (Modified radical mastectomy or Breast conservative surgery with Axillary lymph nodes dissection or Sentinel lymph nodes biopsy).
4. 2-3 grams Sample tissues taken from the core of the tumour, peripheral normal breast tissue and immediately placed into 10% formalin.
5. After completion of the Histopathological examination and ER, PR, Her-2-neu evaluation the blocks are sent for quantitative evaluation of Survivin and Caspase-3.

Procedure: - All specimens were fixed by 10% formalin and the paraffin embedded tissue sections (4µm thick) were stained with rabbit anti human Livin polyclonal antibody, mouse anti human Caspase 3 polyclonal antibody and mouse anti human Survivin polyclonal antibody respectively. These were incubated overnight after being deparaffinised in xylene and rehydrated in ethanol at 500C.To perform heat induced antigen retrieval, the sections were placed in 10mM citrate buffer (pH-6.0) and heated to boil. Endogenous peroxidase function was quenched using peroxidase blocking solution. After PBS washing; the sections were incubated with streptavidin horse radish peroxide (SA-HRP) conjugated goat anti rabbit secondary antibodies for 30 mins and treated with DAB chromogen substrate buffer for time periods determined by the response of antigen and antibody. The sections were counter stained with hematoxylin for 4-5 mins, washed, dehydrated in ethanol and xylene and the mounted on slides.

Evaluation Criterion-

Cells which stain yellowish-brown were considered to be positive.

Numbers of positive cells were used for quantitative analysis.

For both survivin and caspase

- <30%stained cells was considered 1+
- 30-60% was considered 2+

- >60% was considered 3+

Statistical analysis was done by SPSS software of all the data obtained to find out any significant correlation among the different data entries.

Primary outcome-

Outcome was defined in the terms of correlation between levels of survivin, caspase-3 and the different characteristics, receptor patterns, molecular signatures and NPI of breast cancer. Cells in which Survivin and Caspase-3 are present stained yellowish brown in cytoplasm or cell nucleus were considered to be positive. Numbers of positive cells were used for quantitative analysis.

For regular clinical use, Nottingham Prognostic Index gives a decent idea regarding the aggressiveness of the breast tumour. It is calculated by the formula of: NPI = tumour size (S) × 0.2 + lymph node involvement (LN = 1, 2, or 3) + histological grade (H = 1, 2, or 3) (10).

Here , we will find the correlation between the levels of survivin and casepase-3 in different types of breast cancer and will also evaluate these proteins as markers for prognostication of breast cancer by correlating these quantitative measures with molecular subtypes of breast cancers and NPI (Nottingham Prognostic Index) score of the tumour. In this way a more cost-effective tool can be produced compared to the conventional tools for prognostication of breast cancer.

Secondary outcome-

- Assessment of clinical tumour characteristics
- Assessment of hormone receptor status of tumour
- Assessment of NPI of the patient
- Nottingham Prognostic Index gives a decent idea regarding the anticipated aggressiveness of the breast tumour. It is calculated by the formula of:
- NPI = tumour size (S) × 0.2 + lymph node involvement (LN = 1, 2, or 3) + histological grade (H = 1, 2, or 3(8).
- Assessment of levels of survivin and caspase-3 in the post-operative biopsy

Statistical Analysis:

Data were entered into Microsoft Excel and analyzed using SPSS (version 27.0) and GraphPad Prism (version 5). Numerical variables were summarized as means and standard deviations, and categorical variables as counts and percentages. Two-sample t-tests were used for comparing independent groups, while paired t-tests were used for correlated data. Chi-square tests (χ^2) were employed for categorical variables, with Fisher's exact test used when appropriate.

Statistical significance was determined using a p-value ≤ 0.05, which indicated rejection of the null hypothesis.

Result

Table 1: Association between Stage, T-size, Nodal Status and Grade: Caspase-3 Score (+)

	CASPASE-3 SCORE (+)						
		1	**2**	**3**	**TOTAL**	**Chi-square value**	**p-value**
Stage	**IA**	5	10	1	16	33.2453	0.0002
	Row %	31.3	62.5	6.3	100		
	Col %	13.9	14.5	0.9	7.3		
	IIA	13	29	28	70		
	Row %	18.6	41.4	40	100		
	Col %	36.1	42	24.6	32		
	IIB	12	10	27	49		
	Row %	24.5	20.4	55.1	100		
	Col %	33.3	14.5	23.7	22.4		
	IIIA	5	12	37	54		
	Row %	9.3	22.2	68.5	100		
	Col %	13.9	17.4	32.5	24.7		
	IIIB	1	6	14	21		
	Row %	4.8	28.6	66.7	100		
	Col %	2.8	8.7	12.3	9.6		
	IIIC	0	2	7	9		
	Row %	0	22.2	77.8	100		
	Col %	0	2.9	6.1	4.1		
	TOTAL	36	69	114	219		
	Row %	16.4	31.5	52.1	100		
	Col %	100	100	100	100		
T-size	**T1**	5	12	6	23	26.3934	0.0009
	Row %	21.7	52.2	26.1	100		

	Col %	13.9	17.4	5.3	10.5		
	T2	26	38	48	112		
	Row %	23.2	33.9	42.9	100		
	Col %	72.2	55.1	42.1	51.1		
	T3	4	12	43	59		
	Row %	6.8	20.3	72.9	100		
	Col %	11.1	17.4	37.7	26.9		
	T4b	1	7	16	24		
	Row %	4.2	29.2	66.7	100		
	Col %	2.8	10.1	14	11		
	T4d	0	0	1	1		
	Row %	0	0	100	100		
	Col %	0	0	0.9	0.5		
	TOTAL	36	69	114	219		
	Row %	16.4	31.5	52.1	100		
	Col %	100	100	100	100		
Nodal Status	**N0**	19	40	37	96	15.5283	0.0165
	Row %	19.8	41.7	38.5	100		
	Col %	52.8	58	32.5	43.8		
	N1	15	21	53	89		
	Row %	16.9	23.6	59.6	100		
	Col %	41.7	30.4	46.5	40.6		
	N2	2	6	17	25		
	Row %	8	24	68	100		
	Col %	5.6	8.7	14.9	11.4		
	N3	0	2	7	9		
	Row %	0	22.2	77.8	100		
	Col %	0	2.9	6.1	4.1		
	TOTAL	36	69	114	219		
	Row %	16.4	31.5	52.1	100		
	Col %	100	100	100	100		
Grade	**1**	0	2	2	4	13.6702	0.0084
	Row %	0	50	50	100		
	Col %	0	2.9	1.8	1.8		
	2	32	60	78	170		
	Row %	18.8	35.3	45.9	100		
	Col %	88.9	87	68.4	77.6		
	3	4	7	34	45		
	Row %	8.9	15.6	75.6	100		
	Col %	11.1	10.1	29.8	20.5		
	TOTAL	36	69	114	219		
	Row %	16.4	31.5	52.1	100		
	Col %	100	100	100	100		

Table 2: Association between Stage, T-size, Nodal Status and Grade: Survivin Score (+)

	SURVIVIN SCORE (+)						
	Stage	**1**	**2**	**3**	**TOTAL**	**Chi-square value**	**p-value**
Stage	**IA**	13	2	1	16	28.6861	0.0014
	Row %	81.3	12.5	6.3	100		
	Col %	14.8	2.2	2.5	7.3		
	IIA	33	30	7	70		
	Row %	47.1	42.9	10	100		
	Col %	37.5	33	17.5	32		
	IIB	23	17	9	49		
	Row %	46.9	34.7	18.4	100		
	Col %	26.1	18.7	22.5	22.4		
	IIIA	13	28	13	54		
	Row %	24.1	51.9	24.1	100		

	Col %	14.8	30.8	32.5	24.7		
	IIIB	4	10	7	21		
	Row %	19	47.6	33.3	100		
	Col %	4.5	11	17.5	9.6		
	IIIC	2	4	3	9		
	Row %	22.2	44.4	33.3	100		
	Col %	2.3	4.4	7.5	4.1		
	TOTAL	88	91	40	219		
	Row %	40.2	41.6	18.3	100		
	Col %	100	100	100	100		
T-size	**T1**	15	6	2	23	28.6278	0.0004
	Row %	65.2	26.1	8.7	100		
	Col %	17	6.6	5	10.5		
	T2	56	41	15	112		
	Row %	50	36.6	13.4	100		
	Col %	63.6	45.1	37.5	51.1		
	T3	13	32	14	59		
	Row %	22	54.2	23.7	100		
	Col %	14.8	35.2	35	26.9		
	T4b	4	11	9	24		
	Row %	16.7	45.8	37.5	100		
	Col %	4.5	12.1	22.5	11		
	T4d	0	1	0	1		
	Row %	0	100	0	100		
	Col %	0	1.1	0	0.5		
	TOTAL	88	91	40	219		
	Row %	40.2	41.6	18.3	100		
	Col %	100	100	100	100		
Nodal Status	**N0**	48	38	10	96	16.8617	0.0098
	Row %	50	39.6	10.4	100		
	Col %	54.5	41.8	25	43.8		
	N1	33	39	17	89		
	Row %	37.1	43.8	19.1	100		
	Col %	37.5	42.9	42.5	40.6		
	N2	5	10	10	25		
	Row %	20	40	40	100		
	Col %	5.7	11	25	11.4		
	N3	2	4	3	9		
	Row %	22.2	44.4	33.3	100		
	Col %	2.3	4.4	7.5	4.1		
	TOTAL	88	91	40	219		
	Row %	40.2	41.6	18.3	100		
	Col %	100	100	100	100		
Grade	**1**	1	2	1	4	12.3488	0.0149
	Row %	25	50	25	100		
	Col %	1.1	2.2	2.5	1.8		
	2	78	67	25	170		
	Row %	45.9	39.4	14.7	100		
	Col %	88.6	73.6	62.5	77.6		
	3	9	22	14	45		
	Row %	20	48.9	31.1	100		
	Col %	10.2	24.2	35	20.5		
	TOTAL	88	91	40	219		
	Row %	40.2	41.6	18.3	100		
	Col %	100	100	100	100		

Table 3: Distribution of mean NPI: Caspase-3 Score (+) and Survivin Score (+)

		Number	Mean	SD	Minimum	Maximum	Median	p-value
NPI	**Caspase-3 Score (+) 1**	36	3.5422	0.8951	2.38	6	3.5	<0.0001
	Caspase-3 Score (+) 2	69	3.9574	1.5002	1.56	7.8	3.54	
	Caspase-3 Score (+) 3	114	4.8481	1.7024	2	8	4.4	
NPI	**Survivin Score (+) 1**	88	3.7873	1.2795	1.56	7.8	3.52	<0.0001
	Survivin Score (+) 2	91	4.4411	1.6071	2	7.84	4	
	Survivin Score (+) 3	40	5.396	1.7812	1.8	8	5.2	

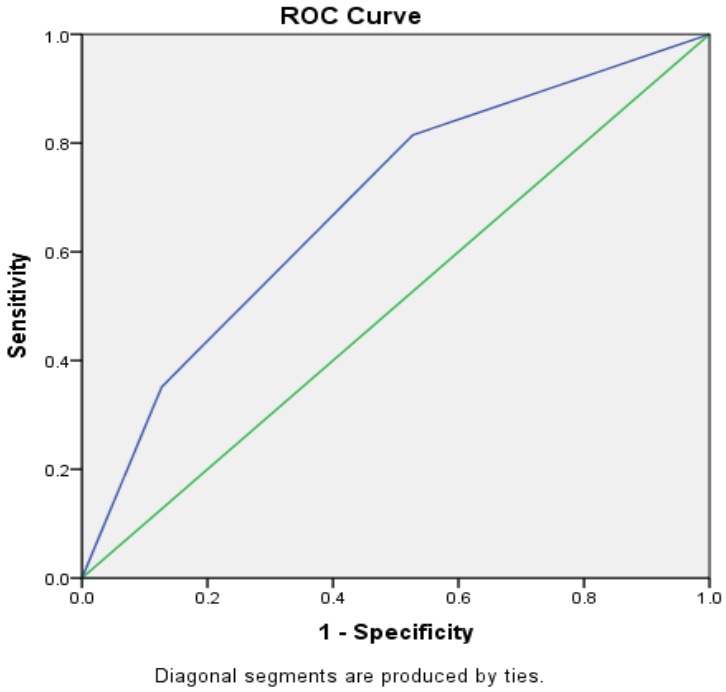

Figure 1: NPI Receiver Survivin Score

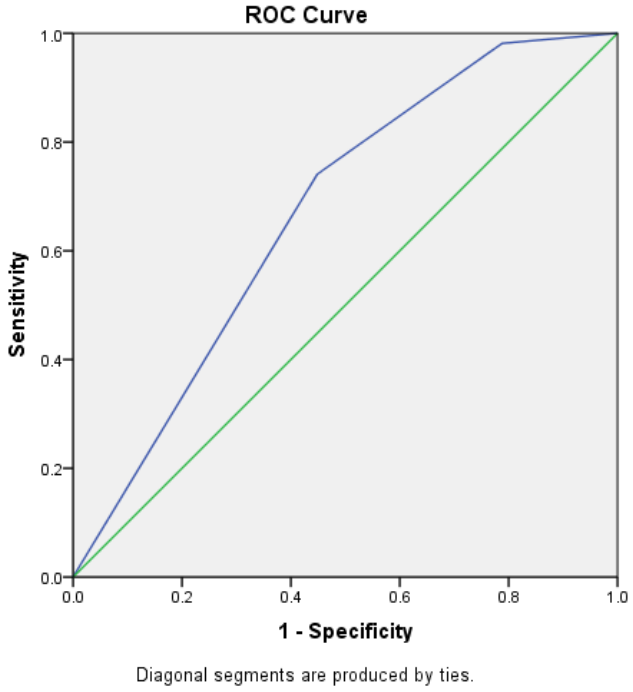

Figure 2: NPI Receiver Caspase-3 Score (+)

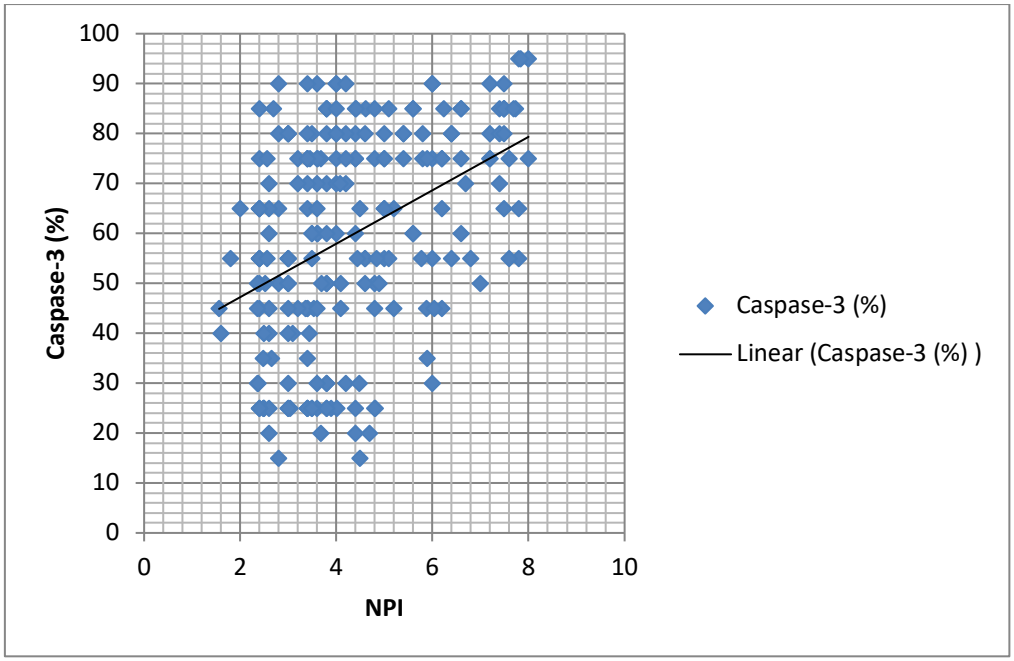

Figure 3: Caspase-3 vs NPI

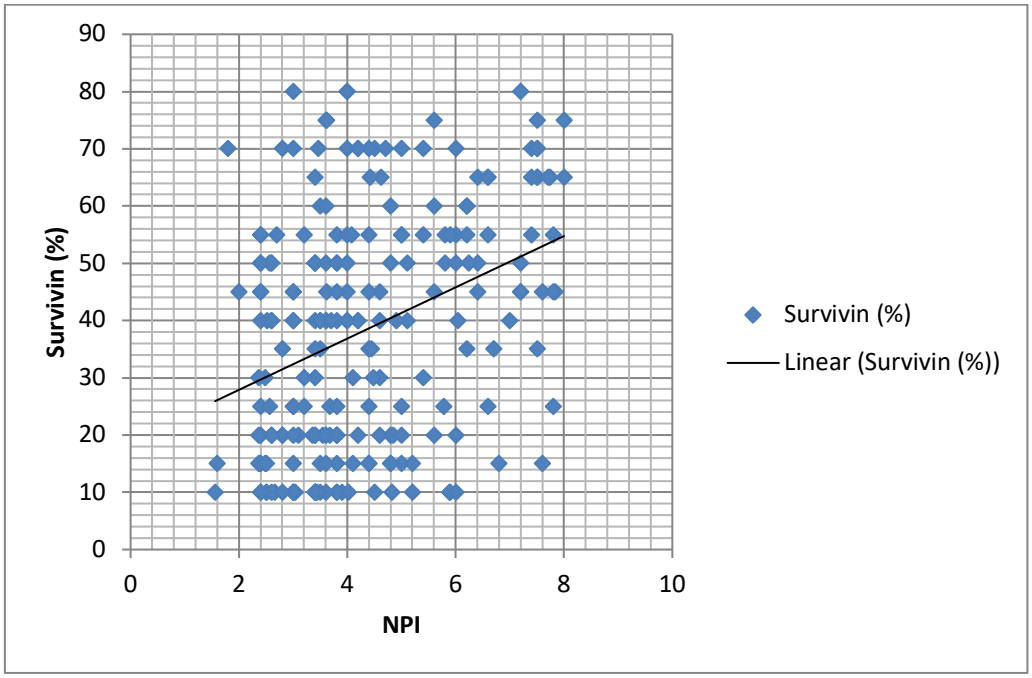

Figure 4: Survivin vs NPI

In Caspase-3 Score (+) 1, 5 (13.9%) patients had IA, 13 (36.1%) patients had IIA, 12 (33.3%) patients had IIB, 5 (13.9%) patients had IIIA and 1 (2.8%) patient had IIIB Stage. In Caspase-3 Score (+) 2, 10 (14.5%) patients had IA, 29 (42.0%) patients had IIA, 10 (14.5%) patients had IIB, 12 (17.4%) patients had IIIA, 6 (8.7%) patients had IIIB and 2 (2.9%) patients had IIIC Stage. In Caspase-3 Score (+) 3, 1 (0.9%) patient had IA, 28 (24.6%) patients had IIA, 27 (23.7%) patients had IIB, 37 (32.5%) patients had IIIA, 14 (12.3%) patients had IIIB and 7 (6.1%) patients had IIIC Stage. Association of Stage with Caspase-3 Score (+) was statistically significant (p=0.0002). In Caspase-3 Score (+) 1, 5 (13.9%) patients had T1, 26 (72.2%) patients had T2, 4 (11.1%) patients had T3 and 1(2.8%) patient had T4b T-size. In Caspase-3 Score (+) 2, 12 (17.4%) patients had T1, 38 (55.1%) patients had T2, 12 (17.4%) patients had T3 and 7 (10.1%) patients had T4b T-size. In Caspase-3 Score (+) 3, 6 (5.3%) patients had T1, 48 (42.1%) patients had T2, 43 (37.7%) patients had T3, 16 (14.0%) patients had T4b and 1 (0.9%) patient had T4d T-size. Association of T-size with Caspase-3 Score (+) was statistically significant (p=0.0009). In Caspase-3 Score (+) 1, 19 (52.8%)

patients had N0, 15 (41.7%) patients had N1and 2 (5.6%) patients had N2 Nodal Status. In Caspase-3 Score (+) 2, 40 (58.0%) patients had N0, 21 (30.4%) patients had N1, 6 (8.7%) patients had N2 and 2 (2.9%) patients had N3 Nodal Status. In Caspase-3 Score (+) 3, 37 (32.5%) patients had N0, 53 (46.5%) patients had N1, 17 (14.9%) patients had N2 and 7 (6.1%) patients had N3 Nodal Status. Association of Nodal Status with Caspase-3 Score (+) was statistically significant (p=0.0165).

In Caspase-3 Score (+) 1, 32 (88.9%) patients had Grade 2 and 4 (11.1%) patients had Grade 3. In Caspase-3 Score (+) 2, 2 (2.9%) patients had Grade 1, 60 (87.0%) patients had Grade 2 and 7 (10.1%) patients had Grade 3. In Caspase-3 Score (+) 3, 2 (1.8%) patients had Grade 1, 78 (68.4%) patients had Grade 2 and 34 (29.8%) patients had Grade 3. Association of Grade with Caspase-3 Score (+) was statistically significant (p=0.0084). In Survivin Score (+) 1, 13 (14.8%) patients had Stage IA, 33 (37.5%) patients had Stage IIA, 23 (26.1%) patients had Stage IIB, 13 (14.8%) patients had Stage IIIA, 4 (4.5%) patients had Stage IIIB and 2 (2.3%) patients had Stage IIIC.

In Survivin Score (+) 2, 2 (2.2%) patients had Stage IA, 30 (33.0%) patients had Stage IIA, 17 (18.7%) patients had Stage IIB, 28 (30.8%) patients had Stage IIIA, 10 (11.0%) patients had Stage IIIB and 4 (4.4%) patients had Stage IIIC. In Survivin Score (+) 3, 1 (2.5%) patient had Stage IA, 7 (17.5%) patients had Stage IIA, 9 (22.5%) patients had Stage IIB, 13 (32.5%) patients had Stage IIIA, 7 (17.5%) patients had Stage IIIB and 3 (7.5%) patients had Stage IIIC. Association of Stage with Survivin Score (+) was statistically significant (p=0.0014) In Survivin Score (+) 1, 15 (17.0%) patients had T1, 56 (63.6%) patients had T2, 13 (14.8%) patients had T3 and 4 (4.5%) patients had T4b T-size. In Survivin Score (+) 2, 6 (6.6%) patients had T1, 41 (45.1%) patients had T2, 32 (35.2%) patients had T3, 11 (12.1%) patients had T4b and 1 (1.1%) patient had T4b T-size. In Survivin Score (+) 3, 2 (5.0%) patients had T1, 15 (37.5%) patients had T2, 14 (35.0%) patients had T3 and 9 (22.5%) patients had T4b T-size.

Association of T-size with Survivin Score (+) was statistically significant (p=0.0004). In Survivin Score (+) 1, 48 (54.5%) patients had N0, 33 (37.5%) patients had N1, 5 (5.7%) patients had N2 and 2 (2.3%) patients had N3 Nodal Status. In Survivin Score (+) 2, 38 (41.8%) patients had N0, 39 (42.9%) patients had N1, 10 (11.0%) patients had N2 and 4 (4.4%) patients had N3 Nodal Status.

In Survivin Score (+) 3, 10 (25.0%) patients had N0, 17 (42.5%) patients had N1, 10 (25.0%) patients had N2 and 3 (7.5%) patients had N3 Nodal Status. Association of Nodal Status with Survivin Score (+) was statistically significant (p=0.0098). In Survivin Score (+) 1, 1 (1.1%) patient had Grade 1, 78 (88.6%) patients had Grade 2 and 9 (10.2%) patients had Grade 3. In Survivin Score (+) 2, 2 (2.2%) patients had Grade 1, 67 (73.6%) patients had Grade 2 and 22 (24.2%) patients had Grade 3. In Survivin Score (+) 3, 1 (2.5%) patient had Grade 1, 25 (62.5%) patients had Grade 2 and 14 (35.0%) patients had Grade 3. Association of Grade with Survivin Score (+) was statistically significant (p=0.0149).

In Caspase-3 Score (+) 1, the mean NPI (mean± s.d.) of patients was 3.5422± .8951. In Caspase-3 Score (+) 2, the mean NPI (mean± s.d.) of patients was 3.9574± 1.5002. In Caspase-3 Score (+) 3, the mean NPI (mean± s.d.) of patients was 4.8481± 1.7024. Distribution of mean NPI with Caspase-3 Score (+) was statistically significant (p<0.0001). In Survivin Score (+) 1, the mean NPI (mean± s.d.) of patients was 3.7873± 1.2795. In Survivin Score (+) 2, the mean NPI (mean± s.d.) of patients was 4.4411± 1.6071. In Survivin Score (+) 3, the mean NPI (mean± s.d.) of patients was 5.3960± 1.7812. Distribution of mean NPI with Survivin Score (+) was statistically significant (p<0.0001). NPI Receiver Caspase-3 Score (+) area was .669 with 95% confidence interval .593 to .744, though it was statistically significant (p<.0001). NPI Receiver Survivin Score (+) area was .685 with 95% confidence interval .603 to .766, though it was statistically significant (p<.0001). NPI Receiver Caspase-3 Score (+) area was .669 with 95% confidence interval .593 to .744, though it was statistically significant (p<.0001). The value of Pearson Correlation Coefficient (r) was .349**. The positive correlation was found between Caspase-3 vs NPI. The P-Value was <0.0001. The result was statistically significant. The value of Pearson Correlation Coefficient (r) was .407**. The positive correlation was found between Survivin vs NPI. The P-Value was <0.0001. The result was statistically significant.

Discussion

Stage in CASPASE-3 SCORES

As the illness stage advances, there is a discernible shift in the distribution of CASPASE-3 values toward higher scores. The percentage of patients with a high score (3) rises dramatically to 32.5% and 12.3%, respectively, in more advanced stages such as IIIA and IIIB, whereas 42% of patients in Stage IIA had a moderate score (2). This raises the possibility that elevated CASPASE-3 levels are linked to advanced disease, underscoring the potential use of this protein as a prognostic indicator for the advancement of disease.

T-size in CASPASE-3 SCORES

The information provided investigates the connection between a clinical cohort's tumor size

(T-stage) and CASPASE-3 score. It demonstrates that greater tumor sizes are mostly linked to higher CASPASE-3 scores, with the highest frequencies of positive CASPASE-3 scores occurring in T2 and T3 stages (55.1% and 42.1%, respectively).

Nodal Status in CASPASE-3 SCORES

As nodal involvement increases, CASPASE-3 expression gradually shifts, according to an examination of CASPASE-3 scores across various nodal statuses. Moderate CASPASE-3 scores (2) are more common in patients with N0 status (no regional metastases) (58%), but higher CASPASE-3 scores (3) are more common in patients with N1 and higher stages, especially in N1 (46.5%) and N2 (14.9%) groups.

Grade in CASPASE-3 SCORES

A significant relationship between higher tumor grades and enhanced apoptotic activity is suggested by the distribution of CASPASE-3 scores across tumor grades. CASPASE-3 expression is low in grade 1 tumors, with most instances having scores of 1 or 2. In contrast, grade 3 tumors had much higher CASPASE-3 scores, especially score 3 (29.8%).

Stage in SURVIVIN SCORE (+)

A trend toward increased SURVIVIN expression as the cancer stage advances may be seen in the data on SURVIVIN scores across different stages. While the majority of cases in early-stage tumors (IA) have lower SURVIVIN scores (1), the incidence of higher scores (2 and 3) increases in more advanced stages (IIIA, IIIB, IIIC), especially in stage IIIA, where 32.5% of cases have a score of 3.

T-size in SURVIVIN SCORE (+)

There is an obvious correlation between larger tumors and higher SURVIVIN expression, as evidenced by the distribution of SURVIVIN scores across tumor sizes (T-stages). While most T1 tumors have lower SURVIVIN scores (1), there is a discernible rise in higher SURVIVIN scores in T3 and T4b stages, especially in T4b, where 22.5% of patients had a score of 3. This implies that SURVIVIN may be upregulated when tumors grow in size, which may help tumor cells survive longer and be more resistant to apoptosis, especially in more advanced stages.

Nodal Status in SURVIVIN SCORE (+)

A tendency of rising SURVIVIN expression with increased nodal involvement is shown by the distribution of SURVIVIN scores across various nodal statuses. The majority of patients in N0 (no nodal metastases) had lower SURVIVIN scores (1 and 2), whereas the percentage of cases with moderate to high SURVIVIN scores increases noticeably in N1 and higher stages, especially in N1, where 42.5% of cases have a score of 3.

Grade in SURVIVIN SCORE (+)

The information demonstrates a direct correlation between SURVIVIN expression and tumor grade, with higher grades being associated with higher SURVIVIN levels. While a considerable percentage of Grade 3 tumors have higher SURVIVIN scores (2 and 3), especially with 35% showing a score of 3, the majority of Grade 1 tumors have low to moderate SURVIVIN scores (1 and 2).

NPI in SURVIVIN SCORE (+)

According to the analysis, when CASPASE-3 scores rise, the Nottingham Prognostic Index (NPI) increases statistically significantly ($p < 0.0001$). For CASPASE-3 score 1, the mean NPI value is 3.5422; for score 2, it rises to 3.9574, and for score 3, it rises to 4.8481. As evidenced by the higher NPI values, this tendency implies that worse prognostic outcomes are linked to greater CASPASE-3 scores, which are a sign of improved apoptotic resistance. There is a clear pattern of rising NPI values as SURVIVIN expression increases, and the data shows a substantial correlation between SURVIVIN scores and the Nottingham Prognostic Index (NPI) ($p < 0.0001$). The anti-apoptotic protein Survivin is associated with more aggressive tumor behavior and a worse prognosis; the mean NPI for Survivin score 1 is 3.7873, for score 2, it is 4.4411, and for score 3, it is 5.396.

Our study showed that, NPI vs Caspase-3 Score (+) area was .669 with 95% confidence interval .593 to .744 [Sensitivity = 74.1% and Specificity =44.8%] and in our study, NPI vs Survivin Score (+) area was .685 with 95% confidence interval .603 to .766 [Sensitivity = 81.5% and Specificity =52.7%] but these were statistically significant ($p<.0001$).

In our study, NPI vs Caspase-3 ($.349^{**}$) and Survivin ($.407^{**}$) were positive correlation. The findings show that the expression levels of Survivin ($r = 0.407$, $p < 0.001$) and Caspase-3 ($r = 0.349$, $p < 0.001$) in breast cancer tissues significantly correlate positively with the Nuclear Proliferation Index (NPI). These results imply that the expression of Caspase-3 and Survivin, which are both involved in controlling apoptosis and cell survival, rises in tandem with an increase in the NPI.

Conclusion

According to statistical analysis, Pearson Correlation Coefficient (r) value was higher in Survivin as compared to Caspase-3. Prognostic significance was more specific in Survivin as compared to Caspase-3 which was statistically

significant. Survivin expression implies aggressive tumour biology in breast cancer and it can predict tumours likely to have poor prognosis. Patients with Survivin positive tumours need to be treated aggressively. In conclusion, our study sheds light on the intricate mechanisms of apoptosis and antiapoptosis within the microenvironment of breast cancer. We have identified several pathways and cellular interactions that contribute to tumor aggresiveness, highlighting potential targets for therapeutic intervention. Understanding these s is crucial for developing effective immunotherapies and personalized treatment strategies.

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