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RESEARCH ARTICLE

AUTOPHAGY BIOMARKERS SQSTM1/P62 AND BECLIN-1 EXPRESSION IN BREAST CARCINOMA AND THEIR CLINICOPATHOLOGICAL SIGNIFICANCE.

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Keywords:-
Breast carcinoma, Autophagy, SQSTM1/p62, Beclin-1, immunohistochemistry

Abstract

Background: Clarifying the different mechanisms of molecular carcinogenesis of breast carcinoma could enable its better management, improving its prognosis and decreasing patient's mortality. Autophagy is the process of lysosomal degradation which could remove the damaged components so as to preserve cells homeostasis. Autophagy had a complicated role in cancer as it might inhibit or stimulate cancer progression which depend on the type of cancer. Several proteins which control autophagy had been discovered in human cells such as Beclin-1and SQSTM1/p62 (Sequestosome-1) that played important roles in autophagy.

Aim of the work: This study aimed to assess expressions of autophagy markers SQSTM1/p62 and Beclin-1 in breast carcinoma comparing expression of both markers with clinicopathological parameters of that type of cancer.

Patients and methods: SQSTM1/p62, Beclin-1 expression was evaluated using immunohistochemistry on sixty paraffin blocks of breast carcinoma. Then correlations between their expression levels and clinicopathological parameters were done.

Results: SQSTM1-p62 overexpression in breast carcinoma was strongly related to higher grade (=0.002) and American Joint Committee on Cancer staging system (AJCC stage) of the tumor, aggressive molecular type (=0.009), presence of lymph node metastases (=0.041), high KI67 index (p<0.001), negative ER& PR hormonal receptors (=0.01), Her2 neu expression (=0.03), presence of distant metastasis (p=0.011). The sensitivity of p62 over-expression as a predictor for advanced stage of breast carcinoma was 72.4 and the specificity was 97.9. Beclin-1 low expression was significantly correlated with aggressive molecular type (=0.004), higher grade (p<0.001) and AJCC stage of the tumor (p=0.002), presence of lymph node metastases (=0.041), high KI67 index (p<0.001), negative ER& PR hormonal receptors (=0.003), high Her2 neu expression (=0.006), presence of distant metastasis (p=0.011). But it had no significant correlation with histopathological subtype of breast cancer. The sensitivity of low **Beclin-1** expression as a predictor

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For advanced stage of breast carcinoma was 85.5 and the specificity was 97.9. We found an inverse relationship between p62 and Beclin-1 (Spearman's r= – 0.806),

Conclusion: SQSTM1-p62 over expression is a marker of poor prognosis, but Beclin-1 overexpression was a marker of good prognosis in breast carcinoma.

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Introduction:-

Breast **carcinoma** is the commonest malignancy and the 2nd cause of cancer related mortality in females all over the world (**Siegel et al., 2014).** It is the commonest among Egyptian female's cancers forming 34.26% of females' malignant tumors **(Mokhtar et al., 2007).** Clarifying the different mechanisms of molecular carcinogenesis of breast carcinoma could enable its better management improving its prognosis and decreasing patient's mortality **(Jemal et al., 2010).** Autophagy is the process of lysosomal degradation which could remove the damaged components so as to preserve cells homeostasis **(Young et al., 2009).** Autophagy had a complicated role in cancer as it might inhibit or a stimulate cancer progression which depend on the type of cancer **(Mathew and White, 2011).** Autophagy could allow cancer cells to resist stressful conditions like radiotherapy and chemotherapy, or it could inhibit cancer growth by removing oncogenic components and destroyed organelles **(White, 2012).** Several proteins which control autophagy had been discovered in human cells **(Weidberg et al., 2011), e.g.** Beclin-1 (Cao and Klionsky, 2007), (**Kondo et al., 2005) and** SQSTM1/p62 (Sequestosome-1) **(Lamark et al., 2009),** that played important roles in autophagy in normal and malignant cells. Many previous studies assessed their expression in cancer but the results are still conflicting.

Aim of the work this study aimed to assess expressions of autophagy markers SQSTM1/p62 and Beclin-1 in breast carcinoma comparing expression of both markers with clinicopathological parameters of that type of cancer.

Patients and methods:-

We have included sixty formalin fixed paraffin-embedded blocks of breast carcinoma of different histological subtypes were collected from the Pathology Department, Faculty of Medicine, **Beni-Suef** University and Pathology Department, Kasr Al Aini Hospital.. We used the American Joint Committee on Cancer staging system (AJCC) classification 7th edition carcinoma staging **(Edge and Compton, 2010)** and Nottingham (Elston–Ellis) modified Scarff– Bloom–Richardson grading system for carcinoma grading **(Elston, 2002).** We detected age of the patient, cancer size, histolopathological subtype, grade, stage, lymph node, and distant metastasis by examination of the patient's and the slide files in Pathology Department.

Immunohistochemical staining:-

Immunohistochemical analysis was done by using the streptavidin–biotin immunoperoxidase method (Hsu et al., 1981), then we had incubated slides with monoclonal. Anti-SQSTM1-p62 antibody [ab56416] (Abcam-Cambridge-Massachusetts- USA) diluted 1: 200 and primary rabbit polyclonal Anti-Beclin-1 ab ab55878 (Abcam) was diluted 1: 50 in blocking solution. We used tonsils and rat brain sections as a positive control for SQSTM1-p62 and for Beclin-1respectively.

Evaluation of immunohistochemical expressions of both Beclin-1 and SQSTM1/p62 proteins:-

We have evaluated both extent and the intensity of stain for both markers. The extent had been graded as zero (negative), one (<30% positive) and two (>30% positive) and we have graded the intensity was as zero (negative), one (weak), two (moderate) and three (strong). Then we have multiplied both scores to have a total final score: negative (from zero to one), low (from two to four) and high (five and six) **(Won et al., 2009),** score less than four was considered as low expression and scores more than four as high expression.

Statistical analysis:-

Continuous variables were expressed as the mean±SD and median (range) and the categorical variables were expressed as a number (%).A P value less than 0.05 was considered statistically significant. Percent of categorical variables were compared using Pearson's w2-test or Fisher's exact test when appropriate. The strength of the relationship between SQSTM1-p62, Beclin-1 and clinicopathological features was determined by computing Spearman's correlation coefficient.

Results:-

Sixty females' patients were included in our study, with age ranged from 39-77 years (Mean ± SD: 56.35±10.99). Demographic data of all patients were detailed in **table (1).**

SQSTM1/p62 expression in relation to clinicopathological features:-

SQSTM1/p62 overexpression in breast carcinoma was significantly correlated with older age of the patients, higher grade (=0.002) and AJCC stage of the tumor, aggressive molecular type (=0.009), presence of lymph node metastases (=0.041), high KI67 index (p<0.001), negative ER& PR hormonal receptors (=0.01),, high Her2 neu expression (=0.03), and presence of distant metastasis (p=0.011). But it had no significant correlation with histopathological subtype of breast cancer. **Tables 2& 4; fig 1**

The sensitivity of high p62 expression as a predictor for advanced stage of infiltrating duct carcinoma (IDC) was 72.4 and the specificity was 97.9

Beclin-1 immunoexpression and its correlation with clinicopathological features:-

Beclin-1 was cytoplasmic and its low expression was significantly correlated with older age of the patients, aggressive molecular type (=0.004), higher grade (p<0.001) and AJCC stage of the tumor (p=0.002), presence of lymph node metastases (=0.041), high KI67 index (p<0.001), negative ER& PR hormonal receptors (=0.003), high Her2 neu expression (=0.006), presence of distant metastasis (p=0.011). But it had no significant correlation with histopathological subtype of breast cancer. **Tables 3& 4; fig 2**

The sensitivity of low **Beclin-1** expression as a predictor for advanced stage of IDC was 85.5 and the specificity was 97.9 **Table 5**

Correlation between immunohistochemical expression of SQSTM1/P62, Beclin-1, in IDC:-

We found an inverse relationship between **SQSTM1/P62** and Beclin-1 expression (Spearman's r= – 0.806). **Table 4**

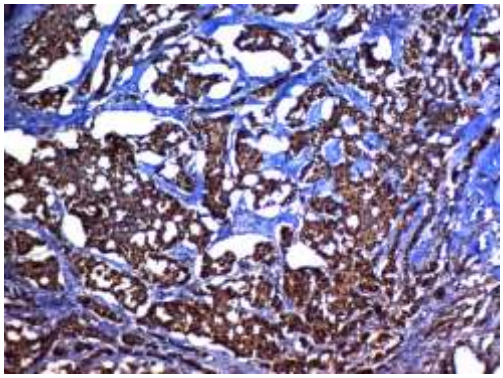

Fig 1 A

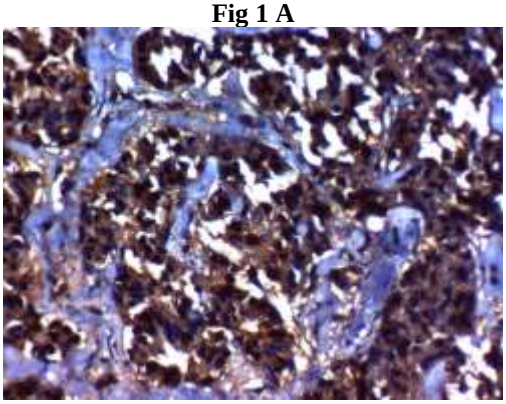

Fig 1 B

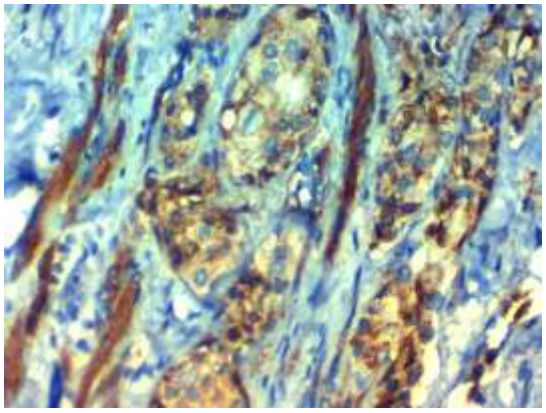

Fig 1 C

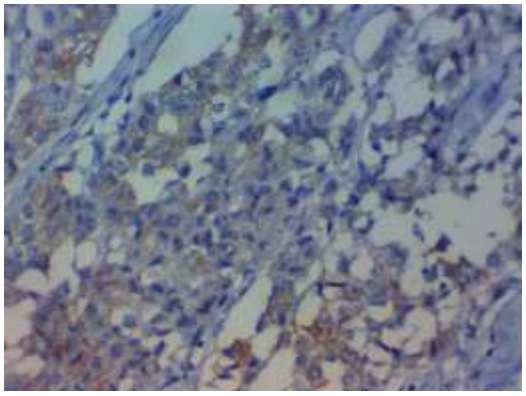

Fig 1 D

Figure 1:- Immunohistochemical staining carcinoma of SQSTM1/p62 expression in carcinoma of the breast: (A) High expression in the cytoplasm of high grade infiltrating duct carcinoma of the breast stage IV x400. (B) High expression in the cytoplasm of high grade infiltrating duct carcinoma stage III x400 (C) Low expression in the cytoplasm of low grade infiltrating duct carcinoma of the breast stage IIx400 (D) Low expression in the cytoplasm of low grade infiltrating duct carcinoma stage I x400.

Note: high SQSTM1/p62 immunohistochemical expression (in the cytoplasm) in high grade and stage IDC

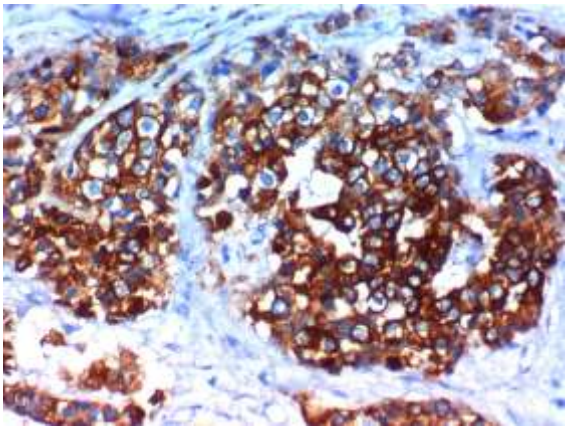

Fig 2 A

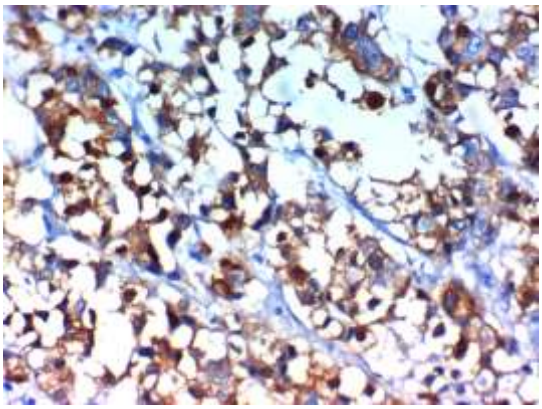

Fig 2 B

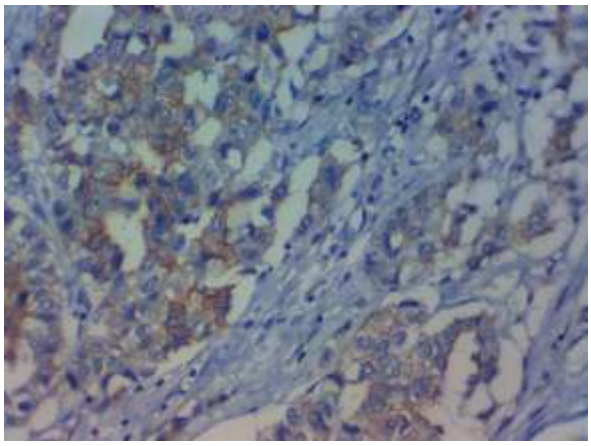

Fig 2 C

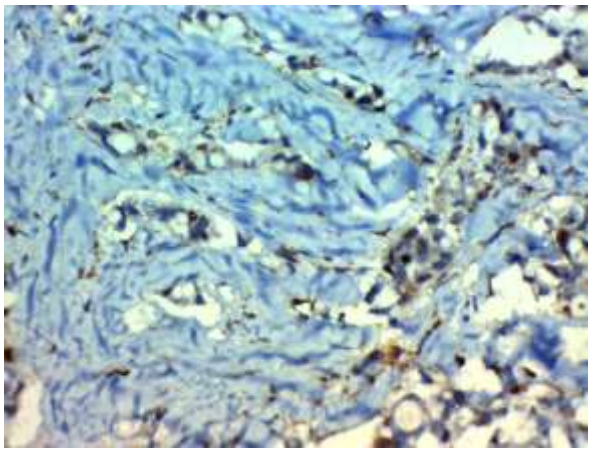

Fig 2 D

Figure 2:- Immunohistochemical staining carcinoma of Beclin-1 expression in carcinoma of the breast the breast: (A) High expression cytoplasm of low grade infiltrating duct carcinoma breast stage I x400. (B) High expression of low grade infiltrating duct carcinoma stage II x400 (C) Low expression in the cytoplasm infiltrating duct carcinoma of the breast x400 expression in the cytoplasm of high grade infiltrating duct carcinoma of the breast stage IIIx400. (D) Low expression in the cytoplasm infiltrating duct carcinoma of the breast x400 expression in the cytoplasm of high grade infiltrating duct carcinoma of the breast stage IVx400

Note:- low beclin-1 immunohistochemical expression (in the cytoplasm) in high grade and stage IDC

Table 1:- Clinicopathological features and immunohistochemical staining in our patients

Characteristics	Number	Percent	Characteristics	Number	Percent
Age (years)			T		
Mean ± SD	56.35 ±10.99		T1	13	21.7%
Median Range	57 (39-77)		T2	11	18.3%
≤ 55 years	24	40%	T3	22	36%
≥ 55 years	36	60%			
Size			T4	14	23.3%
Mean ± SD	6.33 ± 3.59				
Median (Range)	7 (1 – 13)				
≤ 5cm	24	40%			
≥ 5 cm	36	60%			
Pathological type			Lymph node		
IDC	49	81.7%	Negative	19	31.7%
Other	11	18.3%	Positive	41	68.3%
Grade			N		
Grade I	10	16.7%	N0	19	31.7%
Grade II	15	25%	N1	7	11.7%
Grade III	35	58.3%	N2	21	35%
			N3	13	21.7%
ER			M		
Negative	24	40%	M0	44	78.3%
Positive	36	60%	M1	16	21.7%
PR			AJCC Stage group		
Negative	24	40%	Stage I	9	15%
Positive	36	60%	Stage II	14	23.3%
HER2/neu			Stage III	21	35%
Negative	35	58.3%	Stage IV	16	26.7%
Positive	25	41.7%			
Ki-67			P62		
Negative	23	38.3%	Low	28	46.7%
Positive	37	61.7%	High	32	53.3%
ER/PR			Beclin-1		
Positive/Positive	32	53.3%	low	32	53.3%
Positive/Negative	4	6.7%	high	28	46.7%
Negative/Positive	4	6.7%			
Negative/Negative	20	33.3%			
Molecular type					
Luminal A	25	41.7%			
Luminal B	10	16.7%			
HER2 amplified	15	25%			
Triple -ve	10	16.7%			

Categorical variables were expressed as number (percentage).
Continuous variables were expressed as mean ± SD & median (range).

Table 2:- correlation between clinicopathological features and P62 expression in our patients

Characteristics	All (N=60)		P62 Low (N=28)		P62 High (N=32)		p-value
	No.	(%)	No.	(%)	No.	(%)	
Age (years)							
Mean ± SD	56.35	±10.99	51.60	±9.01	60.50	±11.01	0.002
Median (Range)	57	(39-87)	50	(40-76)	60	(39-87)	
≤ 55 years	24	(40%)	18	(75%)	6	(25%)	0.004‡

> 55 years	36	(60%)		10	(27.8%)		26	(72.2%)	
Pathological type									
IDC	49	(81.7%)		24	(49%)		25	(51%)	0.448‡
Other	11	(18.3%)		4	(36.4%)		7	(63.6%)	
Grade									
Grade I	10	(16.7%)		8	(80%)		2	(20%)	0.002§
Grade II	15	(25%)		8	(53.3%)		7	(46.7%)	
Grade III	35	(58.3%)		12	(34.3%)		23	(65.7%)	
ER									
Negative	24	(40%)		1	(4.2%)		23	(95.8%)	0.01‡
Positive	36	(60%)		27	(75%)		9	(25%)	
PR									
Negative	24	(40%)		1	(4.2%)		23	(95.8%)	0.01‡
Positive	36	(60%)		27	(75%)		9	(25%)	
ER/PR									
Positive/Positive	32	(53.3%)		27	(84.4%)		5	(15.6%)	0.01§
Positive/Negative	4	(6.7%)		0	(0%)		4	(100%)	
Negative/Positive	4	(6.7%)		0	(0%)		4	(100%)	
Negative/Negative	20	(33.3%)		1	(5%)		19	(95%)	
HER2/neu									
Negative	35	(58.3%)		27	(77.1%)		8	(22.9%)	0.03‡
Positive	25	(41.7%)		1	(4%)		24	(96%)	
Ki-67									
Negative	23	(38.3%)		20	(87%)		3	(13%)	<0.001‡
Positive	37	(61.7%)		8	(21.6%)		29	(78.4%)	
Molecular type									
Luminal A	25	(41.7%)		25	(100%)		0	(0%)	0.009‡
Luminal B	10	(16.7%)		1	(10%)		9	(90%)	
HER2 amplified	15	(25%)		0	(0%)		15	(100%)	
Triple -ve	10	(16.7%)		2	(20%)		8	(80%)	
T									
T1	13	(21.7%)		10	(77%)		3	(23%)	0.002§
T2	11	(18.3%)		10	(90%)		1	(10%)	
T3	22	(36%)		8	(36.4%)		14	(63.6%)	
T4	14	(23.3%)		0	(0%)		14	(100%)	
N									
N0	19	(31.7%)		16	(84.2%)		3	(15.8%)	0.006§
N1	7	(11.7%)		5	(71.4%)		2	(28.6%)	
N2	21	(35%)		8	(38%)		13	(62%)	
N3	13	(21.7%)		0	(0%)		13	(100%)	
Lymph node									
Negative	19	(31.7%)		16	(84.2%)		3	(15.8%)	0.041‡
Positive	41	(68.3%)		12	(29.3%)		29	(70.7%)	
M									
M0	44	(78.3%)		24	(54.5%)		21	(47.7%)	0.011‡
M1	16	(21.7%)		3	(18.8%)		13	(53.3%)	
AJCC Stage group									
Stage I	9	(15%)		8	(89%)		1	(11%)	0.009§
Stage II	14	(23.3%)		13	(92.8%)		1	(7.2%)	
Stage III	21	(35%)		4	(19%)		17	(81%)	
Stage IV	16	(26.7%)		2	(12.5%)		14	(87.5%)	
Beclin-1									
Mean ± SD	40	±33.94		63.63	±27.53		11.11	±10.28	<0.001‡

Median (Range)	25	(0-90)		80	(0-90)		12	(0-25)			
Low	32	(53.3%)		5	(15.2%)		27	(100%)		<0.001§	
High	28	(46.7%)		28	(84.8%)		0	(0%)		(HS)	

Categorical variables were expressed as number (percentage), continuous variables were expressed as mean ± SD & median (range);
Mann Whitney U test; ‡ Chi-square test; § Chi-square test for trend; p<0.05 is significant.

Table 3:- correlation between clinicopathological features and Beclin-1 expression in our patients

Characteristics	All		Beclin-1				p-value
			Low			High	
	(N=60)		(N=32)			(N=28)	
	No.	(%)	No.	(%)		No.	(%)	
Age (years)								
Mean ± SD	56.35	±10.99	60.50	±11.01		51.60	±9.01	0.004
Median (Range)	57	(39-87)	60	(39.87)		50	(40-76)	
≤ 55 years	24	(40%)	6	(25%)		18	(75%)	0.005‡
> 55 years	36	(60%)	26	(72.2%)		10	(27.8%)	
Pathological type								
IDC	49	(81.7%)	25	(51%)		24	(49%)	0.448‡
Other	11	(18.3%)	7	(63.6%)		4	(36.4%)	
Grade								
Grade I	10	(16.7%)	3	(25%)		9	(75%)	<0.001§
Grade II	15	(25%)	4	(23.5%)		13	(76.5%)	
Grade III	35	(58.3%)	14	(77.8%)		4	(22.2%)	
ER								
Negative	24	(40%)	23	(95.8%)		1	(4.2%)	0.003‡
Positive	36	(60%)	9	(25%)		27	(75%)	
PR								
Negative	24	(40%)	23	(95.8%)		1	(4.2%)	0.003‡
Positive	36	(60%)	9	(25%)		27	(75%)	
ER/PR								
Positive/Positive	32	(53.3%)	5	(15.6%)		27	(84.4%)	0.002§
Positive/Negative	4	(6.7%)	4	(100%)		0	(0%)	
Negative/Positive	4	(6.7%)	4	(100%)		0	(0%)	
Negative/Negative	20	(33.3%)	19	(95%)		1	(5%)	
HER2/neu								
Negative	35	(58.3%)	8	(22.9%)		27	(77.1%)	0.006‡
Positive	25	(41.7%)	24	(96%)		1	(4%)	
Ki-67								
Negative	23	(38.3%)	3	(13%)		20	(87%)	<0.001‡
Positive	37	(61.7%)	29	(78.4%)		8	(21.6%)	
Molecular type								
Luminal A	25	(41.7%)	0	(0%)		25	(100%)	0.004‡
Luminal B	10	(16.7%)	9	(90%)		1	(10%)	
HER2 amplified	15	(25%)	15	(100%)		0	(0%)	
Triple -ve	10	(16.7%)	8	(80%)		2	(20%)	
T								
T1	13	(21.7%)	6	(40%)		9	(60%)	0.002§
T2	11	(18.3%)	8	(34.8%)		15	(65.2%)	
T3	22	(36%)	11	(73.3%)		4	(26.7%)	
T4	14	(23.3%)	7	(100%)		0	(0%)	
N								
N0	19	(31.7%)	3	(15.8%)		16	(84.2%)	<0.001§
N1	7	(11.7%)	5	(45.5%)		6	(54.5%)	

N2	21	(35%)	13	(68.4%)		6	(31.6%)					
N3	13	(21.7%)	11	(100%)		0	(0%)					
Lymph node												
Negative	19	(31.7%)	3	(15.8%)		16	(84.2%)		<0.001‡			
Positive	41	(68.3%)	29	(70.7%)		12	(29.3%)					
M												
M0	47	(78.3%)	21	(44.7%)		26	(55.3%)		0.011‡			
M1	13	(21.7%)	11	(48.6%)		2	(15.4%)					
AJCC Stage group												
Stage I	9	(15%)	3	(25%)		9	(75%)		0.002§			
Stage II	14	(23.3%)	4	(23.5%)		13	(76.5%)					
Stage III	21	(35%)	14	(77.8%)		4	(22.2%)					
Stage IV	16	(26.7%)	11	(84.6%)		2	(15.4%)					
p62												
Mean ± SD	40.10	±32.15	64.65	±22.64		12.03	±11.81		<0.001‡			
Median (Range)	28	(0-90)	70	(20-90)		10	(0-29)					
Low	28	(55%)	5	(15.6%)		23	(100%)		<0.001§			
High	32	(45%)	30	(84.4%)		2	(0%)					

Categorical variables were expressed as number (percentage), continuous variables were expressed as mean ± SD & median (range); • Mann Whitney U test; ‡ Chi-square test; § Chi-square test for trend; p<0.05 is significant.

Table (4): Association & correleation between p62, Beclin-1 and clinicopathological parameters in our patients

	p62		**p62 (%)**		**Beclin-1**		**Beclin-1 (%)**	
	r	**p-value**	r	**p-value**	r	**p-value**	r	**p-value**
Age (years)	+0.713	0.009	+0.726	0.008	-0.499	0.04	-0.700	0.03
Size	+0.770	0.005	+0.747	0.002	-0.654	0.03	-0.741	0.02
Grade	+0.750	0.01	+0.728	0.02	-0.528	0.02	-0.674	0.03
T	+0.860	0.003	+0.795	0.004	-0.732	0.004	-0.806	0.005
N	+0.843	0.002	+0.833	0.003	-0.916	0.005	-0.861	0.005
Stage	+0.868	0.002	+0.862	0.003	-0.907	0.006	-0.867	0.004
p62	---	---	---	---	-0.846	0.001	-0.759	0.001
p62 (%)	---	---	---	---	-0.787	0.001	-0.806	0.001
Beclin-1	-0.846	<0.001	-0.787	<0.001	---	---	---	---
Beclin-1 (%)	-0.759	<0.001	-0.806	<0.001	---	---	---	---

r correleation coefficient; p<0.05 is significant.

Table 5:- Diagnostic performance of immunohistochemical markers as a predictor for advanced stage breast carcinoma.

Markers	TP No (%)	FP No (%)	TN No (%)	FN No (%)	SN % (95% CI)	SP % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Accuracy (95% CI)
P62 (High)	32 (45%)	0 (0%)	23 (38.3%)	9(16.7%)	72.4% (58.2-86.6)	97.9% (92.2-100)	98.2% (93.3-100)	69.1% (53.6-84.6)	82.2% (72.6-91.7)
Beclin-1 (Low)	32 (53.3%)	0 (0%)	23 (38.3%)	5 (8.3%)	85.5% (74.3-96.7)	97.9% (92.2-100)	98.5% (94.3-100)	81% (66.8-95.3)	90.3% (82.9-97.6)

TP: True positive; FP: False positive; TN: True negative; FN: False negative; SN: Sensitivity; SP: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value, 95%CI: 95% Confidence Interval; p< 0.05 is significant.

Discussion:-

Clinicopathological role of autophagy in cancer is still a point of research that could be due to variability in its role in carcinogenesis **(White, 2012).** In this study, we found that SQSTM1-p62 overexpression was correlated positively with a poor clinical behavior of cancer such as large size, higher incidence of positive lymph nodes, higher grade, and advanced stage and high incidence of distant metastases occurrence in patients with breast carcinoma (P<0.001).

Chen et al., 2013; Luo et al., 2013, proved similar findings to us that SQSTM1-p62 over expression in carcinoma of the breast was related to bad clinicopathological criteria, on the contrary **Jiang et al. (2012)** proved different findings to us. **Sakakura et al. (2015)** explained such conflicting results to differences in evaluation of positive areas in carcinoma tissues in between the peripheral and the central area, that were different in composition, while most researches had not analyze which positive part that they have found in cancer tissue. Many conflicting results had been discovered regarding clinicopathological role of Beclin-1, that is important the autophagy-related protein, expression in breast carcinoma. We declared that Beclin-1 expression in breast carcinoma was strongly negatively correlated to size, grade and stage of the tumor, the presence of nodal and/or distant metastasis. Beclin-1 overexpression was strongly positively correlated with good clinicopathological criteria in breast carcinoma. **Dong et al., 2013 proved s**imilar to our results that elevated Beclin-1 expression in carcinoma of the breast was an indicator of good clincopathological criteria. On the contrary, **He et al. 2014** meta-analysis results and Choi et al., 2014 results have detected no characteristic relation between overexpression of Beclin-1 and clinicopathological parameters of breast carcinoma. Moreover, **Won et al., 2009**, had declared no relation was found between expression of Beclin-1 and prognosis of breast cancer patients. **Ahn et al. 2007** explained the absence of such a relation between the expression of Beclin-1 and clinic-pathological characteristics that Beclin-1 could be able to play a role in malignant initiation but had no role in carcinoma progression. Similar tour results in breast cancer **Qiu et al. (2014**, had found that over expression of Beclin-1 in liver carcinoma was strongly related to good prognosis. **He et al. 2014** meta-analysis had showed that Beclin-1 increased expression could be able to be a protective factor in stomach cancer and lymphoma that was similar to our results in cancer breast, but it had no clinicopathological relation to colon or lung cancers. On the contrary to our results, **Han et al. 2014** had found that Beclin-1 over expression in cancer colon was associated with increased incidence of L.N, blood metastasis and associated with poor prognosis. So it had been found that both Beclin-1 increased or decreased expression were detected in human malignancies; as it may had a cancer suppressor role, by interacting with bcl-2 protein members (**Cao and Klionsky, 2007).** Otherwise the tumor stimulatory role of Beclin-1 overexpression in which it was associated with cancers aggressive behavior was done by anti-apoptotic machinery potentiation **(Koukourakis et al. 2010),** and another mechanism Beclin-1 overexpression during adverse cancer environmental conditions like hypoxia and increased acidity, so as to allow cancer cells to overcome such conditions by increasing autophagic cancer cells activity to recycle un needed proteins and damaged organelles to increased their survival **(Samokhvalov et al., 2008).** However Beclin-1 over expression could be able to delay cancer progression by decreasing chromosomal instability and the occurrence of more mutation **(Mathew et al., 2007).** That may be due to Beclin-1-dependent autophagy which induced immunological response **(Xu et al., 2008).**

In summary, in our study we proved that SQSTM1/p62 expressions were strongly positively related to bad clinicopathological parameters of cancer breast patients and its expression was negatively correlated to Beclin-1 expression, which was proved to be a marker of favorable prognosis for cancer breast patients. There are conflicting results on the prognostic value of both markers in cancer breast. We recommended to do more studies confirm their role as clinical predictors of poor or good clinicopathological criteria and different outcome for breast cancer patients that may help to detect novel therapeutic targets to for them improving their prognosis.

Conclusion:-

SQSTM1/p62 is markers of a poor prognosis, while Beclin-1 is a marker of a good prognosis in in breast cancer.

Conflicts of interest
None declared.

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