



Syndecan-1 could be added to hormonal receptors and HER2/neu in routine assessment of invasive breast carcinoma, relation of its expression to prognosis and clinicopathological parameters

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ABSTRACT

Introduction: Syndecan-1 is heparan sulfate proteoglycans (HSPGs) that is used as coreceptors for signaling of growth factors. The comprehensive effect of syndecan-1 is to augment receptor stimulation at little ligand concentrations.

The goal of this research: is to study syndecan-1 expression in breast carcinoma and its value in predicting the prognosis in comparison to other clinicopathological parameters.

Material & methods: immunohistochemistry study for syndecan-1 is done on 103 cases of invasive breast carcinoma. Its expression is assessed and correlated to other clinicopathological parameters and prognosis.

Results: overexpression was significantly related to high histologic grade ($p = 0.001$), large tumor size ($p = 0.043$), HER2-positive status ($p = 0.001$), and ER&PR-negative status ($p = 0.001$). It was also have a negative impact on the overall survival ($p = 0.012$) and disease free survival ($p = 0.009$). Syndecan-1 expression showed weak positive correlation with Her 2 expression (Correlation Coefficient (co): 0.332, $p = 0.001$).

Conclusion: syndecan-1 is a good predictor of poor overall survival and recurrence/ metastasis free survival. It is associated with aggressive phenotype as HER2 enriched and Triple negative rather than luminal subtypes of breast carcinoma. So it can be added to the hormonal receptors and HER 2 assay in the routine management of invasive breast cancer after confirmation on a more larger study.

1. Introduction

Globally, breast cancer is the most common cause of cancer-related death in women [1]. It has distinct molecular subtypes associated with variable clinical outcomes [2]. Breast neoplasms have a lot of histopathological variants but > 95% of them are either ductal or lobular carcinoma [3,4].

Failure of management occurs in 30% of the cases [5]. A reasonable explanation for this failure of treatment is an incomplete picture of the biologic natural history of breast cancer. So, there is a bad need to find sensitive and specific biomarkers for prognosis of breast cancer beyond the classic clinical and pathologic parameters that have important clinical implications [6,7]. One of these markers is the c-erbB-2 receptor, which has a role not only in breast oncogenesis but also as a therapeutic target. C-erbB-2 activation occurs by binding to the growth factor family. Heparan sulfate proteoglycans (HSPGs) are used as coreceptors for these growth factors. Syndecans and glypicans are the main

cell-associated HSPGs [6,8–11].

Syndecan proteoglycans are four-member family of transmembrane receptors that regulate cell adhesion, proliferation, and differentiation. So it is considered as key regulators of tumor invasion and metastasis [8,12].

Syndecan-1, like all HSPGs, is essential for HBGF/HBGF receptor signaling. It increases the local concentration of GFs and protects GFs from proteolytic cleavage (e.g. transforming growth factor). It participates in the internalization of ligands. Also it is regulating cellular responses to mitogenic and angiogenic growth factors. So the comprehensive effect of syndecan-1 is to augment receptor stimulation at little ligand concentrations [10,11]. However Nguyen et al.2013 reported that, syndecan-1 can suppress or promote oncogenesis according to the model used or tissue examined [8].

Syndecan-1 is expressed in epithelial cells of ducts and lobules and transiently in myoepithelial cells [12,13]. Myoepithelial cells showed absolute strong staining whereas luminal cells showed reactivity along

Abbreviation: HSPGs, heparan sulfate proteoglycans

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the basolateral cell membrane, and occasionally cytoplasmic staining [6].

The expression of syndecan-1 in invasive breast carcinoma is doubtful up till now. Enhanced expression of syndecan-1 at the RNA level and protein level is obvious in cell lines and epithelial or stromal cells of tumor tissue [6]. Its expression predicts an aggressive behavior of the tumor and bad clinical outcome [7,14,15]. In the opposite side, an earlier study revealed diminished syndecan-1 expression in breast carcinoma cells [16]. Another member of Syndecan (syndecan-4) is demonstrated in most cell lines of breast carcinoma, but with unobvious role in oncogenesis [12,17,18]. Barbareschi et al. 2003 confirmed that syndecan-1 expression had self-determining prognostic value, particularly in the estrogen-independent tumors [6]. This finding raises clinical concern to examine the prognostic value of the combined steroid receptor/ syndecan-1.

The goal of this research is to study syndecan-1 expression in breast carcinoma and its value in predicting the prognosis in comparison to other clinicopathological parameters.

2. Material & methods

103 cases of invasive cancer breast were collected retrospectively from Mansoura University, Faculty of Medicine, Oncology Center, Egypt between January 2013 and December 2015. All patients treated surgically by modified radical mastectomy and received postoperative hormonal, chemotherapy, or radiotherapy. The data were collected from the sheets of the patients till August 2018. Follow-up period ranged within 32–68 months, with a median follow-up of 37 ± 20.51 months. The ethics committee of Mansoura University approved the research idea. Hematoxylin and eosin-stained slides (cut from formalin fixed, paraffin wax-embedded specimens) were retrieved from the archive of the oncology center and reviewed. WHO 2014 was used to classify the cases [19]. A total of 98 (95.1%) cases were invasive ductal carcinoma (IDC), not otherwise specified (NOS). four (3.9%) cases were invasive lobular carcinoma. One case was mucinous carcinoma (1%). Nottingham Grading System was used to grade the tumor [20].

Manual tissue microarray (TMA) was prepared by pencil tip [21–24]. Triplicate 1-mm cores/ case were constructed. Normal breast tissue was used as an internal control. The adequacy of tumor samples was tested by examination of H&E-stained sections of the final microarray blocks.

Immunodetection was performed using Ventana Benchmark Ultra machine automated staining system. The primary antibodies used were: ER (clone SP1 rabbit monoclonal primary antibody) (Ventana, Tucson, AZ, USA), PR (clone 1E2, rabbit monoclonal primary antibody) (Ventana, Tucson, AZ, USA), HER2/neu (clone 4B5, rabbit monoclonal primary antibody) (Ventana, Tucson, AZ, USA), and primary antibody of CD138/Syndecan-1(clone B-A38, mouse monoclonal primary antibody) (Ventana, Tucson, AZ, USA). Internal positive controls were normal breast duct epithelia. Positive external controls were ER, PR, and HER2/neu-positive breast carcinomas for ER, PR, and HER2/neu, respectively. Negative controls were done via replacing primary antibody with PBS.

2.1. IHC evaluation

The positive of ER and PR is considered when at least 1% of the tumor cells showed definite nuclear staining according to ASCO/CAP guidelines [25]. HER2/neu was scored according to the pattern of membranous staining and percentage of stained malignant cells as follows: 0, no staining or faint incomplete staining in < 10% of cells; 1, faint incomplete staining in > 10% of cells; 2, weak to moderate complete staining in > 10% of cells; and 3, strong complete staining in > 10% of cells. Only score 3 was considered positive [26]. The level of syndecan-1 expression was interpreted by 2 pathologists unaware of the clinical data of the cases. The intensity of the membranous and

cytoplasmic staining was scored as follow: (0+ membranous) Fig. 3A,B; (1+ membranous) Fig. 3C; (2+ membranous) Fig. 3D; (3+ membranous) Fig. 3E; and (cytoplasmic staining) Fig. 3F. In this study we assess the intensity of staining and proportion of the stained tumor cells semiquantitatively. Then we consider cases with more than 10% immunoreactive cells as high syndecan-1 expression [6,8]. Molecular classification of the study cases depend up on the results of hormonal receptor and HER2 status. Tumors were defined as luminal (ER + and/ or PR +), HER2 (ER-/PR-/HER2+), or triple-negative carcinoma (ER-/PR-/HER2-) [27,28].

2.2. Statistical analysis

All statistical analyses were done with the SPSS version 20 software program. Categorical data obtained were statistically evaluated using the χ^2 test. Whereas continuous data were evaluated using the One way ANOVA test. The tests were considered statistically significant when the P value less than 0.05. Overall survival (from date of diagnosis to death) and disease-specific free survival were determined by Kaplan-Meier test. The log-rank test was used to compare groups. Cox regression analysis of the pathological parameters affecting the prognosis of cancer breast was done. Also Correlation between CD138, hormonal receptor (ER,PR) and Her 2 in breast carcinoma was done using bivariate Pearson correlation test.

3. Results

This study was achieved retrospectively on 103 patients with invasive breast carcinomas. The mean age of the patients was 54.56 ± 12.19 years, with an age range of 31–88 years. The different clinicopathological features of cases are displayed in Table 1. The significant association of these clinicopathological characteristics with the Syndecan-1 expression was illustrated in Table 2. The relative significance of either Syndecan-1 expression or other clinicopathological

Table 1
Clinicopathological characteristics of the study cases.

Age	54.56 ± 12.19		
ER	N	50	48.5%
	P	53	51.5%
PR	N	47	45.6%
	P	56	54.4%
Her2	N	82	79.6%
	P	21	20.4%
Tumor grade	GI	29	28.2%
	GII	50	48.5%
	GIII	24	23.3%
Tumor size	≤ 2cm	5	4.9%
	> 2cm	98	95.1%
Lymph Node status	N	25	24.3%
	P	78	75.7%
Tumor Stage	Stage I	2	1.9%
	Stage II	47	45.6%
	Stage III	54	52.4%
Time of follow up	37.49 ± 20.16		
	Live or dead	77	74.8%
Metastasis or recurrence	dead	26	25.2%
	Absent	71	68.9%
Histological type	Present	32	31.1%
	IDC	98	95.1%
Molecular type	ILC	4	3.9%
	Mucinous	1	1.0%
	HER 2	15	14.6%
	Luminal A	42	40.8%
Syndican-1	Luminal B	21	20.4%
	Triple -ve	25	24.3%
	N	58	56.3%
	P	45	43.7%

N: negative, P: positive, G: Grade.

Table 2
The association of syndecan-1 expression with the clinicopathological characteristics of the study cases.

		CD138		P value		
		N	P			
Tumor grade	GI	24	82.8%	5	17.2%	0.000*
	GII	28	56.0%	22	44.0%	
	GIII	6	25.0%	18	75.0%	
Tumor size	≤ 2cm	5	100.0%	0	0.0%	0.043*
	> 2 cm	53	54.1%	45	45.9%	
Lymph node status	N	18	72.0%	7	28.0%	0.069
	P	40	51.3%	38	48.7%	
Tumor stage	Stage I	2	100.0%	0	0.0%	0.334
	Stage II	28	59.6%	19	40.4%	
	Stage III	28	51.9%	26	48.1%	
Histological type	IDC	55	56.1%	43	43.9%	0.395
	ILC	3	75.0%	1	25.0%	
	MUCINOUS	0	0.0%	1	100.0%	
ER	N	20	40.0%	30	60.0%	0.001*
	P	38	71.7%	15	28.3%	
PR	N	18	38.3%	29	61.7%	0.001*
	P	40	71.4%	16	28.6%	
HER2	N	53	64.6%	29	35.4%	0.001*
	P	5	23.8%	16	76.2%	
Molecular type	Her2	2	13.3%	13	86.7%	0.009*
	luminal A	29	69.0%	13	31.0%	
	luminal B	15	71.4%	6	28.6%	
	triple -ve	12	48.0%	13	52.0%	
Live or dead	Live	49	62.0%	28	38.0%	0.012*
	dead	8	37.5%	17	62.5%	
Metastasis/recurrence	N	46	64.8%	25	35.2%	0.009*
	P	12	37.5%	20	62.5%	

P value ≤ 0.05 is significant N: negative, P: positive, G: Grade.

Table 3
Cox regression multivariable analysis of factors affecting prognosis.

A : Overall survival		B: Disease free survival	
	Sig.		Sig
Tumor Grade	0.838	Tumor Grade	0.658
Tumor Size	0.416	Tumor Size	0.504
Lymph Node Status	0.641	Lymph Node Status	0.598
Tumor Stage	0.013	Tumor Stage	0.003
Molecular Type	0.246	Molecular Type	0.190
Histological Type	0.752	Histological Type	0.980
CD138	0.096	CD138	0.355

parameters of invasive breast carcinoma in predicting the prognosis is shown in [Table 3](#).

3.1. Clinicopathological characteristics of the study cases

[Table 1](#) showed that among the 103 cases, 98 cases were IDC NOS (95.1%), 4 cases were invasive lobular carcinoma (ILC) (3.9%), and only 1 case was mucinous carcinoma (1%). Approximately 48.5% of the cases were grade II, and 95.1% of the cases displayed tumor size of more than 2 cm. 75.7% of the patients exhibited pathologically positive lymph nodes, and 52.4% of the cases were in stage III. Additionally, 31.1% of the cases developed distant metastasis and recurrence, and 23.3% of the cases were dead. ER + and PR + rates were 51% and 54%, respectively. 20% of cases were HER2+ (score, 3+). According to this immunophenotyping, the cases of this study were classified as luminal A, luminal B, HER2, and Triple-negative in 40.8%, 20.4%, 14.6%, and 24.3% of the cases, respectively. Syndecan-1 expression as determined by CD138 was positive with different scores in 43.7% of the cases [Fig. 3](#).

3.2. The association of Syndecan-1 expression with the clinicopathological characteristics of invasive breast carcinoma

[Table 2](#) showed that syndecan-1 overexpression was significantly related to clinicopathological parameters associated with an aggressive phenotype, including high histologic grade (p = 0.001), large tumor size (p = 0.043), HER2-positive status (p = 0.001), and ER&PR-negative status (p = 0.001). It was also have a negative impact on the overall survival (p=0.012) and disease free survival (p = 0.009). In addition high Syndecan-1 expression was not significantly associated with invasive ductal carcinoma as compared to invasive lobular or mucinous carcinoma. Only one case of lobular carcinoma show weak cytoplasmic expression of syndecan-1

3.3. Syndecan-1 Expression and Survival of invasive breast carcinoma

Positive syndecan-1 was significantly associated with overall survival (95% confidence interval [CI], 51.6–62.4; P = .021) and disease-free survival (95% CI, 51.6–57.7; P = .036) on univariate analysis (Kaplan-Meier test) ([Fig. 1](#)) but multivariate analysis (cox Regression) failed to identify syndecan-1 as an independent factor of either over-all survival nor disease free survival among the clinicopathological parameters analyzed ([Table 3](#)).

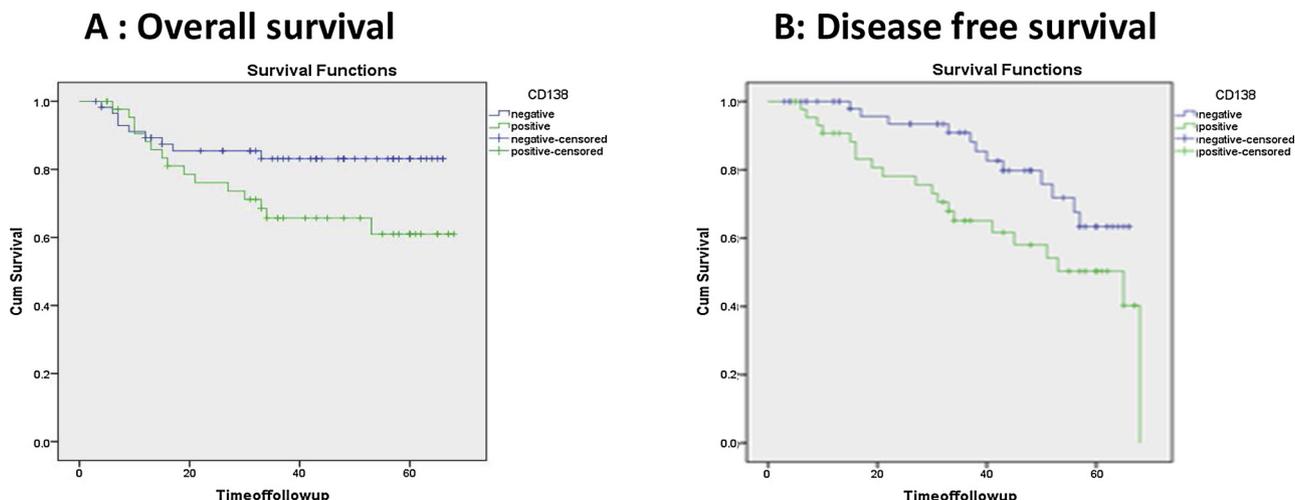


Fig. 1. survival curve of breast carcinoma (Kaplan-Meier test): Test of equality of survival distributions for the different levels of CD138.

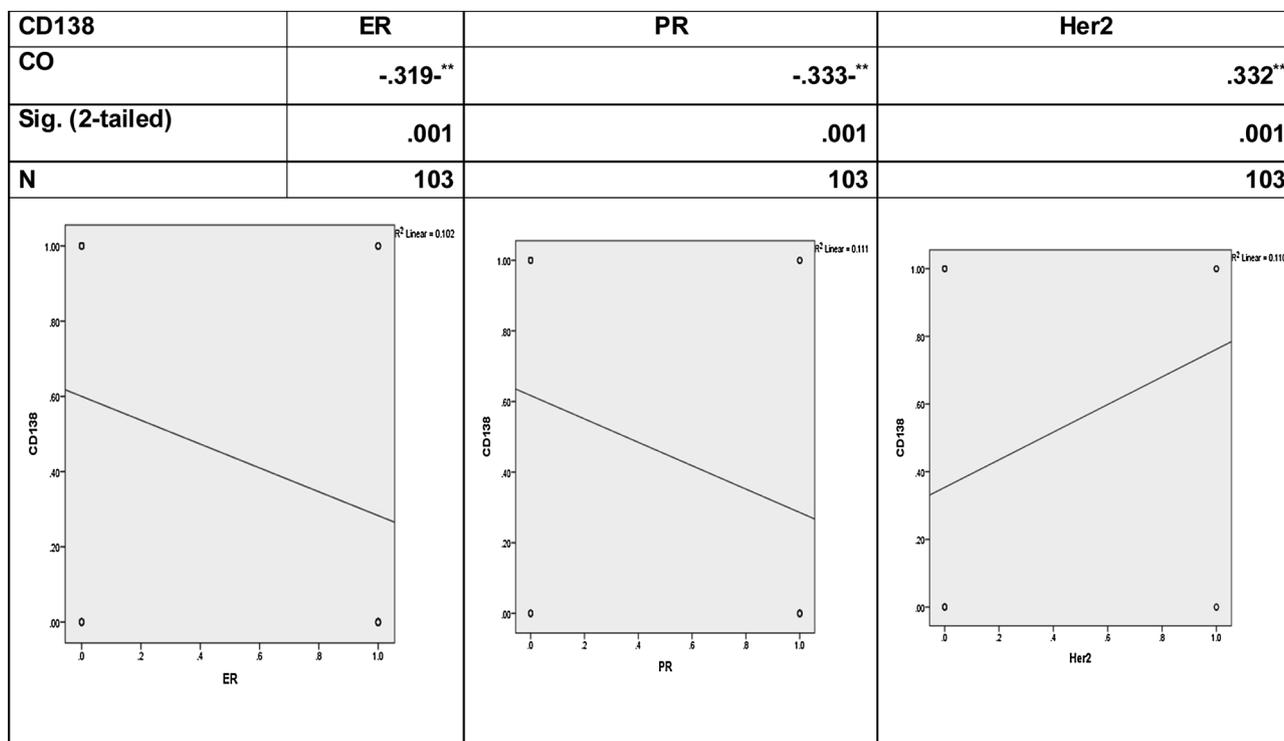


Fig. 2. Correlation between CD138 and hormonal receptor (ER, PR) and Her2 in breast carcinoma Co, Pearson correlation coefficient (*correlation is significant at the 0.01 level; **correlation is strongly significant).

3.4. Syndecan-1 expression correlation with hormonal receptor and Her2 expression

Syndecan-1 expression showed weak negative correlation with ER and PR expression (Correlation Coefficient (co): -0.319, -0.333 respectively p = 0.001 in both) and weak positive correlation with Her 2 expression (Correlation Coefficient (co): 0.332, p = 0.001) Fig. 2.

4. Discussion

Syndican-1 as a characteristic member of HSP mediates cell adhesion, migration, and regulates the cell response to growth factors and angiogenic factors. So it has a major role in proliferation, differentiation, oncogenesis and metastasis [8,10–12]. High mortalities and resistance to different therapeutic modalities are the main motivation to search for new markers that add to genetic and molecular phenotyping of breast carcinoma which in turn allow selective therapeutic applications [6,7]. Gene profiling studies have identified various subtypes of breast carcinomas with prognostic significance. It has now evidenced that the overall survival of patients with HER2 and basal-like subtypes is poorest compared with those with luminal subtypes [29,30].

The clinicopathological and prognostic value of SDC1 expression patterns in breast cancer patients remains controversial [7]. Over-expression was related to clinicopathological parameters associated with an aggressive phenotype, including high histologic grade, large tumor size, and ER-negative status [6,12,15,31]. Further, SDC1 was found to be an independent prognostic factor for overall survival in one model of Barbareschi et al. 2003 study [6]. In contrast, syndecan-1 expression in carcinoma cells was observed to be reduced in 2 additional studies [14,16]. The aim of this study is to examine syndecan-1 expression in breast carcinoma, its correlation with the hormonal receptors, and prognostic significance and then its pertinence to which molecular phenotype.

In the present study, we noticed that syndican-1 overexpression was significantly related to clinicopathological parameters associated with an aggressive phenotype, including high histologic grade (p = 0.001),

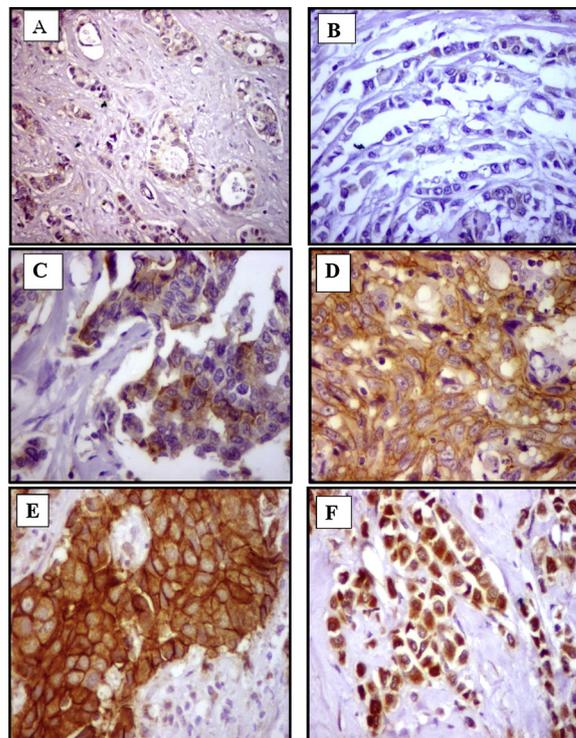


Fig. 3. Immunohistochemical staining pattern of syndecan-1 in invasive breast carcinoma. A) Negative IDC (x400), B) Negative ILC (x400), C) + 1 membranous staining (x400). D) + 2 membranous and cytoplasmic staining (x 400). E) + 3 membranous staining (x 400). F) Cytoplasmic staining of invasive lobular carcinoma (x 400).

large tumor size (p = 0.043), HER2-positive status (p = 0.001), and ER & PR-negative status (p = 0.001). It was also have a negative impact on the overall survival (p=0.012) and disease free survival (p = 0.009).

These results confirm the fact that syndecan-1 regulate cell proliferation, differentiation, oncogenesis, angiogenesis and invasion. Also these results were in agreement of most of the studies [6,12,15,31]. However, they were at variance to the results of earlier studies that concluded lower syndecan-1 expression in breast carcinoma [14,16]. Our explanation for this feature is the limited number of cases in these studies that's why they can't examine the exact role and expression of syndecan-1 in breast carcinoma. In our study, Syndecan-1 expression in ductal carcinoma is more in invasive ductal carcinoma than in invasive lobular carcinoma but not to the degree to be significant by Chi Square test and also the limited number of invasive lobular carcinoma may be a cause. This result reflects its role in cell adhesion.

Univariate analysis of the positivity of syndecan-1 by using Kaplan-Meier test revealed that, it significantly predicts the overall survival and recurrence/ metastasis free survival. And positive syndecan-1 was associated with poor outcome of breast carcinoma. These results were in agreement of many studies [6,12,15,31]. However multivariate analysis (Cox Regression) failed to identify syndecan-1 as an independent factor of either overall survival or disease free survival among the other clinicopathological parameters analyzed. Only tumor stage in this study was the only predictor of the outcome of breast carcinoma among the different clinicopathological parameters including syndecan-1 expression (Table 3). Also these results were in harmony with the Pearson correlation test result that revealed syndecan-1 expression was inversely correlated with steroid receptors (ER, PR) and positively correlated with HER 2 expression thus confirming that syndecan-1 expression is associated with the aggressive phenotype HER2 type and Triple negative subtypes rather than luminal subtypes of breast carcinoma.

5. Conclusion

By pooling of the currently available data and the result of our study, we conclude that, High Syndecan-1 expression is significantly associated with the aggressive clinicopathological parameters of invasive breast carcinoma as high grade, large tumor size, and advanced tumor stage. Also it is a good predictor of poor overall survival and recurrence/ metastasis free survival. It is associated with aggressive phenotype HER2 enriched and Triple negative subtypes rather than luminal subtypes of breast carcinoma. Much larger study with more variety of histological subtypes is needed to confirm the impact of syndecan-1 on the survival of breast carcinoma patients. Then it can be added to the hormonal receptors and HER 2/neu assay in the routine assessment of breast carcinoma and make a beneficial change in the therapeutic modalities of cancer breast.

Conflicts of interest and sources of funding

There are no conflicts of interest to disclose. There are no financial/ personal interests or belief that could affect the research objectivity,

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