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Original Article

Prognostic Significance of Beclin1 and TGF-β1 in Ovarian Cancer

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ABSTRACT

Ovarian cancer is a gynecological malignancy with a high mortality rate. Autophagy is lysosomal degradation of damaged subcellular structures which is known as type II programmed cell death. Autophagy was initially thought to be a tumor-suppression mechanism and dysregulation of autophagy is suggested to be involved in tumor genesis. BECN1 is a tumor suppressor gene involved in the initiation of autophagy. It encodes Beclin-1 protein, which inhibits tumor growth, there is wide controversy about its role in initiation, promotion of tumor and prognostic importance of autophagic molecules. Transforming growth factor β1 induce process of epithelial-mesenchymal transition (EMT), keeping, epithelial cells more motile and invasive leading to cancer progression and metastasis.

Material and methods: Fifty Blocks of paraffin-embedded ovarian tissue were selected, representing cases diagnosed as ECO. The immunohistochemical staining procedure was done using Beclin 1 and TGF-β to detect their expression and to correlate it with the different clinical parameters**.**

Results: Positive Beclin1 expression was observed in 54% and positive TGF-β1 staining was observed in 70 % of patients tissue samples. Beclin1 significantly correlated with lower tumor grade (P<0.031) and lower FIGO stage, P=0.01, a significant association was observed between higher FIGO stage and TGF-β1 expression. All metastatic cases were positive for TGF-β1 versus 27.3% of metastatic cases positive for beclin. Beclin1 showed significant correlation with non-recurring disease, P=0.005 and was associated with less mortality P=<0.001. TGF-β1 was significantly associated with higher mortality rates and relapsing disease, P=0.015, P=0.005

Conclusion: Beclin1 protein could be considered a good prognostic factor in OC cases while Tgf-β1 considered adverse factor which could be of benefit in OC molecular targeting therapy

Keywords
Beclin 1,
Tgf-β1,
Ovarian Cancer

INTRODUCTION

Ovarian cancer is a gynecological malignancy with a high mortality rate. It occurs most frequently in postmenopausal period (Reidet al 2017).

Patients at diagnosis mostly present with advanced stage disease (Jemalet al 2010).

Treatment strategy include cytoreductive surgery combined with platinum based chemotherapy , however drug resistance is common which decrease treatment efficacy and lead to poor prognosis. (Yinget al2015).

Autophagy is lysosomal degradation of cellular large molecules and damaged subcellular structures which is known as type II programmed cell death (White 2015)

Autophagy was initially thought to be a tumor-suppression mechanism and dysregulation of autophagyis suggested to be involved in tumor genesis (Korenand Kimchi 2012).

Autophagy deficiency causes oxidative stress, and genome instability leading to cancer initiation and progression (White 2015)
Loss of autophagy in liver is toxic, producing chronic damage of hepatocytes and inflammation ,which are known to cause liver cancer (Sun and Karin 2013)

In some circumstances, autophagy mediates the cytotoxic effect of anticancer agents, in which blockade of autophagy abolishes the therapeutic actions (Wu et al 2012)
Beclin1promotes apoptosis of tumor cells induced by the chemotherapeutic drugs by increasing caspase 9 activity.) Huanget al 2010)
Autophagy was also shown to correlate with clinico-pathologic parameters and disease outcomes including overall survival in cancer patients Which highlight the possibility of using autophagy-associated molecules as novel prognostic markers in clinical settings (Wu et al 2012)
BECN1 is a tumor suppressor gene involved in the initiation of autophagy. It encodes Beclin-1 protein, which aids in the formation of phages and inhibits tumor growth (Yinget al2015).
It was identified that BECN1autophagic gene was downly regulated or lost in prostate, breast, and ovarian cancers (Choi et al2013).
Altered expression of several autophagy markers such as Beclin 1 and microtubule associated protein light chain 3 (LC3) was found in brain, colon, gastric, liver, pancreatic cancers, osteosarcoma and melanoma.(Wuet al 2012)
It is well known that decreased autophagy leads to carcinogenesis, suggested studies other, however opposite role of BECN1In genetically engineered mouse models (GEMMs) for breast cancer, loss of BECN1wasfound to promote p53 activation and reduces tumor genesis (Huo et al 2013)
Beclin 1 is expressed in many tumors, other tumors showed Beclin 1 down regulation However its role in ovarian tumor genesis and prognosis is still controversial (Yinget al 2015).

Now, it is suggested that autophagy has a pivotal role in tumor genesis and there is wide controversy about its role in initiation, promotion of tumor and prognostic importance of autophagic molecules

In this study, we investigated the relationship between Beclin 1 expression and epithelial ovarian cancer as regarding prognosis and its relation to patient survival and tumor relapse.
Tumor formation requires multiple pathways, as cell proliferation, inhibition of apoptosis, and tumor vessel formation. which are regulated by many factors such as transforming growth factorβ1TGF-β1.which has dual action, it inhibits tumor cell proliferation in early carcinogenesis, However, in later stages of cancer it promotes cancer progression by enhancing cell motility, angiogenesis and immune evasion (Caoet al2012)

Transforming growth factor β1 induce process of epithelial-mesenchymal transition (EMT), which is characterized by breakdown of cell junction skeeping, epithelial cells more motile and invasive leading to, cancer progression and metastasis (Satpathyet al., 2009)

A critical step of EMT is loss of type I cadherins that maintain stable cell–cell junction. Down regulation of E-cadherin leads to adoption of a mesenchymal behavior (Dumontet al., 2003).

Activation of SMADs by TGF-β1recruits transcriptional repressors like Snail, Zeband Twist which repress E-cadherin (Vincent et al., 2009),

The present study investigate the expression of TGF-β1in the ovarian cancer cells, to detect the association between its expression, and the invasion, metastasis and prognosis of ovarian cancer.

Material and Methods

Human tissue samples

This study was performed in Gynecology and Obstetrics, General Surgery ,Pathology and Clinical Oncology departments, faculty of medicine, Zagazig University. Fifty patients were registered in the period from May 2013 to May 2018. Fifty tissue samples were obtained from patients with ovarian cancer who had undergone surgical resection at Surgical Oncology Hospital unit between May 2013 and May 2018, Fifty Blocks of paraffin-embedded ovarian tissue were prepared at the Pathology department, representing cases diagnosed as ECO were examined by two independent pathologists, classified and graded according to the World Health Organization (WHO) grading system (Kurman et al., 2014)and staged according to criteria of the International Federation of Gynecology and Obstetrics (FIGO) (Part, 2014) . Clinical, radiological and pathological data were abstracted from files of the corresponding departments. Clinical follow-up for EOC was done every three months to all cases and information concerning follow up was abstracted from hospital records or patient contact .Other types of ovarian cancers rather than EOC were rejected.

Immunohistochemistry (Hsu et al 1981)

The immunohistochemical staining procedure was done using streptavidin–biotin immunoperoxidase technique (Dako-Cytomation, Glostrup, Denmark). Sections of 3–5 μm from the formalin-fixed-paraffin-embedded blocks were cut and mounted on positively charged slides then de-paraffinized by xylene, and rehydrated in graded alcohol. Thereafter, sections were boiled in buffered citrate (pH 6.0) for about 20 minutes then washed in PBS (pH 7.3). Then, endogenous peroxidase activity was blocked with 6% H2O2 in methanol. The slides were incubated overnight with Beclin 1 Mouse Monoclonal antibody, diluted at 1:200. Catalog Number GTX34055GeneTex (1-877-436-3839) and mouse monoclonal antibody to transforming growth factor β1(TGF-β1,diluted at 1:200,catalog numberNBP2-22114,SNF Medical corporation, Novus ,USA) After rinsing in PBS, the slides were immersed with a biotin-conjugated secondary antibody (Lab Vision Corporation, Fermont, USA). DAB was used as a chromogen and Mayer's Hematoxylin was used as a counter stain, and then the slides were washed with distilled water and PBS.
Immunohistochemical scoring:
Beclin-1-positive cells were identified by brown particles distrib-

uted throughout the cytoplasm and to less extent in nucleus, . The percentage of positive cells to total cells was counted according to the following criteria: <10%, 0 points; 10-20%, 1 point; 21-50%, 2 points; and >50%, 3 points. The intensity of staining was also awarded points according to the following system: no color, 0 points; pale yellow, 1 point; brown, 2 points; and tan, 3 points. The total score for each case was the sum of the points for the percentage of positive cells and the staining intensity. A score of ≤3 was regarded as negative for Beclin-1 expression, whereas a score of >3 was regarded as positive for Beclin-1 expression.(Yinget al 2015).

TGF-β1 positive cells were identified by brown cytoplasmic granules cored as 0, 1, 2, or 3 based on the stain intensity 0; nopigmentation,1;light yellow, 2 ; yellow or3;brown, the positivity scored as:%5> positive cells scored 0; 5-25% positive cells scored 1; 26-50%positive cells scored 2; 51-75% positive cells scored 3; and >75%positive cells scored 4. The two scores were then multiplied whereby 0-2 corresponds
to (-), 3-4 to (+), 5-8 to (++) and 9-12 to (+++) (Wang, *et al*2012).

Statistical analysis

Continuous variables were expressed as the mean ± SD & median (range), and the categorical variables were expressed as a number (percentage). Continuous variables were checked for normality by using Shapiro-Wilk test. Mann Whitney U test was used to compare between two groups of non-normally distributed variables. Kruskal Wallis H test was used to compare between more than two groups of normally distributed variables. Percent of categorical variables were compared using Pearson's Chi-square test or Fisher's exact test when was appropriate. Disease Free Survival (DFS) was calculated as the time from date of surgery to relapse or the most recent follow-up in which no relapse was detected. Overall Survival (OS) was calculated as the time from diagnosis to death or the most recent follow-up contact (censored). Stratification of DFS and OS was done according to immunohistochemical markers. These time-to-event distributions were estimated using the method of Kaplan-Meier plot, test. p-value <0.05 was considered significant. All statistics were performed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) and MedCalc windows (MedCalc Software bvba 13, Ostend, Belgium).

Results

Patients' characteristics.

Fifty tissue samples were obtained from patients with ovarian cancer who had undergone surgical resection at Zagazig university Hospital between May 2013 and May 2018. The cases were classified as serous carcinoma (n=23), mucinous carcinoma (n=20) and endometrioid carcinoma (n=7). The age range of the patients was(36-69) years old, with a median age of 55 years old. The pathological surgical staging was performed according to the 2009 International Federation of Gynecology and Obstetrics system as follows: Stage I, 8 cases; stage II,15cases; stage III, 16 cases; and stage IV, 11 cases .Table (1)

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

Table 1. Clinicopathological features, immunohistochemical markers and outcome of 50 patients with ovarian carcinoma.

Variable	N=50	%
Histological type of malignancy		
Serous type	23	46
Mucinous type	20	40
Endometroid type	7	14
Grading		
I	13	26
II	18	36
III	19	38
Distant metastasis		
Absent	39	78
Present	11	22
Staging		
I	8	16
II	15	30
III	16	32
IV	11	22
Relapse n=39		
Absent	9	23.1
Present	30	76.9
Death		
Absent	21	42
Present	29	58
Beclin		
Negative	23	46
Positive	27	54
TGF-β1		
Negative	15	30
Positive	35	70
Age		
X±SD	54.4±8.34	
Median (Range)	(36-69)55	
Disease free survival		
X±SD	44.44 ± 13.81	
Median (Range)	(16-60)46	
Overall survival		
X±SD	47.48±15.15	
Median (Range)	(16-60)55	

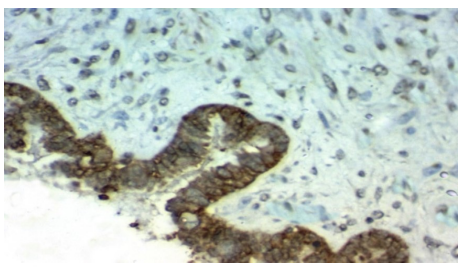
Association of Beclin1 and TGF-β1expression with clinicopathological parameters (Table 2, Fig1 and 2)

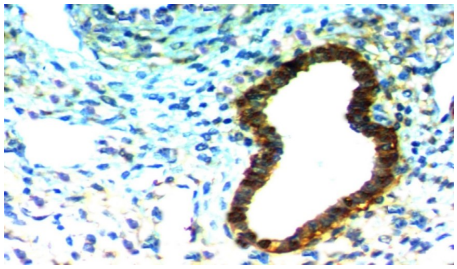
Positive Beclin1 expression was observed in 54% of the patients tissue samples and Positive TGF-β1IHC staining was observed in70 %. Beclin1was stained in the cytoplasm and nuclei of cancer cells (Fig1) while TGF-β1was stained in the cytoplasm of cancer cells (Fig2). Beclin1 significantly correlated with lower tumor grade (P< 0.031). In addition, A significant association between lower FIGO stage and Beclin1 IHC staining was observed, in which (36.4)% of the patients with stage IV had positive staining versus75 % of the patients with stage I disease P =0.01.,Conversely a significant association was observed between higher FIGO stage and TGF-β1 IHC staining, with100 % of the patients with stage IV disease having a positive TGF-β1 staining versus 25% of patients with stage I disease (P <0.003).No significant association was detected between TGF-

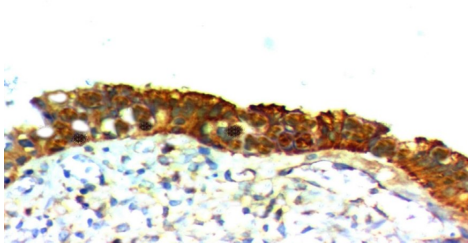
Table 2. Relation between clinicopathological features and immunohistochemical staining for Beclin and TGF-β1in 50 patients with ovarian carcinoma

Variable	Beclin		p	TGF-β1		p
	Negative(23)	Positive(27)		Negative(15)	Positive(35)	
	No(%)	No(%)		No(%)	No(%)	
Histological type of malignancy						
Serous type		10(43.5)		6(26.1)	17(73.9)	
Mucinous type	13(56.5)	13(65)		5(25)	15(75)	0.239§
Endometroid type	7(35)	4(57.1)	0.363§	4(57.1)	3(42.9)	
	3(42.9)					
Grading						
I	3(23.1)	10(76.9)		6(46.1)	7(53.9)	
II	7(38.9)	11(61.1)	0.031§	6(33.3)	12(66.7)	0.171§
III	13(68.4)	6(31.6)		3(15.8)	16(84.2)	
Distant metastasis						
Absent	15(38.5)	24(61.5)	0.016‡	15(38.5)	24 (61.5)	0.021‡
Present	8(72.7)	3 (27.3)		0(0)	11 (100)	
Staging						
I	2 (25)	(75) 6		(75) 6	2 (25)	
II	4 (26.7)	11(73.3)	0.015§	6(40)	9(60)	0.003§
III	10(62.5)	6 (37.5)		2(12.5)	14(87.5)	
IV	7(63.6)	4 (36.4)		0(0)	11 (100)	
Age						
X±SD	54.13±8.46	54.63±8.39	0.836*	53.53±7.65	54.77±8.7	0.636*
Median (Range)	55(39-69)	55(36-67)		54(39-65)	55(36-69)	
Disease free survival						
X±SD	34.81±11.74	51.13±11	<0.001•	51.73±12.26	39.88±12.92	<0.007*
Median (Range)	33.5(16-58)	55(24-60)		58(16-60)	38.5(21-60)	
Overall survival						
X±SD	40.87±17.38	53.11±10.27	0.005*	56.93±7.33	43.43±15.88	<0.001*
Median (Range)	45(16-60)	59(24-60)		60(33-60)	48(16-60)	

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







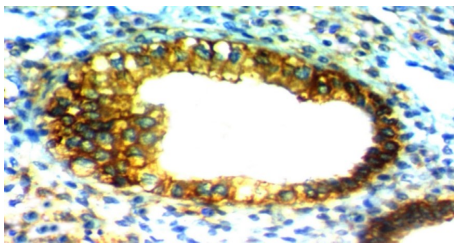


fig1 a, b: low grade serous carcinoma of ovary with strong beclin stain in cytoplasm and to lesser extent in nucleus

Fig1c: low grade endometroid carcinoma positive for beclin cytoplasmic and nuclear stain

Fig1d: low grade mucinous carcinoma of ovary positive for cytoplasmic beclin stain

fig 1e,f : high grade serous ovarian carcinoma show negative beclin staining

Table 3. Relation between clinicopathological features and relapse in 50 patients with ovarian carcinoma

Variable	Relapse		p
	Absent	Present	
	No 9(%)	No 30 (%)	
Age			
X±SD	55.33±7.79	54.43±8.6	0.78*
Median (Range)	55(44-65)	55(36-67)	
Histological type of malignancy			0.309 §
Serous type	2(13.3)	13(86.7)	
Mucinous type	4(23.5)	13(76.5)	
Endometroid type	3(42.9)	4(57.1)	
Grading			
I	5(38.5)	8(61.5)	
II	4(28.6)	10(71.4)	0.062 §
III	0(0)	12(100)	
Staging			
I	5(62.5)	3(37.5)	
II	3(20)	12(80)	0.008§
III	1(6.2)	15(93.8)	
Death			
Absent	9(42.9)	12(57.1)	0.002¥
Present	0(0)	18(100)	
Beclin			
Absent	0 (0)	16(100)	0.005¥
Present	9(39.1)	14(60.9)	
TGF-β1			
Absent	7(46.7)	8(53.3)	0.015¥
Present	2(8.3)	22(91.7)	

Categorical variables were expressed as number (percentage).
Continuous variables were expressed as mean ± SD & median (range).
*Independent samples Student's test; ‡ Chi-square test; § Chi-square test for trend; ¥ Fisher exact test, p<0.05 is significant

Table 4. Relation between presence of Beclin and TGF-β1in 50 patients with ovarian carcinoma

Varia-ble	Beclin		p	TGF-β1		p
	Negative (23)	Positive (27)		Negative (15)	Positive (35)	
	No (%)	No (%)		No(%)	No(%)	
Beclin						
Negative				5(21.7)	18(78.3)	0.239‡
Positive				10(37)	17(63)	
TGBF						
Negative	5(33.3)	10(66.7)	0.239‡			
Positive	18(51.4)	17(48.6)				

Categorical variables were expressed as number(percentage).
‡ Chi-square test;; p<0.05 is significant

Table 5. Relation between outcome of ovarian carcinoma and presence of Beclin and TGF-β1

Variable	Beclin		p	TGF-β1		p
	Negative	Positive		Negative	Positive	
	No(%)	No(%)		No(%)	No(%)	
Relapse	**No=16**	**N=23**		**N=15**	**N=24**	
n=39	0(0)	9(100)	0.005¥	7(77.8)	2(22.2)	0.015
Absent	16(53.3)	14(46.7)		8(26.7)	22(73.3)	¥
Present						
Death	**No=23**	**N=27**		**N=15**	**N=35**	
Absent	3(14.3)	18(85.7)	<0.001¥	11(52.4)	10(47.6)	0.005
Present	20(68)	9(32)		4(13.8)	25(86.2)	¥

Categorical variables were expressed as number (percentage).
¥ fisher exact test;; p<0.05 is significant

β1 and tumor grade. All metastatic cases were positive for TGF-β1 versus27.3%of metastatic cases positive for Beclin

Association of Beclin1 and TGF-β1expression with outcome of ovarian carcinoma (Tables 3, 5 , 6, Fig 3):

Among 50 patients thirty nine (39) presented with early stage disease and underwent radical surgery followed by adjuvant chemotherapy (for stage IC and higher or high grade tumors). On the other hand, eleven (11) of EOC patients presented with advanced bulky disease with significant comorbidities which precluded primary debulking surgery. These patients had received neoadjuvant chemotherapy (non of them had achieved complete response to chemotherapy) and the comorbidities precluded surgical intervention. The median follow-up time was 55 months (range16-60 months), and during the follow-up period, 9 (23.1%) of the patients were disease-free without relapse. Recurrence occurred in 30 (76.9%) of the patients, and (58%)of the patients 29/50 died during follow-up. The clinicopathological characteristics of the 50 patients with EOC were summarized in Table1.

Beclin1 showed significant correlation with non recurring disease with 100 % of non relapsing cases showed beclin positivity while 46.7% of relapsing cases did, P=0.005.beclin1 was associated with less mortality

P=<0.001.TGF-β1 was significantly associated with higher mortality rates and relapsing disease state.%3.73of recurring disease and 86.2% of died cases were positive for tgfb P = 0.015, P =0.005.

Discussion

Ovarian cancer is a malignancy with a high mortality rate, due to lack of effective screening methods, late diagnosis and drug resistance which cause us to seek new directions in prevention and treatment Winkler et al.2015

Autophagy means lysosomal degradation of excessive proteins and subcellular structures, this process is involved in tumor formation and development Caiet al.2014

Beclin 1 mediates proteins which regulate autophagy. The expression of Beclin 1 and its role in tumor genesis had great interest. Caiet al.2014

In this study, we used 50 tissue samples from zagazig university hospitals, we detected positive expression of Beclin1 in(54%)of ovarian epithelial cancers

Huanget al 2010 revealed that Beclin1 expression levels differed between types of tumor cell. The expression of Beclin1 was down regulated in a variety of tumor cells, such as those of breast, ovarian and prostate cancers, and gliomas

Ahn*et al* 2007foundthathigh levels were maintained in colorectal

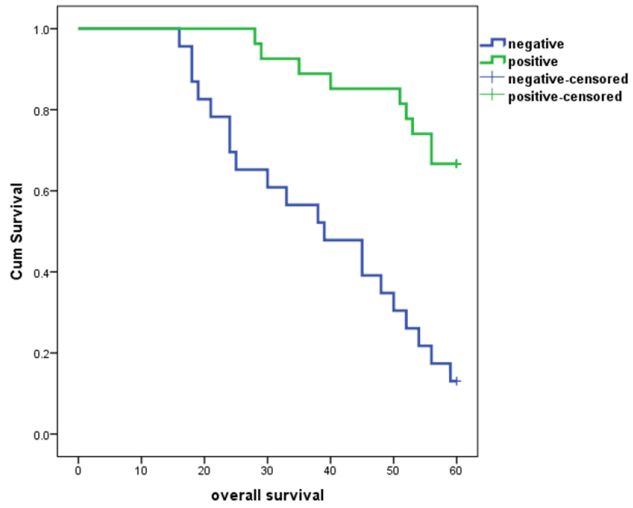


Figure (3a) Kaplan Meniere graph showing overall survival in cases with beclin positive and negative

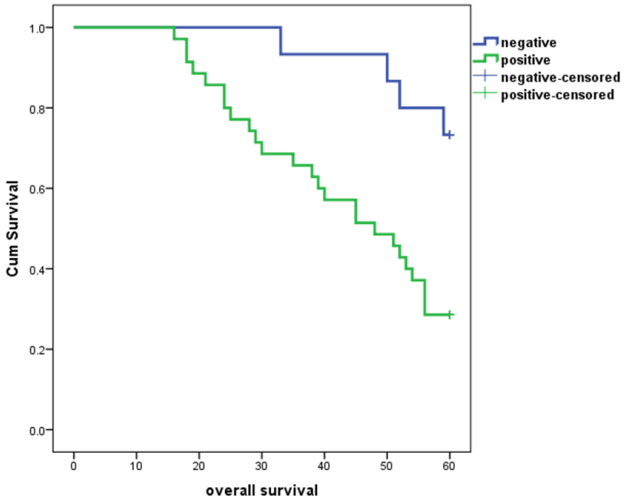


Figure (3b) Kaplan Meniere graph showing overall survival in cases with tgfb positive and negative

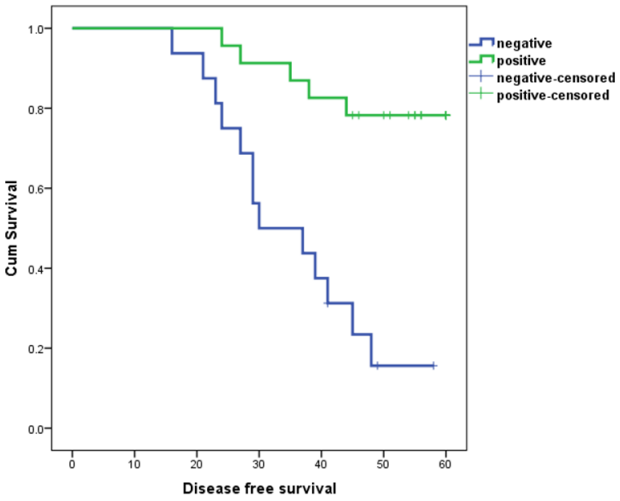


Figure (3c) Kaplan Meniere graph showing disease free survival in cases with beclin positive and negative

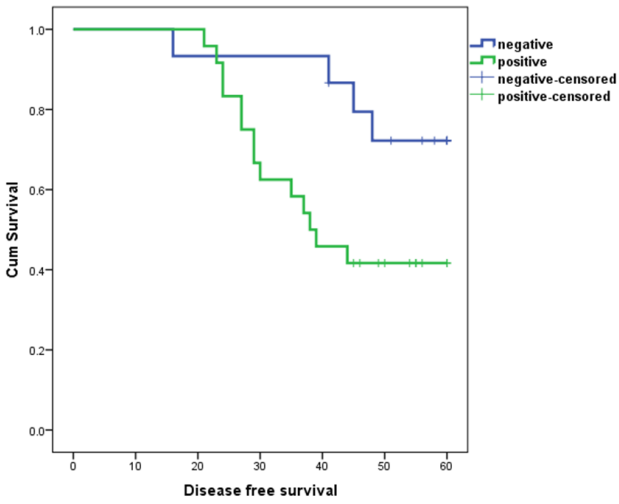


Figure (3d) Kaplan Meniere graph showing disease free survival in cases with tfgb positive and negative

cancer and gastric cancer**with** little expression was revealed in normal tissues.

Kimmelman2011 suggested that autophagy initially acts as a barrier to prevent the initiation of tumors, but following the formation of lesions, it promotes tumor maintenance.

In the present study**,**Beclin1 significantly correlated with lower tumor grade (P< 0.031)and lower FIGO stage, P =0.01 .beclin 1 showed correlation with non recurring disease with 100 % of nonrelapsing cases were beclin positive while 46.7 % of relapsing cases did ,P =0.005 .

patients with high Beclin 1 expression showed asignificantly longer survival with less mortality than those with low Beclin 1 expressionP = <0.001 ,which mays uggest that Beclin 1 expression largely affect tumor prognosisandBECN1 geneis tumor suppressor gene

Similar resultshave also been reported by**Ahn,et al 2007**and-**Dong,et al2011**, for gastric cancer, in trahepatic cholangio carcinoma, and stage IIIB cancer colon through promoting both autophagic tumor death andapoptosis induced by the chemotherapeutic drug

Tonget al2013 showed that the up regulated Beclin 1significantly decreased leukemia cells proliferation by enhanced autophagy

Caiet al, 2014studywas near to our results which showed that high Beclin 1 levels were associated with a lower mortality rates in ovarian cancer patients. Their possible suggested mechanisms included: (1) Beclin 1 induces autophagy in ovarian cancer cells lacking apoptotic ability; (2) Beclin 1 reduces the frequency of additional gene mutations and (3) Beclin 1 overexpression inhibits cell proliferation, and promotes autophagy and apoptosis

Table 6.Relation between clinicopathological features and mortality in 50 patients with ovarian carcinoma

Variable	Death		p
	Absent	Present	
	No 21(%)	No 29(%)	
Age			
X±SD	54.29±7.58	54.48±8.99	0.935*
Median (Range)	55(39-65)	54(36-69)	
Histological type of malignancy			
Serous type	7(30.4)	16(69.6)	0.148§
Mucinous type	9(45)	11(55)	
Endometroid type	5(71.4)	2 (28.6)	
Grading			
I	9(69.2)	4 (30.8)	
II	9(50)	9(50)	0.007§
III	3(15.8)	16(84.2)	
Distant metastasis			
Absent	20 (51.3)	19(48.7)	0.003¥
Present	0(0)	11(100)	
Staging			
I	6(75)	2(25)	
II	10(66.7)	5(33.3)	0.001§
III	531.3 ()	11(68.7)	
IV	0(0)	11(100)	
Relapse (39)			
Absent	9 (100)	0 (0)	0.002¥
Present	12 (40)	18 (60)	
Beclin			
Absent	3(13)	20(87)	<0.001¥
Present	18(66.7)	9(33.3)	
TGF-β1			
Absent	11(73.3)	4(26.7)	<0.005¥
Present	10(28.6)	25(71.4)	

Categorical variables were expressed as number (percentage).
Continuous variables were expressed as mean ± SD & median (range).
*Independent samples Student's test; ‡ Chi-square test; § Chi-square test for trend; ¥ fisher exact test, p<0.05 is significant

Results of current study are consistent with YINGet al , 2015whorevealed that Beclin1 protein expression was significantly lower in the drug resistant group of ovarian cancer patients compared with the drug sensitive group, suggesting that lower Beclin1 expression may be associated with chemotherapy resistance and poor prognosis and higher expression of Beclin1 may increase the sensitivity to chemotherapy, which could improve treatment efficacy in ovarian cancer patients
to opposite are study Results of current **Zhao et al. 2013**who concluded that Beclin 1 is not an independent prognostic factor for ovarian carcinoma. However, follow-up period in their study was only one month. in our study median follow-up period was more long.

Further studies arerequired to investigate the possibility of targeting Beclin 1 in the treatment of ovarian cancer.

Table 7. mean overall survival in both arms (Beclin positive and negative).

Measure	Negative	Positive	P
Mean overall survival	38.87	**54.82**	<0.001

Table 8. mean overall survival in both arms (TGF-β1positive and negative)

Measure	Negative	Positive	P
Mean	56.93	43.43	0.004

Ovarian cancer has closely associated tumor stroma, during tumor genesis, the stroma surrounding epithelial cells is activated, forming a cancer-associated stroma, subsequently promoting cancer development CHENet al, 2014
Inactivated tumor stroma, fibroblasts are transformed into CAFs (Cancer associated fibroblasts) ,this conversion is stimulated byTGF-β1 leading to formation of cancer-promoting stromal environment (Micke and Ostman 2004)

In the currents study, Positive TGF β1IHC staining was observed in70 % of patients. significant association was observed between higher FIGO stage and TGF β1IHC staining, with100 % of the patients with stage IV disease having a positive staining versus 25% of the patients with stage I disease (P <0.003).so,TGF β1is important for promoting cancer N. ear results are obtained by Rosenthal et al. 2004 who added that TGF-β1 up regulates the expression of CAFs, and reduces E-cadherin enhancing cell motility. also TGF-β1 promotes the expression of matrix metalloproteinase-2factorsMMP-2 degrades the intercellular matrix and basement membrane.
.
On the contrary, Chen et al 2014 declared that the expression of TGF-β1 in advanced stage and poorly-differentiated epithelial ovarian cancer was significantly lower than that in early stage and well-differentiated tumors, that may be due to difference in number of cases studied

Woo et al 2001 are also contradicting to current results who found that lymphocytes release TGF-β1 cytokine, which suggested suppressor function of TGF-β1 in the course of neoplastic diseases that may be due to using different staining protocols.
In the current study, all metastatic cases showed positive TGF β1 IHC staining denoting that it is vital for metastatic occurrence, which assessed by Chen et al 2014 who concluded that TGF-β1promotes angiogenesis and suppression of immune response, providing suitable microenvironment for cancer cells to

accelerate growth and metastasis.

Cao et al, 2012 also demonstrated that activation of TGF β1 signaling in OC cells induces the expression and function of enzymes linked to OC metastasis

The current results provide strong suggestion for the involvement of TGF β1in OC invasion and tumor progression. These results point to possible therapeutic interventions targeting TGF β1to stop OC tumor progression through application of a TGF-β1 antibody

However, further studies regarding the association between TGF-β1and the initiation and development of ovarian cancer may be needed for the diagnosis and treatment of the disease.

Conclusion

Beclin1 protein could be considered a good prognostic factor in OC cases while TGF-β1 considered adverse factor which could be of benefit in OC molecular targeting therapy.

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