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Survivin Expression in Breast Carcinoma and It's Correlation with Clinicopathological Features: Immuohistochemical Study

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Abstract: Background: Breast cancer (BC) is the most common type of cancer among women world-wide and a leading cause of cancer related deaths. Survivin is the smallest member in the inhibitor of apoptosis (IAP) gene family. It has a potential dual role in inhibition of apoptosis by inhibiting caspase-9 and cell proliferation via regulation of mitosis. Survivin overexpression in cancer promotes survival of aneuploid cells, facilitates bypassing of cell cycle checkpoints and increases angiogenesis, thereby using its cytoprotective character to ensure tumor progression. **Objectives:** Investigation of immunohistochemical expression of survivin in breast carcinomas and correlation with the clinico-pathological aspects & molecular subtypes of the tumors. **Materials and methods:** 60 formalin fixed paraffin embedded BC tissue sections were randomly collected. Both epidemiological data and molecular subtypes were collected from the patients' reports. The paraffin blocks were sectioned, stained with hematoxylin & eosin stains for histologic evaluation. Additional sections were immune stained with survivin. **Results:** Survivin expression was detected in 58.3% of cases and showed statistically significant correlation with higher tumor grade, large tumor stage, cases with lymphovascular invasion (LVI),Oestrogen, progesterone receptor negativity, high Ki-67 index, Human epidermal growth factor receptor type 2 (HER-2/Neu) enriched subtype and Triple negative subtype (TN) (P value < 0.05). **Conclusion:** Survivin expression is associated with poor prognostic factors & triple negative subtype and HER-2/Neu enriched molecular subtype.

[Hany Mahmoud Khattab, Alaa Saad Abd El Hamid, Mohammed Mahmud Abdellah Ali, Reham Shehab El Nemr Ismael. **Survivin Expression in Breast Carcinoma and It's Correlation with Clinicopathological Features: Immuohistochemical Study.** *Nat Sci* 2020;18(5):7-27]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 2. doi:10.7537/marsnsj180520.02.

Key words: Breast cancer - Surviving - Triple negative.

1. Introduction

The World Health Organization has ranked breast cancer as the most common type of cancer among women world-wide. The incidence rates of breast cancer vary worldwide, with higher rates in North America, Northern and Western Europe; intermediate rates in South America and Southern Europe; and lower rates in Africa and Asia **(Ferlay et al., 2010).**

In Egypt, Breast cancer accounts for 34.26 % of all female cancers according to the National Cancer Institute registry, Cairo University **(Mokhtar et al., 2007).**

Epithelial tumors (carcinomas) are the most frequent type of breast tumors which may be confined to the glandular component of the organ (in situ carcinoma) or invading the stroma (invasive carcinoma). The term invasive carcinoma encompasses numerous entities differing from each other by morphological characteristics. The most common form (about 75% of cases) is ductal invasive Carcinoma. The second large category is lobular carcinomas (about 10–15% of cases). Other rare types have been described, each accounting for less than 5% of the total including metaplastic carcinoma, mucinous carcinoma and tubular carcinoma **(Salamon et al., 2003).**

Gene expression microarrays have allowed researchers to carry out simultaneous expression analyses of thousands of genes in a single experiment in order to create the molecular profile of a tumor. The intrinsic subtype classification groups breast tumors into five molecular subtypes that also correlate to prognosis. Recently, new intrinsic subtypes have been added ***(Eroles et al., 2012)***.

Survivin regulates the G2/M phase of the cell cycle by associating with mitotic spindle microtubules. Survivin exists in 2 subcellular pools (cytoplasmic and nuclear), consistent with its function in the regulation of both cell viability and cell division **(Alberts B,. et al. 2010)**. In the majority of cancers studied to date, survivin is associated with poor prognosis. Survivin is over expressed in most human tumors including bladder **(Swana et al., 1999)**, blood **(Adida et al., 2000)**, colon **(Sarela et al., 2000)**, liver **(Ito et al., 2000)**, brain **(Islam et al., 2000)**, lung ***(*Monzo et al., 1999*)***, pancreas ***(*Satoh et al., 2001*)***,

prostate **(Xinget al., 2001)**, and kidney **(Takamizawa et al., 2001).**

Multiple studies has reported significant relationship between survivin expression in breast cancer and histologic grade ***(*Singh et al., 2004 and, Span et al., 2004)***, lymph node metastasis **(Zhang et al., 2004)**, and advanced tumor stage **(Tsuji et al., 2004)**.

In addition, it is expressed to be significantly associated with negative hormone receptor status. The cancer-specific expression of survivin, coupled with its importance in inhibiting cell death and in regulating cell division, makes it a potential target for novel cancer treatment ***(*Turner, *et al., 2013)***.

Aim of the Work

The aim of this work is to study survivin expression in breast carcinomas and to evaluate its possible correlation with clinicopathological features**.**

2. Material and Methods

A sixty formalin fixed, paraffin embedded full-face tumor tissue sections have been collected from modified radical mastectomy and conservative breast surgery specimens of female patients with BC. The cases were collected from the Pathology Department at the Kasr El Aini Hospital in the time period between December 2017 and October 2018.

The data collected from the pathology reports of the cases included patient's age, tumour's size, number of masses as well as the lymph node status.

An IHC report including the ER, PR, HER-2/Neu and Ki-67 results was obtained for each case.

Exclusion criteria included:

1) Cases with missing data or no available IHC report.

2) Cases who received neo-adjuvant therapy; either hormonal or chemotherapy.

3) Patients performing lumpectomy or simple mastectomy without axillary sampling.

The paraffin blocks of the tumor sections have been serially sectioned at four µm thickness, stained with hematoxylin & eosin stains and survivin (from US Biological life sciences company for pathological examination. The tumors had been histologically typed according to the latest available World Health Organization recommendations ***(Lakhani et al., 2012)***.

The tumor sections were also examined for tumor type, grade, and in situ component, tumors histological grading was performed according to the Nottingham Grading System which is used until now ***(Elston & Ellis, 1991)***.

Tumors staging was performed using the TNM staging system and the cases were further divided into prognostic stages ***(Edge et al., 2010)***.

For further statistical evaluation, Grade1 and 2 cases were considered as low grade, while grade 3 cases were considered as high grade ***(Kim et al., 2017 and Wang et al., 2018).***

Lympho-vascular invasion (LVI):

Lympho-vascular invasion was defined as presence of tumor cells within an endothelial lined space (lymphatic and/or blood vessel) outside the border of the tumor ***(Gujametal., 2014).***

Tumor Infiltrating Lymphocytes (TIL):

Regarding the TILs, they were scored following the recommendations of the International TILs Working Group 2014 **(Kojima YA.,et al 2018),** which evaluate all mononuclear cells in the stromal compartment within the borders of the invasive tumor. The cells are then reported as a percentage value of the stromal area (i.e. % of the stromal area occupied by mononuclear inflammatory cells) and not as a percentage of the stromal cells. TILs outside of the tumor border, around DCIS and normal breast tissue, as well as in areas of necrosis and hyalinosis, if any, were not included in the scoring. The working group recommended that full assessment of average TILs in the tumor area should be used; they don't recommend focusing on 'hot spots' ***(Salgadoetal, 2015).***

Tumors were defined as High-TILs (≥30%) or Low TILs (<30%) ***(Polónia et al., 2017 and Tomioka et al., 2018).***

Staging and Molecular Subtyping

Tumors staging was performed using the TNM staging system. The cases were further divided into anatomic stages and prognostic stages according to the latest edition of the AJCC staging manual ***(Hortobagyi et al., 2017).***

Regarding the prognostic and anatomic stages, The cases were subdivided in the AJCC classification into early stages that included (stage I, and stage II) and advanced stages included (stage III, and stage V) ***(Zhou et al., 2018***). Nine 9 cases were excluded as their prognostic stages were missing.

Regarding the molecular subtyping, the tumors were classified according to the St. Gallen International Expert Consensus 2013 recommendations (as detailed in the review):

Luminal A (ER-positive, PR-positive HER-2/Neu negative, low Ki67), **luminal B –HER-2/Neu negative** (ER-positive, HER-2/Neu negative and either low PR or high Ki67), **'Luminal B-like (HER-2/Neu positive)'**, (ER-positive, HER-2/Neu positive, any Ki-67 and any PR), **HER-2/Neu positive – non luminal** (HER-2/Neu positive and ER and PR negative) and **Triple Negative** (ER, PR and HER-2/Neu negative) ***(Goldhirsch et al., 2013).***

Luminal cases having high histologic grade were considered as Luminal B rather than luminal A according to St. Gallen International Expert Consensus 2017 recommendations ***(Curigliano et al., 2017).***

The tumor sections were also examined for tumor type, grade, and in situ component.

Statistical analysis

- Microsoft excel 2013 was used for data entry and the statistical package for social science (SPSS version 24) was used for data analysis.
- Simple descriptive statistics (arithmetic mean and standard deviation) used for summary of normal quantitative data and frequencies used for qualitative data.
- Bivariate relationship was displayed in cross tabulations and Comparison of proportions was performed using the chi-square and Fisher's exact tests where appropriate.
- Independent T test was used to compare normally distributed quantitative data.
- The level of significance was set at probability (P) value <0.05.

Finally, microscopic photos were taken using a digital camera attached to an Olympus microscope model BX 51.

3. Results

This study is an analytical observational cross sectional study including 60 cases of breast carcinoma obtained from mastectomy specimens. The cases were collected from the Kasr Alainy hospital in the time period between December 2017 and October 2018, where the clinic-pathological data, histological examination and immunohistochemical results are categorized in tables.

Age: The age in this study ranges between 22 and 76 years old with mean age about 53.83 years (**Table 1**).

Table (1): Age in the studied cases:

Age	
Number of cases	60
Mean	55
Standard deviation	12.792
Minimum	22
Maximum	76

Histological Finding of cases

I) Histological type:

The collected cases included 60 cases of invasive breast carcinoma. Cases with IDC-NST subtype were the highest expression subtypes as their included forty one (41) cases (68.3%), and cases with cribriform subtype were the lowest expression subtypes as their included one (1) case (1.7%) case of cribriform subtype. **(Table2)** and the photos were included in **(figures1), (figures 2) and (figures 3).**

Table (2): Histological types in the studied cases:

Tumor type	Number	Percent
IDC-NST	41	68.3%
Papillary (IDC)	2	3.3%
Mucinous (IDC)	2	3.3%
Medullary (IDC)	3	5%
Cribriform Carcinoma	1	1.7%
ILC	8	13.3%
Tubular Carcinoma	3	5%
Total	60	100%

II) Histological Grade: Among the collected cases; The cases with grade II were the highest number as included 29 cases (48.3%), and the cases with grade I were the lowest number as included 4 cases (6.7%). **(Table 3) & (figure 4)**.

Table (3): Tumor grade in the studied cases:

Tumor grade	Number	Percent
Grade I	4	6.7%
Grade II	29	48.3%
Grade III	27	45%
Total	60	100%

III) Stage T stage: Regarding the T stage; The cases with T2 stage were the highest number as included 33 cases (55.0%), the cases with T3 were the lowest number as included 12 cases (20.0%), and no reported cases of stage T4 **(Table 4)**.

Table (4): Tumor stage in the studied cases:

Tumor staging	Number	Percent
T1	15	25%
T2	33	55%
T3	12	20%
Total	60	100%

IV) Nodal stage: Concerning the N stage; 16 cases (26.7%) were Negative nodal stage, and 44 cases (73.3%) were positive nodal stage **(Table 5)**.

Table (5): Nodal stage in the studied cases:

Nodal stage	Number	Percent
N negative	16	26.7%
N positive	44	73.3%
Total	60	100%

V) Anatomic stage in the studied cases (according to the AJCC classification):

In our study, the early stage (I and II) cases (49 cases; 81.6%) were more than the advanced stage (III and IV) cases (11 cases; 18.4%) **(Table 6).**

Table (6): Anatomic stage in the studied cases:

Anatomic stage	Number	Percent
Early stage	49	81.67%
Late stage	11	18.33%
Total	60	100%

VI) Presence of in situ component in invasive breast carcinoma:

Among the collected cases of invasive breast carcinoma; 17 (28.3%) were positive for in situ component and 43 (71.7%) were negative for an in situ component **(Table 7).**

VII) Number of masses:

Regarding the multiplicity of masses; 48 cases (80%) showed one mass, and 12 cases (20%) showed multiple masses. **(Table 8)**.

Table (7): Presence of in situ component in the studied cases:

In situ	Number	Percent
Positive	17	28.3%
Negative	43	71.7%
Total	60	100%

Table (8): Multiplicity of masses in the studied cases:

Number of masses	Number	Percent
Single	48	80%
Multiple	12	20%
Total	60	100%

VIII) Lymph vascular invasion (LVI):

Regarding to LVI of masses; 32(53.3%) cases with LVI, and 28(46.7%) cases without LVI **(Table 9) & (figure 6)**.

Table (9): LVI in the studied cases:

LVI	Number	Percent
Positive	32	53.3%
Negative	28	46.7%
Total	60	100%

IX) Tumor infiltrating lymphocytes (TIL):

Regarding the extent of stromal TILs, 53 cases showed low TILs (88.33%) and 7 cases showed high TILs (11.6%) **(Table 10).**

Table (10): TIL in the studied cases:

TIL	Number	Percent
Low TIL	53	88.33%
High TIL	7	11.67%
Total	60	100%

This classification according to ***(Polónia et al., 2017 and Tomioka et al., 2018)*** as tumors were defined as High-TILs (≥30%) or Low TILs (<30%).

Hormonal receptor status (ER & PR) in studied cases

The collected cases included 40 (66.7%) ER positive cases and 20 (33.3%) ER negative cases. Regarding the PR, 39 cases (65%) were PR positive and 21 cases (35%) were PR negative **(Table 11)**.

Table (11): ER and PR status in the studied cases:

Estrogen receptor		ER	PR
Positive	Count	40	39
	percentage	66.7%	65%
Negative	count	20	21
	percentage	33.3%	35%

HER-2/Neu staining in studied cases

Out of the studied invasive breast carcinoma cases;10 cases (16.7%) were positive over expression for HER-2/Neu and 50 cases (83.3%) were negative over expression for HER-2/Neu. **(Table 12).**

Table (12): HER-2/Neu over expression in the studied cases:

HER-2/Neu	Number	Percent
Positive	10	16.7%
Negative	50	83.3%
Total	60	100%

Negative HER-2/Neu staining included (score 0, and score 1), and positive HER-2/Neu staining included (score2, and score3) according to **Hicks etal.,2011.**

Ki -67 staining in studied cases

As for the Ki-67 proliferation index; 32 cases (53.3%) were considered to have low Ki-67 index and 28 cases (46.7%) were considered to have high Ki-67index **(Table 13)**.

Table (13): Ki-67 proliferation index in the studied cases:

Ki-67inpercent	Number	Percent
Low	32	53.3%
High	28	46.7%
Total	60	100%

According to **(Leung SCY etal.,2016)** low Ki-67 index <20% and high Ki-67 index >20%.

Molecular subtypes in studied cases

The 60 collected cases included 20 (33.3%) Luminal A cases, 20 (33.3%) Luminal B, 6 (10%) HER-2/Neu enriched cases and 14 (23.4%) Triple negative cases **(Table 14)** & **(Graph1).**

Table (14): Molecular subtypes in the studied cases:

Molecular Subtypes	Number	Percent
Luminal A	20	33.3%
Luminal B	20	33.3%
HER-2/Neu enriched	6	10%
Triple negative	14	23.4%
Total	60	100%

Prognostic stage in the studied cases

Regarding the prognostic stage 9 cases (30%) were early stage cases and 42 cases (70%) were advanced stage cases **(Table 15).**

After exclusion of the missed 9 cases according to AJCC staging system ***(Zhou et al., 2018***) due to lacking of data in their reports

Result of survivin immunohistochemical expression cases

Survivin immunohistochemical expression was positive in 35 cases (58.3%) and negative in 25 cases (41.7%) **(Table 16) & (Graph 2).**

Table (15): Prognostic stage in the studied cases:

Prognostic stage	Number	Percent
Early stage	**9**	**17.65%**
Advanced stage	**42**	**82.35%**
Total	**51**	**100%**

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Table (16):Survivin immunohistochemical expression in the studied cases:

Survivin	Number	Percent
Positive	**35**	**58.3%**
Negative	**25**	**41.7%**
Total	**60**	**100%**

Correlation of survivn expression and demographic data

Relationship between the age and survivin expression:

No statistically significant relationship was detected between age and survivin expression.

Table17:Relationship between the age and survivin expression

	Age			
Survivin staining	N	Mean	Std. Deviation	P value
Negative	25	50.68	12.760	0.105
Positive	34	56.15	12.495	

(P value=0.105).

Correlation of survivn expression and Histological finding

I) Relation between histological types and survivn expression:

Regarding the relation between the various histological types and survivin. IDC-NST showed 63.4% positive cases, ILC showed 50 % positive cases, carcinoma with medullary features showed 100% positive cases and the other types showed 83.3% positive cases. Therefore, **carcinoma with medullary features showed the highest rate of survivin expression**. However, this correlation was statistically insignificant (**P value = 0.228) (Table 18)** the photos were included in **(figures1), (figures 2) and (figures 3).**

Table18: Relationship between the histological types and survivin expression

		Histologic types					
Survivin staining		IDC-NST	ILC	Medullary	Mucinous	Papillary	Tubular
Negative	Count	15	4	0	2	1	2
	% within cases	36.6%	50%	0%	100%	50%	66.7%
Positive	Count	26	4	3	0	1	1
	% within cases	63.4%	50%	100%	0%	50%	33.3%
Total	Count	41	8	3	2	2	3
	% within cases	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

(P value= 0.228).

II) Relation between tumor grade and survivin expression:

On studying the relation between tumor grade and survivin expression, **Cases with high grade III showed higher rate of expression (88.9%) than cases with grade II (37.9%). This Correlation was highly statistically significant (P value = 0.001) (Table 19) & (Graph 3) & (Figure 4).**

Table 19: Relationship between the tumor grade and survivin expression:

		Histologic grade		
Survivin staining		Grade I	Grade II	Grade III
Negative	Count	4	18	3
	% within cases	100%	62.1%	11.1%
Positive	Count	0	11	24
	% within cases	100%	37.9%	88.9%
Total	Count	4	29	27
	% within cases	100.0%	100.0%	100.0%

(P value= 0.001)

III) Relation between the T stage and survivin expression:

Concerning the relation between the T stage and survivin expression**,** T1 (40%) positive,T2 (54.5%) positive and T3 (91.7%) No recorded T4 cases. Large tumor size (T3) cases showed higher rate of survivin expression (88.9%), compared to the smaller tumor size (T1 and T2) cases (37.9%). However, this correlation was statistically significant (P value = 0.021) (Table 20) & (Graph 4) and (figure 5).

Table20: Relationship between the tumor stage and survivin expression:

		T stage		
Survivin staining		T1	T2	T3
Negative	**Count**	9	15	1
	% within cases	60%	60%	8.3%
Positive	Count	60	18	11
	% within cases	40%	54.5%	91.7%
Total	Count	15	33	12
	% within cases	100.0%	100.0%	100.0%

(P value= 0.021).

IV) Relation between the nodal stage and survivin expression:

As for the N stage, **survivin was higher expression in nodal status positive cases (59.1%) than nodal status negative cases (56.3%).** However, this correlation was in statistically insignificant (P value = 0.844) **(Table 21).**

Table 21: Relation between the N stage and survivn expression

	N stage		
Survivin staining		Negative nodal stage	Positive nodal stage
Negative	Count	7	18
	% within cases	43.8%	40.9%
Positive	Count	9	26
	% within cases	56.3%	59.1%
Total	Count	16	44
	% within cases	100.0%	100.0%

(P value= 0.844)

Table 22: Relation between the anatomic stage and survivn expression

	Anatomic stage		
Survivin staining		early	late
Negative	Count	21	4
	% within anatomic stage	42.9%	36.4%
Positive	Count	28	7
	% within anatomic stage	57.1%	63.6%
Total	Count	49	11
	% within anatomic stage	100.0%	100.0%

(P value= 0.748)

V) Relation between anatomic stage and the survivin expression:

Regarding the relation between the anatomic stage and survivin expression, **a higher rate of survivin expression was noticed in cases with advanced stage (63.6%), compared to cases with early stage**

(57.1%). However, this correlation was statistically insignificant (P value = 0.748) **(Table 22).**

VI) Relation between the presence of in situ component and the survivin expression:

In this study**, survivin is higher expression in cases without in situ component (60.5%) than cases with in situ component (52.9%)**. This correlation was statistically insignificant (P value = 0.594) **(Table 23).**

Table 23: Relation between in situ component and survivin expression

	In situ component		
Survivin staining		Absent	Present
Negative (25 cases)	Count	17	8
	% within in situ component	39.5%	47.1%
Positive (35 cases)	Count	26	9
	% within in situ component	60.5%	52.9%
Total (60 cases)	Count	43	17
	% within in situ component	100.0%	100.0%

(P value=0.594)

VII) Relation between number of masses and the survivin expression:

In this study**, survivin is equal expression in cases with single mass and cases with multiple masses**. This correlation was statistically insignificant (P value = 1) **(Table 24).**

Table 24: Relation between number of masses and survivin expression

	Number of masses		
Survivin staining		single	multiple
Negative (25 cases)	Count	20	5
	% within number of masses	41.7%	41.7%
Positive (35 cases)	Count	28	7
	% within number of masses	58.3%	58.3%
Total (60 cases)	Count	48	12
	% within number of masses	100.0%	100.0%

(P value=1.000)

VIII) Relation between the LVI and survivin expression

Regarding the relation between the presence of LVI and survivin expression, the rate of survivin expression is higher in cases with LVI (78.1%) than cases without LVI (35.7%). This Correlation is highly statistically significant (P value = 0.001) (Table 25) & (Graph 5) and (Figure 6).

Table 25: Relation between LVI and survivin expression.

	LVI		
Survivin staining		Absent	Present
Negative (25 cases)	Count	18	7
	% within LVI	64.3%	21.9 %
Positive (35 cases)	Count	10	25
	% within LVI	35.7%	78.1%
Total (60 cases)	Count	28	32
	% within LVI	100.0%	100.0%

(P value < 0.001)

Table 26: Relation between Stromal TILs and survivin expression

	TIL		
Survivin staining		Low	High
Negative (25 cases)	Count	24	1
	% within TIL	45.3%	14.3%
Positive (35 cases)	Count	29	6
	% within TIL	54.7%	85.7%
Total (60 cases)	Count	53	7
	% within TIL	100.0%	100.0%

(P value=0.333)

IX) Relation between stromal TIL and the survivin expression

In this study, **cases with high stromal TILs showed higher rate of survivin expression (85.7%) than cases with low stromal TILs (54.7%).** This correlation was statistically insignificant (P value = 0.333) **(Table 26).**

Correlation of survivin expression and hormonal receptors expression

Relation between the ER, PR status and the survivin expression:

On studying the relation between the ER status and survivin expression, **it was found that the rate of survivn expression was higher in cases with ER negative (100%) than in cases with ER positive (37.5%)**. According to PR status and survivin expression, **it was found that the rate of survivin expression was higher in cases with PR negative (95.2%) than in cases with PR positive (38.5%). This Correlation was statistically significant (P value = 0.001) (Table 27) & (Graph 6,7) and (Figure 7).**

Table 27: Relationship between the ER,PR status and survivin expression

			Survivin staining		Total
			Negative	Positive	
ER	Negative	Count	0	20	20
		% within ER negative	0.0%	100.0%	100.0%
	Positive	Count	25	15	40
		% within ER positive	62.5%	37.5%	100.0%
PR	Negative	Count	1	20	21
		% within PR negative	4.8%	95.2%	100.0%
	Positive	Count	24	15	39
		% within PR positive	61.5%	38.5%	100.0%

(P value< 0.001)

Relation between the HER-2/Neu status and the survivin expression

As for the relation between the HER-2/Neu status and the survivin expression, **it was found that the rate of survivin expression was higher in cases with HER-2/Neu positive (70%) than in cases with HER-2/Neu negative (56%).** This correlation was statistically insignificant **(P value = 0.499) (Table 28).**

Table 28: Relation between HER-2/Neu status and survivin expression.

	HER-2/Neu overexpression		
Survivin staining		Absent	Present
Negative (25 cases)	Count	22	3
	% within cases	44%	30%
Positive (35 cases)	Count	28	7
	% within cases	56%	70%
Total (60 cases)	Count	50	10
	% within cases	100.0%	100.0%

(P value = 0.499)

Relation between the Ki-67 status and the survivin expression

Regarding the relation between the Ki-67 proliferation index and the survivin expression, **it was found that the rate of survivin expression was higher in cases with high Ki-67 index (85.7%) than in cases with low Ki-67 index (34.4%). This correlation was statistically significant (P value = 0.001) (Table 29) & (Graph 8) and (figure 8).**

Table 29: Relation between Ki-67 index and survivin expression.

	Ki-67		
Survivin staining		Low	High
Negative (25 cases)	Count	21	4
	% within Ki-67 cases	65.6%	14.3%
Positive (35 cases)	Count	11	24
	% within Ki-67 cases	34.4%	85.7%
Total (60 cases)	Count	32	28
	% within Ki-67 cases	100.0%	100.0%

(P value<0.001)

Relation between the molecular subtypes and the survivin expression

On studying the relation between the molecular subtypes survivin expression. **The HER-2/Neu enriched and Triple negative subtype showed the highest rate of survivin expression (100%). This Correlation was highly statistically significant (P value = 0.001) (Table 30) & (Graph 10) and (figure 9 to figure 12).**

Table 30: Relation between the molecular subtypes and survivin expression.

Molecular subtype					
Survivin staining		luminal A	Luminal B	HER-2/Neu enriched	Triple negative
Negative	Count	16	9	0	0
	% within cases	80%	45%	0%	0.0%
Positive	Count	4	11	6	14
	% within cases	20%	55%	100%	100.0%
Total	Count	20	20	6	14
	% within cases	100.0%	100.0%	100.0%	100.0%

(P value<0.001).

Relation between the prognostic stage and survivin expression

Concerning the relation between the prognostic stage and survivin expression, **a higher rate of survivin expression was noticed in cases with advanced stage (69%), compared to cases with early stage (44.4%).** This correlation was statistically insignificant (P value = 0.249) **(Table 14).**

Table 31. Relation between the Prognostic stage and survivin expression.

	Prognostic stage		
Survivin staining		early	Advanced
Negative (18cases)	Count	5	13
	% within prognostic stage	55.6%	31%
Positive (33 cases)	Count	4	29
	% within prognostic stage	44.4%	69%
Total (51 cases)	Count	9	42
	% within prognostic stage	100.0%	100.0%

(P value= 0.249)

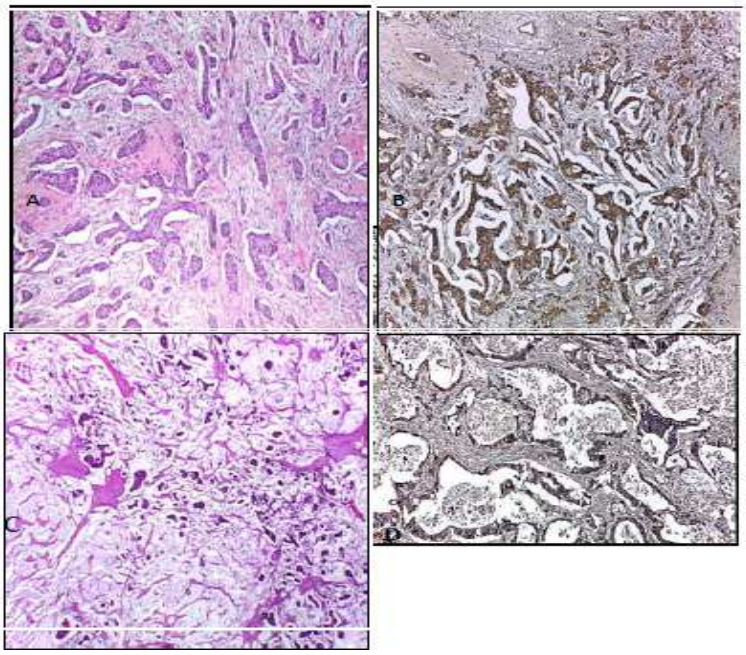

Figure 1. Correlation of survivin and different histological types:
(A) IDC-NST grade II x200 (H & E), **(B)** strong diffuse expression of survivin in IDC-NST x200**, (C)** Mucinous carcinoma grade IIx200**, (D)** moderate diffuse expression of survivin in Mucinous carcinoma x 200.

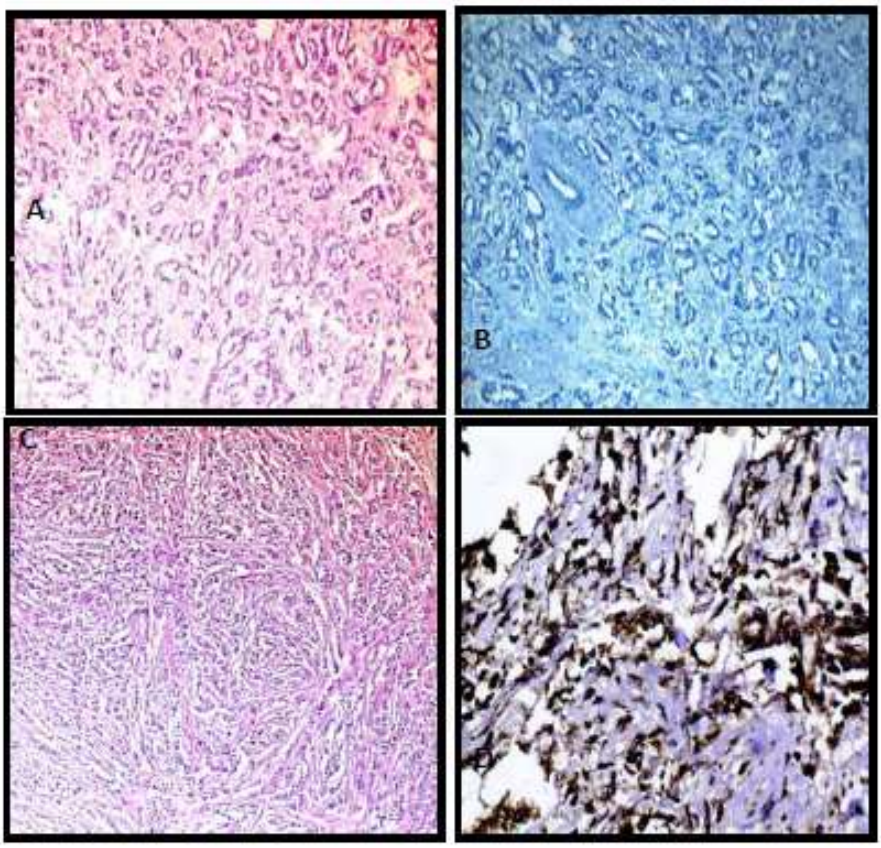

Figure 2. Correlation of survivin and different histological types:
(A) Tubular carcinoma grade II x200(H & E), **(B)** Negative expression of survivin in tubular carcinoma x200, **(C)** Invasive classic lobular carcinoma grade II x200 (H & E), **(D)** Stronge diffuse survivin expression in ILC x200.

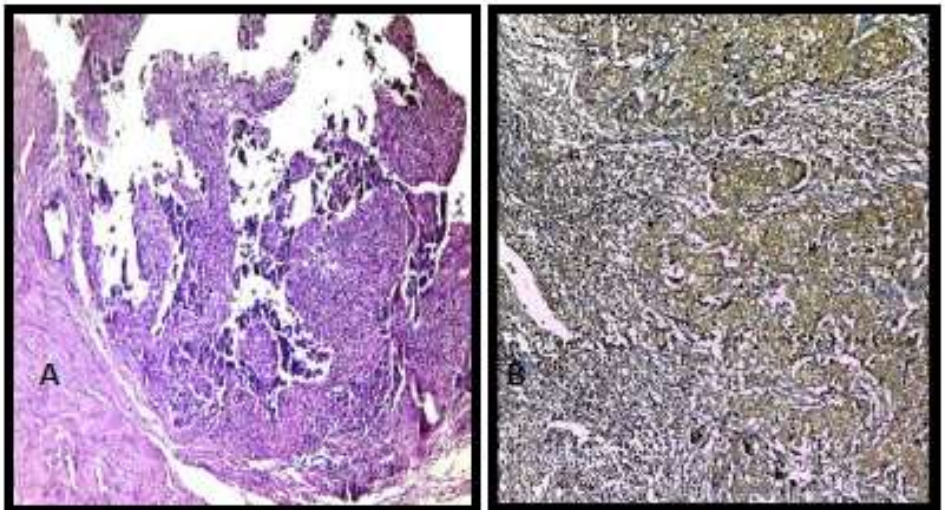

Figure 3. Correlation of survivin and medullary carcinoma subtype:
(A) Medullary carcinoma grade II x200 (H & E), **(B)** strong diffuse survivin expression in medullary carcinoma x200.

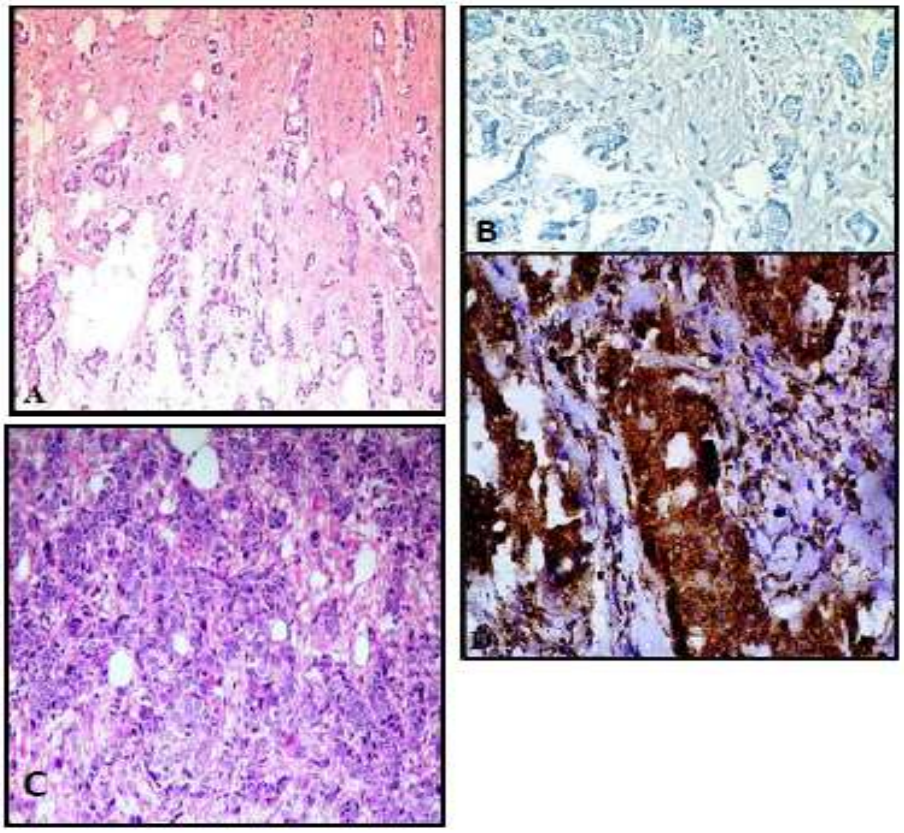

Figure 4. Correlation of survivin expression and tumor grades:
(A) IDC-NST Grade II; Low Grade. The Tumor showed tubules formation and moderately anaplastic nuclei with low mitotic activity **(**H & E X200**), (B)** IDC-NST Grade II; Low grade showed negative expression of survivin x200, **(C)** IDC-NST Grade III; High grade. It showed no tubules formation and markedly anaplastic nuclei (H & E X200), **(D)** IDC-NST Grade III; High grade showed strong diffuse survivin expression x200.

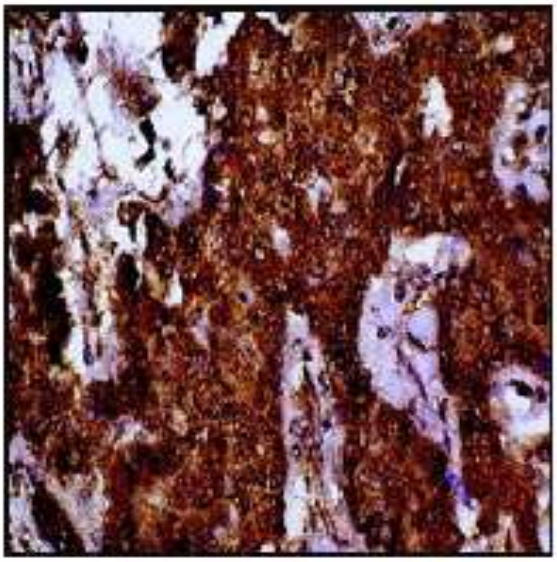

Figure 5. Correlation of survivin expression and tumor stage:
A case of T3 (tumor size>5) showed strong diffuse expression of survivin x200.

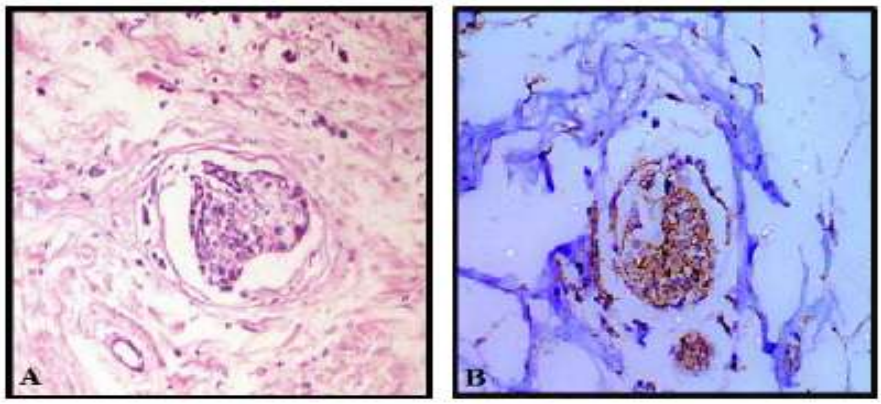

Figure 6. Correlation of survivin expression and LVI in studied case:
(A) Case showing positive LVI (H & E X400), **(B)** It showed positive expression for survivin x400.

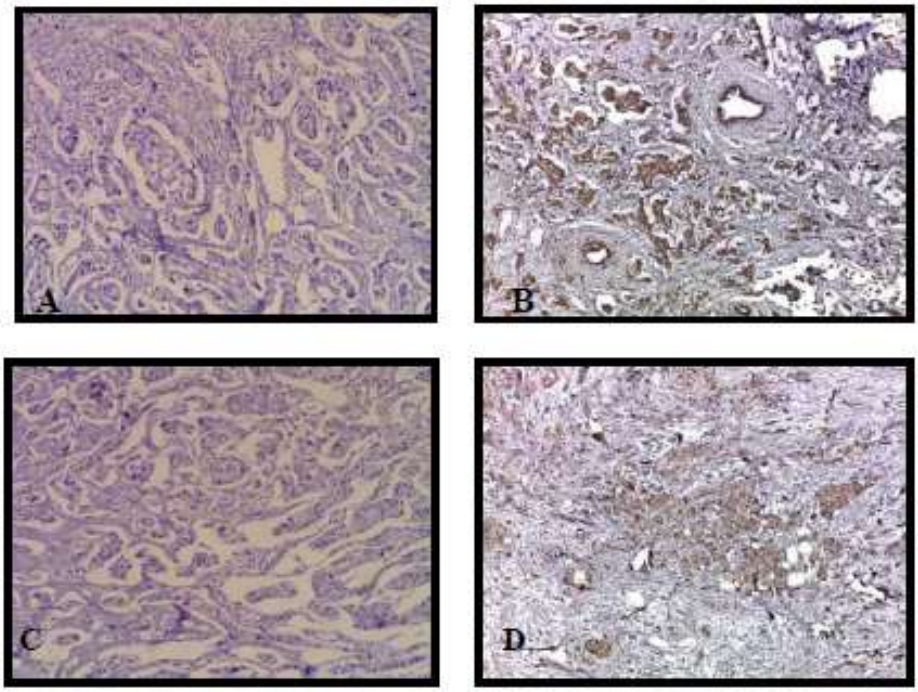

Figure 7 correlation survivin expression and hormonal receptors expression:
(A) Case with ER receptor negative expression x200**, (B)** It showed strong diffuse expression of surviving x200**, (C)** Case with PR negative expression x200**, (D)** It showed strong diffuse expression of survivin x200.

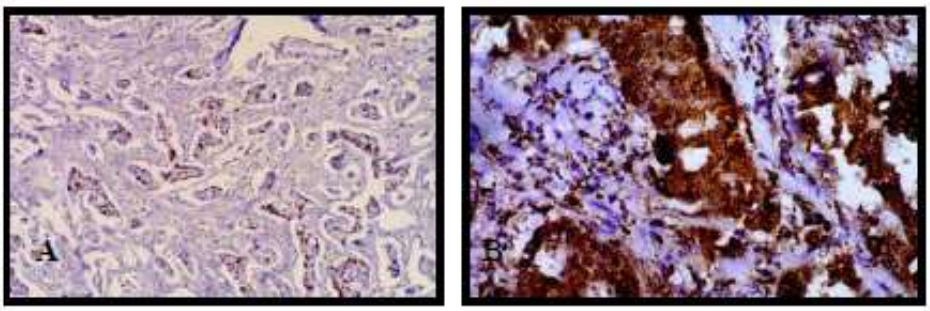

Figure 8. Correlation survivin expression and Ki-67:
(A) A case with high Ki-67 index x200**, (B)** It showed strong survivin expression x 200.

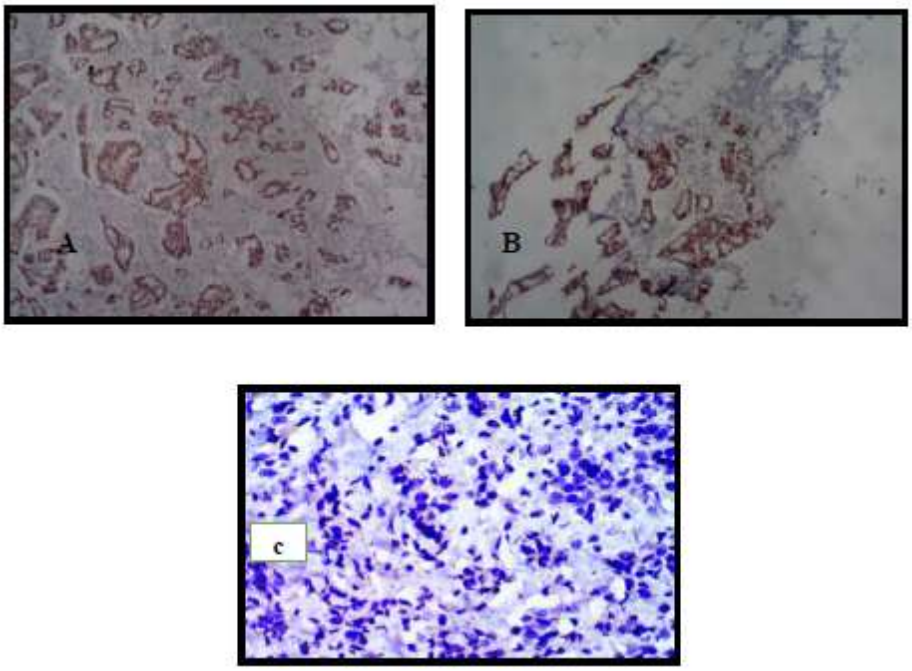

Figure 9. Correlation survivin expression and Luminal A Molecular subtype:
(A) Case with ER positive expression x200**, (B)** It showed PR positive expression x200, (**C)** Survivin negative expression x200.

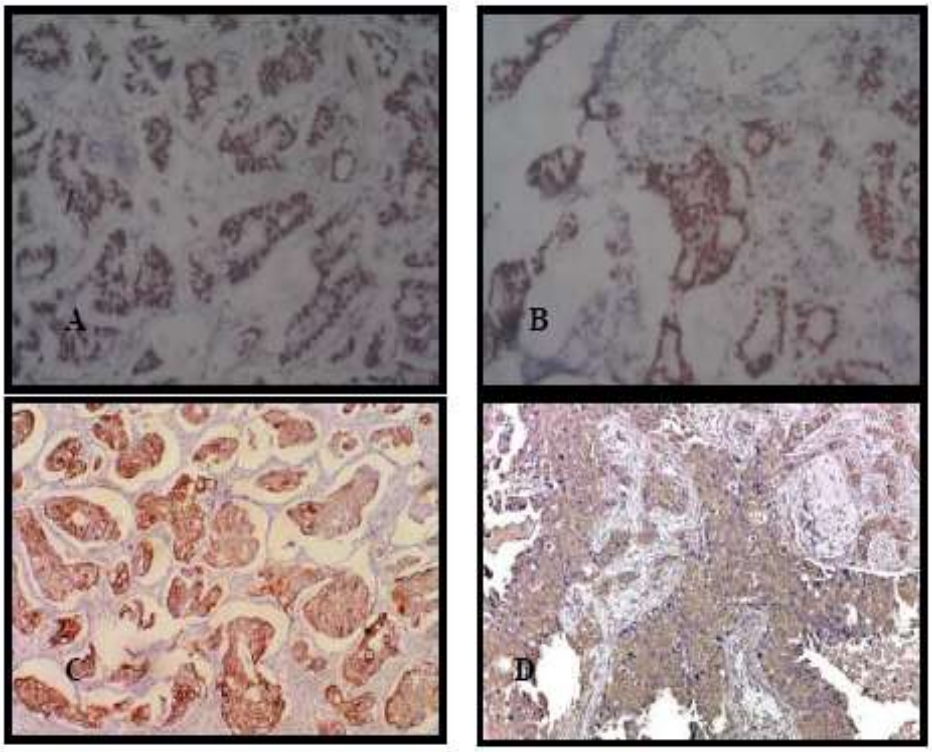

Figure 10. Correlation survivin expression and Luminal B Molecular subtype:
(A) Case with ER positive expression x200**, (B)** It showed PR positive expression x200, **(C)** HER-2/Neu positive expression x200, (**D)** Survivin moderate diffuse expression x200.

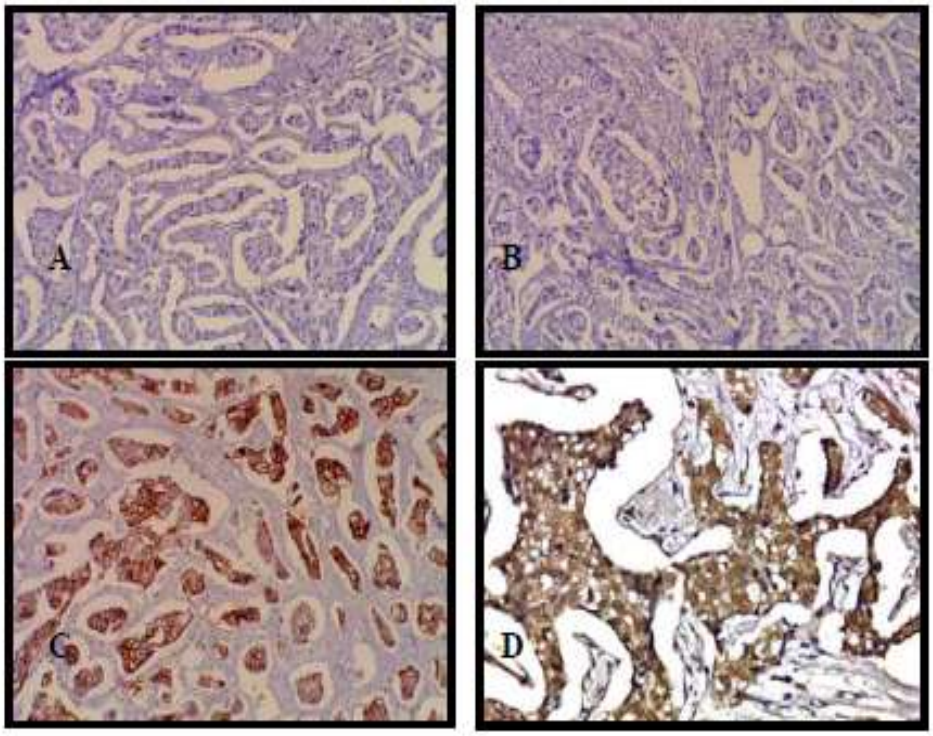

Figure 11. Correlation survivin expression and HER-2/NEU enriched Molecular subtype:
(A) Case with ER negative expression x200**, (B)** It showed PR negative expression x200, **(C)** It showed HER-2/Neu positive expression x200, (**D)** Survivin strong diffuse expression x400.

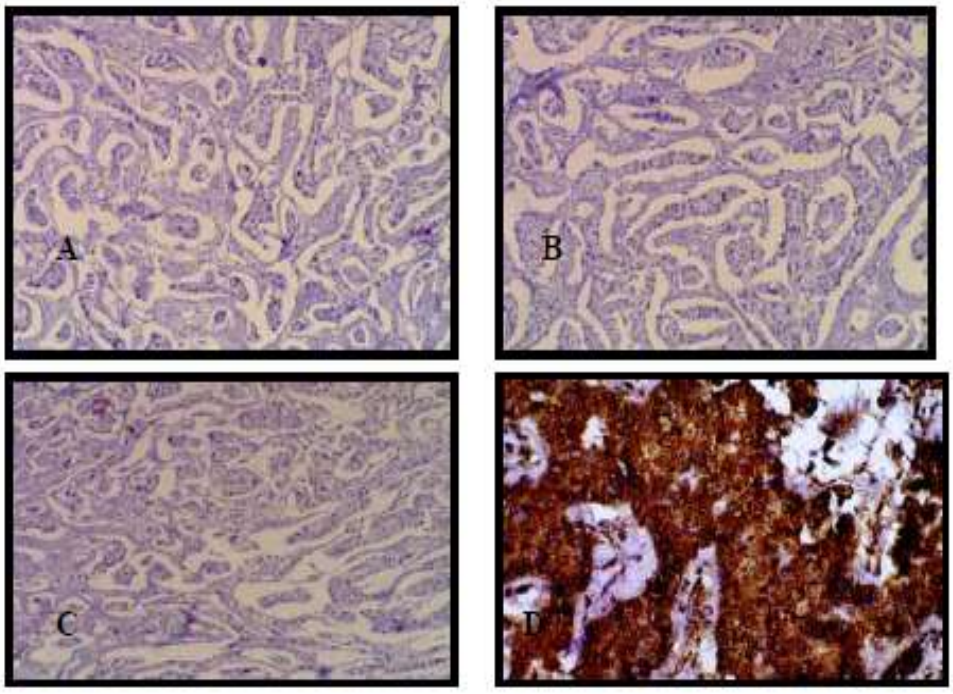

Figure 12. Correlation survivin expression and Triple negative Molecular subtype:
(A) Case with ER negative expression x200**, (B)** It showed PR negative expression x200, **(C)** It showed HER-2/Neu negative expression x200, (**D)** Survivin strong diffuse expression x400 (cystoplasmic and nuclear expression).

4. Discussion

Breast cancer is the most common type of cancer among women world-wide. In Egypt, carcinoma of the breast is the most prevalent cancer among Egyptian women and constitutes 29% of National Cancer Institute cases ***(Helal et al., 2015)*****.**

Survivin is a 16.5-kDa intracellular protein that belongs to the inhibitor of apoptosis (IAP) gene family. Survivin is highly expressed in many embryonic tissues, as well as most human tumors of the lung, colon, breast, stomach, liver, ovary, and prostate. It is not detectable in most differentiated normal adult tissues but is expressed in most human cancer tissues. Its expression in cancer has been shown to be correlated with poor prog NSTis, cancer progression, and drug resistance **(Michiko Shintani et al., 2013).**

This work aimed to studying survivin expression in breast carcinomas and to evaluate its possible correlation with clinicopathological features**.**

In this study, we recruited 60 tumor sections from mastectomy specimens collected from the pathology department at the Kasr el Aini hospital in the period between December 2017 and October 2018.

In the current study, statistically significant correlation was found between the rate of survivin expression and many of the clinico-pathological parameters (p-value < 0.05) as tumor grade, T stage, LVI, ER, PR statuses, Ki-67, and molecular subtypes.

On the other hand, no statistically significant correlation was found between the rate of survivin expression and other clinico-pathological parameters (p-value > 0.05) as age, histologic types, anatomic stage, prognostic stage, TIL, in situ component, number of masses, nodel status and HER-2/Neu.

The mean age in our study was 55 years (ranging between 22-76 years). This is consistent with Egyptian cancer registries, where the mean age at presentation for BC was reported to be 51 years according to the pathology based cancer registry of Ain-Shams faculty of medicine ***(Helal et al., 2015)*** and equal according to Cancer Pathology Registry of the National Cancer Institute ***(Mokhtar et a., 2016)*** also this mean age is so close to that reported by ***(Natalie Reimers et al., 2004)***, who reported a mean age of 50 years, but higher than that reported by ***(Ying Liu et al., 2013)*** which reported a mean age of 35 years.

In this study, survivin positive expression with mean age 56 years that agree with **(Rasoola et al., 2019),** who reported positive survivin in cases with age above 45 years, also agree with **(Shi et al.,2019) and (Dogu et al., 2010)** who reported that survivin higher expression in cases above 50 and menopause cases. But our study was opposite to **(Zhang et al., 2014)** who reported that survivin was positive in the age below 50 years.

The study included 41 cases IDC-NST (68.3%) that was the highest expression subtypes, 8 cases ILC (13.3%) and 3 cases carcinoma with medullary features (5%). The remaining 13.4% were other miscellaneous types including tubular and cribriform carcinomas, as well as a case of papillary carcinoma features, the WHO reported that although classic medullary carcinoma accounts for <1% of all BC, this figure is much higher when the term carcinoma with medullary features, including the atypical medullary carcinoma and carcinoma NST with medullary features is used (***Lakhani et al., 2012)*****.** Our figures of the IDC-NST were slightly lower than other available studies ***(Neri et al., 2015)*** who reported that 75.8% was IDC-NST, but in our study ILC and other types were slightly higher than the available reported as (12.3% ILC and 11.9% other types).

Our study was near to WHO publication which reported that IDC-NST ranging between 40-70% and ILC ranging between 5-10%. Also IDC-NST was higher reported in **(Ishrat Rasoola et al.,2019)** than in this study which (75%), and Lobular (25%). Concerning the studies done by **(Manuela Sarti et al., 2013)** and **(Minghui Zhang et al.,2013)** who reported IDC-NST was higher than our study (90.5%) and (80.9%) respectively, but ILC was lower than our study (9.5%), (10.3%) respectively and other type was lower than our study (8.8%) in **(Minghui Zhang et al., 2013)**.

Regarding the relation between the histologic types of breast carcinoma and survivin expression no statistically significant was seen (p=.228). In this study, medullary subtype showed highest rate of survivin expression (100%) followed by IC-NST (63.4%) cases then ILC (50%) cases and finally other types. These results were opposite to **(Sušac et al., 2019) and (Dogu et al., 2010),** who reported that survivin was higher expression was noticed in IC-NST (81.5%), (86.7%) respectively, followed by medullary subtype and then other subtypes. **Rasoola et al and Zhang et al** reported that survivin showed high expression in IC-NST followed by ILC subtype and then other types **(Rasoola et al.,2019) and (Zhang et al., 2013).** The difference between our study and other studies might be due to small simple size.

This study included 4 cases of grade I (6.7%), 29 cases of grade II (48.3%).The cases with grade II were the highest number of cases, and 27cases of grade III (45%) These results are compatible with many studied which reported Grade II to show the highest prevalence, yet with lower figures than this study; 67.5% ***(Ravikumar and Ananthamurthy, 2014)*** and 62% ***(Jeong et al., 2014)***. ***Natalie Reimers et al., 2004*** showed Grade I (25.6%), Grade II (40.9%) and Grade III (33.3%).

Lim et al., 2014 reported 3.2% Grade I cases, 24.6% Grade II cases and 72.1% Grade III cases. ***Aggarwal et al., 2014*** showed 3.4% Grade I cases, 37.3% Grade II cases and 59.3% Grade III cases. ***Ying Liu et al., 2013*** showed Grade I (13.5%), Grade II (35.7%) and Grade III (50.8%).The difference between the current study and others might be related to this small sample size.

Also these results are compatible to ***(Wang et al., 2018)*** who reported that cases with high grade more than low grade cases were found to be concerned only by TNBC or HER-2/Neu overexpressing BC ***(Niemiec et al., 2018)***.

High grade cases showed statistically significant as survivin was higher expression in it than low grade cases; cases of grade III showed positive survivin expression in 88% of them followed by cases of grade II showed positive survivin expression in 37.9% of them. This is consistent with most of the studies in the literature ***(*Shi et al.,2019, Sušac et al., 2019, Rasoola et al., 2019, Sarti et al., 2013, and Dogu et al., 2010).***

Concerning the T stage, the majority of cases were T2 (33 cases; 55%), followed by T1 (15 cases; 25%), then T3 (12 cases; 20%) and no cases reported in T4. This is consistent with two Saudian and Sudanese studies which reported predominant T2 cases (62%), (47%) respectively ***(Khabaz et al., 2017 and Sengal et al., 2017)***, also compatible with 54.4 % ***(Ren et al., 2014)***, 62.2% ***(Liu et al., 2014)***, 73.7% ***(Zhang et al., 2014)***, 68.3% tumor size >2 reported in **(Ying Liu et al., 2013)**, 45.5% ***(Chen et al., 2011)***, and 47.6 % **(Natalie Reimers et al., 2004).** On the contrary, many Western studies reported predominance of T1 cases ***(Do et al., 2017; Pu et al., 2018 and Agrawal et al., 2018).***

Neri et al., 2015 reported 63.6% T1 cases and ***Bae et al., 2013*** reported 54% T1 cases. Another Egyptian study reported a predominance of T3 cases (48%) ***(Aboulhagag et al., 2018).*** This difference may be related to higher BC awareness and earlier detection in the more developed countries.

In study the relation of survivin expression and tumor size showed statistical significance (p=.021); as T3 showed highest rate (91.7%) followed by T2 (54.5%), larger tumor size cases (T2 and T3) showed higher rate of survivin expression than smaller tumor size cases. This is compatible with **(Rasoola et al., 2019 and Zhang et al.,2013)** as cases of T3 was highest rate followed by cases of T2. **Dogu et al., 2010** reported that cases of T2 & T3 showed higher survivin expression than cases of T1. **Shi et al., 2019** reported that cases of T1,T2 (65.3%) showed higher survivin expression than cases of T3 (34.7%).

The study showed that nodal stage positive (44 cases 73.3%) was higher than nodal stage negative (16 cases 26.7%) that was in details as 16 cases of N0 stage (26.7%), 27 cases of N1 stage (45%), 12 cases of N2 stage (20%), and 5 cases of N3 stage (8.3%). Within the node positive cases in our study, N1 was the commonest this is consistent with most reported studies (***Do et al., 2017; Agrawal et al., 2018 and Louhichi et al., 2018)***.

These results were incompatible with other studies that showed N0 cases were highest rates nodal stage with variable figures; 60.4% ***(Neri et al., 2015)***, 48% ***(Zhang et al., 2014)***, 59.6% ***(Baccelliet al., 2014)*** and 37.5% ***(Mohammadizadeh et al., 2014)***.

Natalie Reimers et al., 2004 showed N0 and N1/2 are both near equal 50%. While our study was compatible with the results of some studies that node positive cases were higher than node negative cases as reported by ***(Ying Liu et al., 2013)*** **and** ***(Wu et al., 2017 and Saponaro et al., 2018),*** others studies reported that highest rate with node negative cases ***(Han et al., 2018; Wang et al., 2018 and Yue et al., 2018).*** This difference may be related to different cultures of different countries, higher BC awareness and earlier detection in the more developed countries.

For statistical purpose the cases were grouped as nodal metastases positive cases (including 44 cases 73.3%) & nodal metastases negative cases (including 16 cases 26.7%).The cases with positive LN metastasis showed higher survivin expression than cases with LN negative metastasis. This agreed with the results of ***(*Shi et al., 2019, Sušac et al., 2019, Sarti et al., 2013, Zhang et al., 2013 and Dogu et al., 2010).***

By grouping the cases into anatomic stages, the early stage (stage I & stage II) were 49 cases (81.67% and advanced stage (stage III & stage V) included 11 cases (18.33%) according to the AJCC classification ***(Zhou et al., 2018***). This was compatible with most of the reported studies ***(Khabaz et al., 2017; Zhou et al., 2018 and Luo et al., 2018).*** Survivin expression was higher in advanced stage than early stage cases (p=.748). This was consistent with the results of **(Shi et al.,2019, Sušac et al., 2019, Zhang et al., 2013 and Dogu et al., 2010)** who reported survivin higher expression in advanced stage and this expression indicate poor prognosis.

An associated in situ component in invasive breast carcinoma was detected in 28.3% of this cases. This is agree with ***Ravikumar et al., 2014*** who reported an in situ component in 38.9 % of the cases and ***Gentilini et al., 2008*** who reported 37.6%. However, ***(Lim et al., 2014)*** reported an in situ component in 91.8 % of the cases. In our study survivin showed higher expression in cases of breast carcinoma without in situ component. This correlation was statistically insignificant.

Regarding the extent of stromal TILs, 53 cases showed low TILs (88.33%) and 7 cases showed high

TILs (11.67%) This classification were taken from ***(Polónia et al., 2017 and Tomioka et al., 2018)*** who reported that tumors were defined as High-TILs (≥30%) or Low TILs (<30%). This was consistent with the results of many studies reporting predominance of low TILs cases, confirming BC as a generally poorly immunogenic malignancy ***(Polónia et al., 2017 and Tomioka et al., 2018).*** Cases with high stromal TILs (≥ 30% of the stroma) showed higher survivin expression than cases with low stromal TILs. This correlation were statistically insignificant.

Concerning the number of masses, 80% of our cases showed single mass, while 20% were more than one. This was consistent with most other studies, as showing; 79.7% by ***(Neri et al., 2015)***, 85.9% by ***(Chung et al., 2012)***, 89% by ***(Cabioglu et al., 2009)*** and 86.9% by ***(Joergensen et al., 2008)***. However, ***Tot et al., 2011*** showed lower than current study 65.7%.

Survivin showed equal expression in cases with single mass and cases with multiple masses. This correlation was statistically insignificant.

To the best of our knowledge none of the previous studies, correlated the expression of survivin and in situ component, stromal TILs, and number of masses.

By studying the cases for evidence of LVI, cases with LVI was 32 cases (53.3%),and cases without LVI was 28 cases (46.7%). This incompatible with many studies, which yet reported higher rates of LVI negativity; 75% by ***(He et al., 2017),*** 82.5% by ***(Wang et al., 2018)*** and 66% by ***(Aboulhagag et al., 2018).*** Survivin expression was higher in cases with LVI than in cases without LVI, this correlation was statistically significant. This agreed with (**Dogu et al., 2010)** who reported that survivin higher expression in cases with LVI, however incompatible with **(Zhang et al.,2013)** who reported that survivin expression was higher in cases without LVI.

Regarding the hormone receptors of the studied cases, 40 cases were positive ER expression (66.7%) and 39 cases were positive PR expression (65%). Regarding ER expression this was consistent with the WHO report ***(Allred et al., 2012)***, 62.8% ***(Zou et al., 2014)***, 71.5% ***(Woo et al., 2014)***, 73% ***(Ieni et al., 2014)***, 68.7% ***(Youssef et al., 2014)***, 76.7% ***(Natalie Reimers et al., 2004)*** and with many studies ***(Inoue et al., 2017 and Litwin et al., 2018)***, other studies performed on different nationalities reported contradictory higher rates of hormone negative cases as reported by (***Aggarwal et al., 2014***, ***Bansal et al., 2017, Sengal et al., 2017 and Mwakigonja et al., 2017).***

For PR expression our results were compatible with almost all the studies which agreed that PR positive cases have the upper hand with mostly close reported figures; 65.1% ***(Neri et al., 2015)***, 56.7% ***(dos-Santos et al., 2014***), 64.2% ***(Youssef et al., 2014)***, 66.3% (***Choi et al., 2014)*** and also with many studies ***(Inoue et al., 2017 and Litwin et al., 2018)***.While other study showed predominance of PR negative 65.7% (***Natalie Reimers et al., 2004)***.

Another Egyptian study reported more ER positive cases and more PR negative cases than ER negative cases and PR positive cases respectively ***(Aboulhagag et al., 2018).*** The common between all those studies was the slightly lower incidence of PR positivity than ER positivity, which is similar with our results.

Concerning our study, Survivin showed higher expression in hormonal receptors negative (ER and PR) than hormonal receptors positive. This correlation was statistically significant (p<.001). Similarly, **Shi et al.,2019, Sušac et al.,2019 and Zhang et al.,2013** who reported statistically significant (p<.001) correlation of survivin expression with hormone negative status. ***Tsuji et al., 2004 and Tanaka et al., 2000*** reported that the rate of survivin mRNA expression was higher in the ER negative cases (64.2%), (75.5%) than in the ER positive cases respectively and higher in the PR negative cases (57.1%) than in the PR positive cases (36.4%). Also, other studies reported a relationship between high survivin expression and ER-negative and PR negative tumors by using mRNA as (**Span et al., 2004**) and using immunohistochemical analysis as ***(Singh et al., 2004***).

On the other hand **(Rasoola et al., 2019)** reported that suvivin expression was high in cases with ER negative but PR positive. This may refer the difference to using different methods for survivin assessment as in their study they measured survivin using mRNA and its two isoform variants (ΔEx3 and survivin-2B mRNA) using real-time PCR (qRT-PCR) method.

In the studied cases, 10 cases only were positive for HER-2/Neu overexpression (16.7%) according to **Hicks et al., 2011** who was scoring HER-2/Neu expression as negative included (score 0,1) and positive HER-2/Neu expression included (score 2,3), which is close to figure reported by WHO (15%) ***(Allred et al., 2012).*** However, variable figures was few lower than ours study (12.8%) ***(Pu et al., 2018)*** and many higher than our study reaching up to 49% ***(Saponaro et al., 2018)***, 82.5% ***(Zou et al., 2014)***, 65% ***(Jeong et al., 2014)***, 72.9% ***(Zhang et al., 2014) and.*** 50.9 % ***(Soliman et al., 2013)***.

No statistical significant relationship between survivin expression and HER-2/Neu status was observed in the current research. This was in concordance with **(Rasoola et al.,2019),** ***(Youssef et al., 2008)*** and ***(***Nassar et al., 2008).

As for the Ki-67 proliferation index, 32 cases (53.3%) of the cases showed low Ki-67 index and 28

cases (46.7%) showed high Ki-67 index, according to **(**L**eung SCY etal.,2016)** who scored Ki-67 into low Ki-67 index <20% and high Ki-67 index >20%. This agreed with many studies which showed high, yet variable rates of low Ki-67 index; 68.1% low Ki-67 cases ***(Luo et al., 2018),*** 63.2% ***(Woo et al., 2014),*** 56% ***(Kim et al., 2014)***, 59.3% ***(dos-Santos et al., 2014)*** and 55.6% ***(Ying Liu etal.2013)***, other studies high lighted High Ki-67 cases to have the upper hand with variable figures; 60% ***(Xie et al., 2014)***, 76.2% ***(Liu et al., 2014)***, 68.9% ***(Ieni et al., 2014)***, and 69.3**% (Shu Zhao et al., *2013)***.

Another studies using a 20% cut off similar to us reported 62.2% and 42.1% low Ki-67 cases respectively ***(Wang et al., 2017 and Saponaro et al., 2018).***

This considerably variable results can be explained by the inter-laboratory differences, inter-observer variability and the different cut off points used, which encouraged some authors for using the more reproducible histological grading as an alternative for Ki-67 index, especially when classifying the Luminal cases into A and B ***(Curigliano et al., 2017)***. Survivin expression observed to be statistically related to high Ki-67 index (p<.001). This was compatible with **(Sušac et al., 2019), (Dalić and Šarčević 2017) and (Sarti et al., 2013)** who reported that survivin higher expression was associated with high proliferative index Ki-67.

Regarding the molecular subtypes of the studied cases, 20 cases were Luminal A (33.3%), 20 cases were Luminal B 20 cases (33.3%), then 14 cases were TN 14 cases (23.4%) and finally 6 cases were HER-2/NEU enriched (10%). Regarding the luminal cases, our results of higher rate in Luminal A and B as both were equal. ***Mo et al., 2017 and Wu et al., 2017*** reported that luminal B higher than Luminal A cases**.** However, other studies reported more Luminal A than B cases ***(Burugu et al., 2017 and Louhichi et al., 2018).***

These differences can be partially explained by the previously mentioned variability in the Ki-67 results and whether the histological grade is used to classify Luminal cases or not. Our results can be explained by the relatively high ratio of high grade and high ki-67 cases in our study. Similarly, our results were consistent with some studies reporting that TN cases was more than HER-2/Neu enriched cases ***(Ni et al., 2017 and Ryspayeva et al., 2017)***, however, other studies reported the reverse ***(Ahmed et al., 2018).***

Finally on grouping our cases into molecular subtypes, the highest rate of survivin expression was noticed in HER-2/Neu enriched subtype and the survivin in the cases with TN subtype expressed mainly nuclear than cytoplasmic that was predictor of worse out come. This correlation is statistically significant (p=.001) and compatible with most of studies (**Dalić, and Šarčević 2017)** ***and (Youssef et al., 2008)*** who reported that survivin expression was high in HER-2/Neu enriched subtype and TN subtype. **Shi et al.,2019, Zhang et al., 2013 and Dogu et al., 2010** reported that survivin showed highest expressed in TN subtype that expression indicate poor prognosis. Also **Manuela et al.,2013** reported that nuclear survivin expression had strong association with TN subtype that worse the outcome as drug resistance and low survival period.

Ni et al., 2017 and Ryspayeva et al., 2017 reported that survivin expression more in TN cases than HER-2/Neu enriched cases. However, other studies reported the reverse ***(Ahmed et al., 2018).***

These differences can be partially explained by the previously mentioned variability in the Ki-67 results and whether the histological grade is used to classify Luminal cases or not.

In our study survivin expression was statistically significant with high tumor grade, T3 stage, cases with LVI, (ER & PR) negative hormone statuses, high KI-67 index and TN, HER-2/Neu enriched molecular subtypes. This finding may be explained based on the functional propriety of survivin as apoptosis regulator **(**Jha K **et al., 2012).** More importantly, cytoplasmic localisation of survivin in non-malignant cells suppresses apoptosis, while nuclear translocation may be important to regulate proliferation **(**Knauer SK **et al., 2007**).

Nuclear survivin is a predictor of worse outcome in breast cancer and a strong association between nuclear survivin and the triple-negative breast cancer subtype **(Rexhepaj E et al.,2010).**

The high frequency of survivin expression in high grade breast carcinomas mainly TN and HER-2/Neu enricherd subtype suggest the potential role of survivin antagonist as apoptosis based therapy in the management of such cases **(Stache C et al.,2016).**

Moreover, as survivin expression was shown to be related to resistance to radiotherapy & chemotherapy in breast cancer **(Chun-Tao Shi et al., 2019)**; Some author's suggests that decreasing the level of survivin using tyrosine kinase inhibitors could be another strategies processing management in cancer **(Stache C et al.,2016).**

In the future prime-based vaccination targeting survivin is supposed to be for potential importance **(Huang et al., 2014).**

Conclusion

- Our results support that positive survivin expression is poor prognostic features of cancer breast being associated with 58.3% of the cases.

- Survivin expression is most associated with both TN subtype and HER-2/Neu enriched molecular subtype.
- The inconsistency and controversy of results between the various studies may be related to heterogeneity of cases included in these studies, technical factors, such as the type of antibody, differences in immunostaining method or immunostaining scoring method and cut-off points for negative/positive.

Recommendations

- Further studies with standardization of methodologies, larger study samples and long term follow up are required to establish the prognostic significance of survivin expression to provide target therapy.
- Extensive molecular studies, both in vivo and in vitro, with simultaneous immunohistochemical studies are required to elucidate the possible mechanistic association of survivin expression with occurrence of recurrence and distant metastases.
- Extended focused studies are needed to evaluate the possible role of anti survivin therapy on TN and HER-2/NEU enriched breast cancer cases.

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4/25/2020