

ORIGINAL PAPER

PROGNOSTIC SIGNIFICANCE OF HYPOXIA-INDUCED AUTOPHAGY RELATED MARKERS IN MALIGNANT SURFACE EPITHELIAL OVARIAN TUMOURSALAA AMER¹, SARA DARWISH², NEHAL HEABAH¹¹Pathology Department, Faculty of Medicine, Tanta University, Tanta, Egypt²Clinical Oncology and Nuclear Medicine Department, Faculty of Medicine, Tanta University, Tanta, Egypt

Bcl-2/adenovirus E1B 19-kDa interacting protein 3 (BNIP3), a pro-apoptotic protein, regulates autophagy. BNIP3 is induced in hypoxic cells, being regulated by hypoxia-inducible factor 1- α (HIF1- α). The role of hypoxia-induced autophagy in surface epithelial ovarian carcinomas (EOC) is not well established. This work aimed to evaluate BNIP3 and HIF1- α immunohistochemical expression and study the relation between their expression and patients' survival in EOC patients.

High BNIP3 and HIF1- α expressions were associated with a high tumour grade ($p = 0.012$ and 0.013), stage III tumours ($p = 0.031$ and 0.035), presence of omental deposits ($p = 0.029$ and 0.004), presence of capsular invasion ($p = 0.010$ and 0.019), and presence of vascular invasion ($p = 0.033$ and 0.007). High BNIP3 and HIF1- α expressions were associated with shortened overall survival (OS) ($p = 0.021$ and 0.029) and poor disease-free survival (DFS) ($p = 0.002$ and 0.005). The relation between BNIP3 and HIF1- α immunohistochemical expression was statistically significant ($p < 0.001$).

High BNIP3 and HIF1- α expressions are related to unfavorable clinicopathological parameters in terms of higher tumour grade, advanced tumours, presence of omental deposits, capsular and vascular invasion, together with poor OS and DFS in malignant surface epithelial ovarian tumour patients.

Key words: BNIP3, HIF1- α , autophagy, ovarian cancer, hypoxia.

Introduction

Ovarian cancer is the 5th main cause of cancer-related deaths among women worldwide. Epithelial ovarian carcinoma (EOC) accounts for about 90% of ovarian cancers [1]. The estimated number of newly diagnosed cases in 2020 was 313,959 with incidence and mortality rates of 6.6 and 4.2 per 100,000 globally, respectively [2]. Being the most lethal gynecological malignancy, EOC is known as the "silent killer" as its silent spread together with the lack of satisfactory screening tests mean that disease progression often occurs before diagnosis [3].

Autophagy is an important cellular homeostasis process, named after the Greek word "self-eating". Autophagy contributes to degradation of damaged intracellular materials and recycles metabolites to provide energy for cellular survival under a variety of stress conditions [4].

In the initial stages of tumorigenesis, autophagy may function as a tumour suppressor by destroying damaged organelles and harmful substances, preventing the spread of DNA-mutated cells. However, in late stages of tumour development, autophagy plays a tumour-promoting mechanism due to its capacity to maintain tumour viability in stressful microenvi-

ronments. Beyond this tumour-promoting activity, autophagy contributes to resistance to therapy [5].

Bcl-2/adenovirus E1B 19-kDa interacting protein 3 (BNIP3) is a mitochondrial pro-apoptotic protein that regulates apoptosis and autophagy. BNIP3 could either promote cell survival or induce cell death. Overexpression of BNIP3 is often observed in human malignancies and linked with aggressive tumour behavior, poor outcomes, and resistance to chemotherapy [6, 7]. However, its role in malignant surface epithelial ovarian tumours needs further description.

BNIP3 plays an essential role in hypoxia-responsive cells, regulated by hypoxia-inducible factor 1- α (HIF1- α). HIF1- α mediates adaptive responses to tissue oxygen level changes. While HIF1- α is rapidly degraded under normal conditions, it is stabilized under hypoxic ones. Under hypoxic conditions, stabilized HIF1- α translocates to the nucleus, activates several hypoxic-response elements, as vascular endothelial growth factor and stimulates essential pathways for tumour progression [8].

This work aimed to evaluate the immunohistochemical expression of hypoxia-induced autophagy related markers, BNIP3 and HIF1- α , in malignant surface epithelial ovarian tumours and also, to study the relation between BNIP3 and HIF1- α expression and the available clinicopathological parameters together with patients' survival in malignant surface epithelial ovarian tumour patients.

Material and methods

This prospective study was conducted in Pathology and Clinical Oncology Departments, Faculty of Medicine, Tanta University, Egypt, between November 2023 and October 2025 on 86 patients with a *de novo* stage I, II and III malignant surface epithelial ovarian tumours.

The clinicopathologic criteria of the studied cases were obtained from the Pathology reports. Cases were classified according to the 2020 World Health Organization (WHO) classification of female genital tumours [9]. Staging was done following the International Federation of Gynecology and Obstetrics (FIGO) staging system [10].

Serous carcinomas were graded as low-grade and high-grade, as reported by the 2020 WHO classification of female genital tumours [9]. Endometrioid carcinoma was classified as grade I, II, and III using the FIGO grading system [11]. Mucinous carcinoma was graded as endometrioid carcinomas [12]. Clear cell carcinomas were high grade by definition [13].

Cases were grouped as: a low-grade group including low-grade serous carcinoma, grades I, II endometrioid and mucinous carcinomas, and a high-grade group including high-grade serous carcinoma, grade III endometrioid and mucinous carcinomas, and clear cell carcinoma.

Inclusion criteria

The study included patients with *de novo* FIGO stage I, II and III EOC proved by histopathological examination, who received either a neoadjuvant or an adjuvant chemotherapy \pm bevacizumab, with good performance status (PS) \leq 1 and surgical resection of the tumour either primary or interval de-bulking (according to the tumour stage and guidelines).

Exclusion criteria

Patients with metastatic ovarian carcinoma, recurrent disease, and patients with poor PS were excluded from this study.

Patient work up

Every patient was subjected to careful history taking, abdominal examination, and investigations including laboratory test (tumour marker CA125), abdominal-pelvic ultrasonography, computed tomography (CT) scan of the abdomen and pelvis with oral and intravenous contrast, chest CT scan, magnetic resonance imaging (MRI), and guided core biopsy of the tumour.

Immunohistochemical procedure

Five μ m-thick sections on positively charged slides were dried at 37°C for 30 minutes. The slides were then placed in Dako PT Link unit for de-paraffinization and antigen retrieval. Application of EnVision FLEX target retrieval solutions was performed, for 20 minutes at 97°C. By using Dako Autostainer (Link-48), slides were placed in peroxidase-blocking reagent for 5 minutes, incubated with anti-BNIP3 antibody (ANa40), a mouse monoclonal antibody (Abcam, ab10433, dilution 1 : 100) and anti-HIF1- α antibody (EP1215Y), rabbit recombinant monoclonal antibody (Abcam, ab51608, dilution 1 : 100) – for 20 minutes. Incubation with a horseradish peroxidase reagent for 20 minutes and di-amino-benzidine chromogen for 10 minutes were done. Slides were finally counterstained with hematoxylin.

Immunohistochemical staining assessment

Three fields were assessed for each case. Areas of tumour necrosis were not considered. The immunohistochemical semiquantitative immunoreactive score (IRS) was evaluated independently by two pathologists.

Assessment of BNIP3 immunohistochemical staining

Cases were classified into two groups: low and high BNIP3 expression according to the IRS obtained by multiplying of the intensity score and the percentage of stained cells. Staining intensity included: 0 (negative

or colorless), 1 (weak or pale yellow), 2 (moderate or light brown), and 3 (strong or dark brown). The percentage of stained cells was evaluated as follows: 0 (no expression), 1 (< 1% of cells), 2 (1–10% of cells), 3 (11–50% of cells), and 4 (\geq 50% of cells). The final IRS ranged 0–12 [14]. A positive control was performed using specimens of normal renal tissue. A negative control was performed by replacing the primary antibody with phosphate-buffered saline (PBS).

Assessment of HIF1- α immunohistochemical staining

HIF1- α expression was assessed by multiplying the staining intensity and the percentage of stained cells. Staining intensity scores were: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The percentages of positive tumor cells were: 0 (< 10%), 1 (10–25%), 2 (26–50%), 3 (51–75%), and 4 (> 75%). The percentage of stained tumor cells was multiplied by the staining intensity score to get a final IRS (0–12); scores \leq 2 were considered low HIF1- α expression and scores \geq 3 were considered high HIF1- α expression [15]. A positive control was performed using specimens of human ovarian carcinoma. A negative control was performed by replacing the primary antibody with PBS.

Survival analysis

Overall survival (OS) was identified as the time from the date of diagnosis to the date of death or the date of last follow-up. Disease-free survival (DFS) was the time from the date of diagnosis to physical, biological, or radiological evidence of disease recurrence. The primary end point of the study was OS while the secondary end point was DFS.

Chemotherapy

The chemotherapy regimen was paclitaxel (175 mg/m² IV) followed by carboplatin (area under curve 5–6 IV) day 1 repeated every 3 weeks. In total 6 cycles were received (in case of neoadjuvant chemotherapy – NAC; 3 cycles preoperatively and 3 cycles were received postoperatively). The response was evaluated by clinical examination, serum CA 125 level, abdominal-pelvic CT scan, and CT chest.

Follow-up

Follow-up visits were performed every 3 months in the first 2 years and every 6 months thereafter. During the follow-up visits, the patients were examined clinically, and serum CA125 was measured. Abdominal-pelvic CT scan was performed routinely every 6 months. When an increased CA125 level was observed, abdominal-pelvic CT scan with oral and intravenous contrast, chest CT scan and MRI were performed.

Statistical analysis

Results were statistically analyzed using Statistical Package for Social Science (version 23, Armonk, NY; IBM Corp). Categorical variables were summarized as numbers and percentages, while numerical variables were presented as range, mean \pm standard deviation, median, and interquartile range. To compare the two studied groups, χ^2 test was used. Fisher exact and Monte Carlo tests were applied whenever needed. Restricted mean survival time (RMST) and Kaplan-Meier survival analysis with number at risk were done using MedCalc® Statistical Software version 23.1.5 (MedCalc Software Ltd., Ostend, Belgium; <https://www.medcalc.org>; 2025). The significance level was set at 5%.

Results

Clinicopathologic characteristics of the studied cases

This study included 86 EOC cases, with a mean age of 54.37 ± 10.80 years and a mean tumor size of 10.77 ± 4.81 cm. High grade serous carcinoma represented the most prominent histologic type (30.2% of cases) (Figure 1). The majority of cases were high-grade tumors (74.4% of cases). Stage III cases accounted for 51.2%. Cases with positive omental deposits accounted for 58.1%. Cases with positive capsular and vascular invasion accounted for 74.4% and 72.1% of the cases, respectively. Thirty eight patients (44.2%) underwent primary surgery, while 48 patients (55.8%) received NAC followed by interval debulking surgery. After cytoreductive surgery, complete resection (R0) was achieved in 64 patients (74.4%) while optimal resection (R1) was achieved in 24 patients (25.6%).

The median follow-up period was 21 months (ranged 6–24 months); mortality was reported in 11 patients (12.8%) while recurrence was reported in 23 patients (26.7%). The clinicopathologic characteristics of the cases involved are summarized in Table I.

Relation between BNIP3 expression and clinicopathologic parameters

High BNIP3 expression was detected in 70.9% of cases (Figure 2). Table II shows a statistically significant association between high BNIP3 expression and high tumor grade ($p = 0.012$), advanced tumor stage ($p = 0.031$), presence of omental deposits ($p = 0.029$) as well as the presence of capsular and vascular invasion ($p = 0.010$ and 0.033 , respectively). Regarding recurrent cases, 87.0% of them showed high BNIP3 expression ($p = 0.048$).

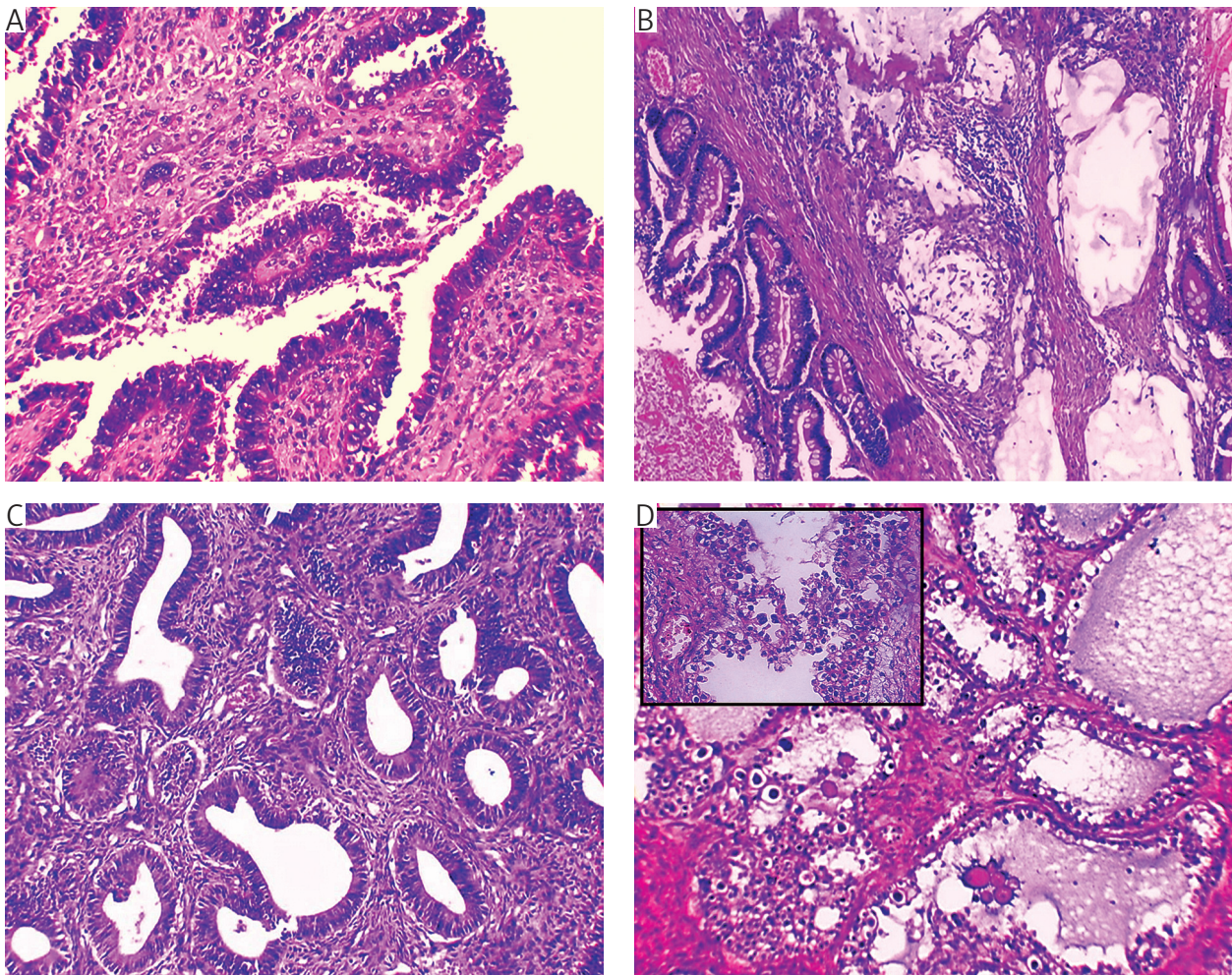


Figure 1. Representative hematoxylin and eosin stained sections of malignant surface epithelial ovarian tumor specimens. **A)** High-grade serous carcinoma showing papillary structure lined by high-grade malignant cells invading the underlying core (400×). **B)** Mucinous carcinoma showing irregular glandular structures lined by mucin secreting cells with lacks of extracellular mucin (100×). **C)** Endometrioid carcinoma showing complex packed malignant glands (200×). **D)** Clear cell carcinoma showing tubulo-cystic spaces lined by one or two cell layers of clear cells with hobnail nuclei (200×, inset 400×)

Relation between HIF1- α expression and clinicopathologic parameters

High HIF1- α expression was detected in 74.4% of cases (Figure 3). Table III shows a significant association between high HIF1- α expression and high tumor grade ($p = 0.013$), advanced tumor stage ($p = 0.035$), presence of omental deposits ($p = 0.004$) as well as the presence of capsular and vascular invasion ($p = 0.019$ and 0.007 , respectively).

The relation between BNIP3 and HIF1- α immunohistochemical expression was statistically significant ($p < 0.001$); as 90.6% of cases with high HIF1- α expression showed also high BNIP3 expression (Table IV).

Restricted mean survival time of the studied cases

Tables V and VI show a significant association between high BNIP3 expression with shortened RMST regarding OS and DFS ($p = 0.021$ and 0.002 , respectively) (Figures 4A, B).

As regards HIF1- α expression, high HIF1- α expression was associated with shortened RMST regarding OS and DFS with a significant difference ($p = 0.029$ and 0.005 , respectively), as shown in Tables VII and VIII, Figures 5A, B.

COX regression analysis for factors affecting overall survival and disease-free survival of the studied cases

The overall survival was significantly affected by high grade serous carcinoma as histopathologic type ($p = 0.008$), stage III tumor ($p = 0.017$) and presence of omental deposits ($p = 0.025$). On performing multivariate analysis, the significant variables were high-grade serous carcinoma ($p = 0.002$) and stage III tumor ($p = 0.045$), as shown in Table IX.

The disease-free survival was significantly affected by the age of the patients ($p = 0.031$), high grade serous carcinoma as a histopathologic type ($p = 0.002$), high BNIP3 expression ($p = 0.006$), high HIF1- α (0.01) as well as the presence of capsular invasion

Table I. Clinicopathologic characteristics of the studied cases ($N = 86$)

PARAMETERS	VALUE
Age [years]	
Minimum–maximum	29.0–74.0
Mean \pm SD	54.37 \pm 10.80
Median (IQR)	55.0 (48.0–63.0)
Size [cm]	
Minimum–maximum	4.0–25.0
Mean \pm SD	10.77 \pm 4.81
Median (IQR)	11.0 (7.0–13.0)
Gross, n (%)	
Cystic	16 (18.6)
Solid	26 (30.2)
Cystic and solid	44 (51.2)
Laterality, n (%)	
Unilateral	46 (53.5)
Bilateral	40 (46.5)
Histopathologic types, n (%)	
HGSC	26 (30.2)
LGSC	12 (14.0)
Endometrioid	16 (18.6)
Clear cell carcinoma	12 (14.0)
Mucinous carcinoma	20 (23.2)
Tumour grade, n (%)	
Low	22 (25.6)
High	64 (74.4)
Stage, n (%)	
I	22 (25.6)
II	20 (23.2)
III	44 (51.2)
Omental deposits, n (%)	
Negative	36 (41.9)
Positive	50 (58.1)
Capsular invasion, n (%)	
Negative	22 (25.6)
Positive	64 (74.4)
Vascular invasion, n (%)	
Negative	24 (27.9)
Positive	62 (72.1)
Follow-up period [months]	
Minimum–maximum	6.0–24.0
Mean \pm SD	19.24 \pm 5.45
Median (IQR)	21.0 (17.0–24.0)
Overall survival, n (%)	
No	75 (87.2)
Death	11 (12.8)
Disease-free survival, n (%)	
No	63 (73.3)
Recurrence	23 (26.7)
Mean \pm SD	19.24 \pm 5.45

HGSC – high grade serous carcinoma, IQR – inter quartile range, LGSC – low grade serous carcinoma, SD – standard deviation

($p = 0.03$). On performing multivariate analysis, the significant variables were the age of the patients ($p = 0.035$) and high-grade serous carcinoma ($p = 0.009$), as shown in Table X.

Discussion

Among females, EOC are relatively common tumours with high mortality rate and worse prognosis among gynecologic malignancies. Like other tumours, EOC are susceptible to autophagy, a cellular adaptive response to various metabolic stresses. Autophagy is up regulated under hypoxic conditions and contributes to tumour cell survival [16].

Being a member of the Bcl-2 family, Bcl-2/E1B-19 kDa interacting protein (BNIP3) acts as a pro-apoptotic protein that activates Bax/Bak apoptotic pathway [17]. BNIP3 is responsive to hypoxic conditions *via* the carboxyl-terminal trans-membrane domain. Subsequent interaction between BNIP3 and microtubule-associated protein light chain 3 (LC3) *via* LC3-interacting regions promotes hypoxia-induced autophagy [18].

In this work, we evaluated the immunohistochemical expression of BNIP3 and HIF1- α as hypoxia-induced autophagy related markers in malignant surface epithelial ovarian tumours and studied the relation between BNIP3 and HIF1- α expression and the available clinicopathological parameters together with patients' survival.

In our study, high BNIP3 expression showed a significant association with high tumour grade, advanced tumour stage, presence of omental deposits, capsular and vascular invasion. A significant association between high BNIP3 expression and poor OS and DFS was observed.

Fan *et al.* [19] demonstrated a statistical difference between low and high BNIP3 expression groups in EOC patients regarding the histopathologic subtypes; being more expressed in serous carcinoma. However, they reported no statistically significant differences between BNIP3 expression and FIGO stage, nodal metastasis, omental deposits and the presence of malignant ascites.

Overall, the relation between BNIP3 expression and prognosis of EOC patients remains unclear, and the function of BNIP3 in such patients needs further description. Jia *et al.* [20] studied the association between BNIP3 levels and OS and PFS in EOC patients. They found that a high BNIP3 level was related to poor OS and progression free survival (PFS), as well as advanced stage ovarian cancer.

In contrast to our results, Fan *et al.* [19] stated that low BNIP3 expression was associated with poor PFS, with no difference regarding OS. They concluded that BNIP3 could be a novel therapeutic target for ovarian carcinoma patients.

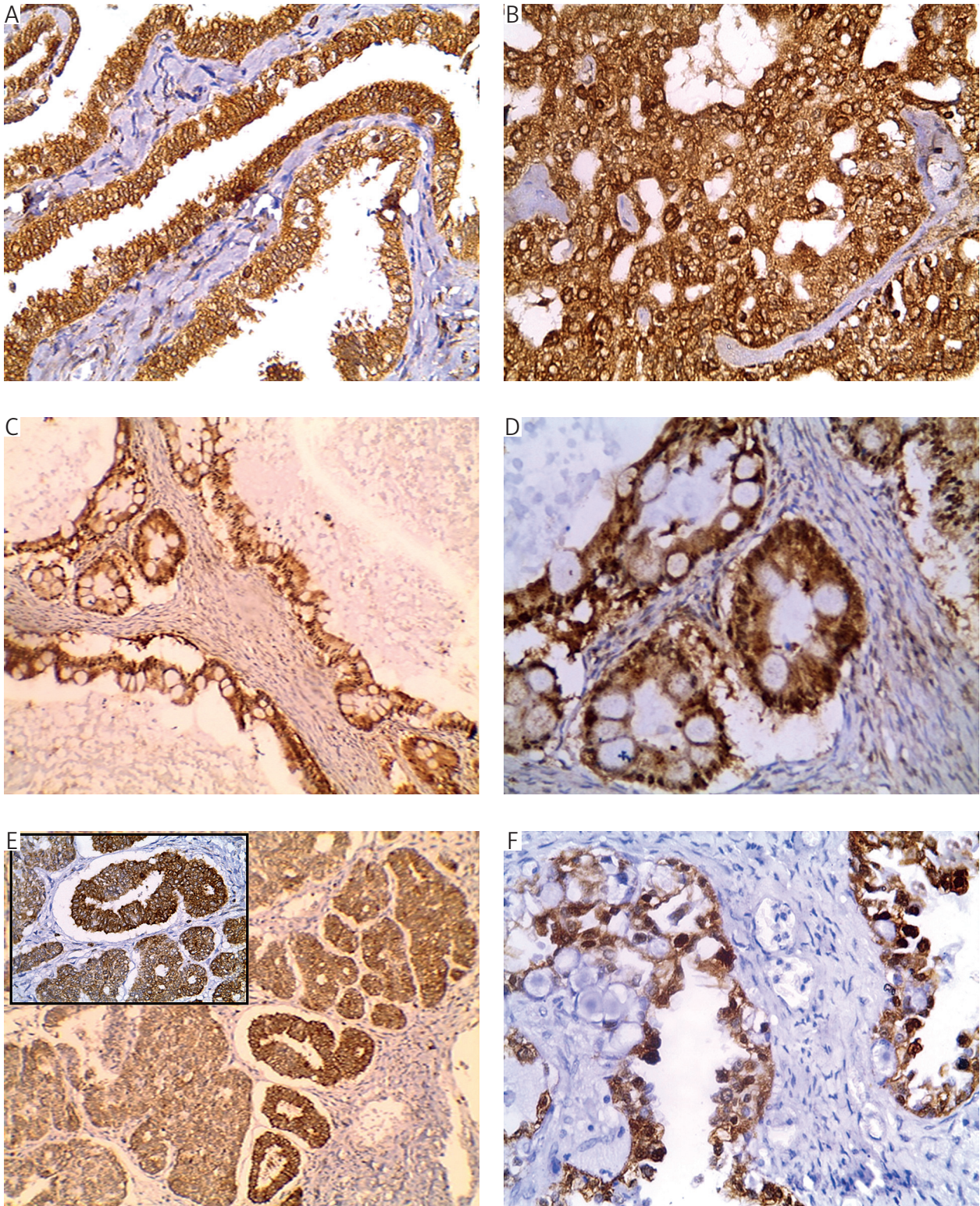


Figure 2. A, B) Bcl-2/adenovirus E1B 19-kDa interacting protein 3 (BNIP3) immunohistochemical expression in malignant surface epithelial ovarian tumor specimens. High-grade serous carcinoma showing high BNIP3 expression (A 200 \times , B 400 \times). C, D) Mucinous carcinoma showing high BNIP3 expression (C 200 \times , D 400 \times). E) Endometrioid carcinoma showing high BNIP3 expression (200 \times , inset 400 \times). F) Clear cell carcinoma showing high BNIP3 expression (400 \times)

Table II. Relation between Bcl-2/adenovirus E1B 19-kDa interacting protein 3 expression and clinicopathologic parameters of the studied cases (N = 86)

PARAMETERS	BNIP3 EXPRESSION		TEST OF SIGNIFICANCE	P-VALUE	
	Low (N = 25)	HIGH (N = 61)			
Age [years]					
Mean \pm SD	51.12 \pm 13.78	55.70 \pm 9.11	$t = 1.532$	0.135	
Median (minimum–maximum)	54.0 (33.0–74.0)	55.0 (29.0–73.0)			
Size [cm]					
Mean \pm SD	10.88 \pm 4.76	10.72 \pm 4.86	$U = 729.50$	0.753	
Median (minimum–maximum)	11.0 (4.0–18.0)	11.0 (4.0–25.0)			
Gross, n (%)					
Cystic	16	10 (62.5)	6 (37.5)	$\chi^2 = 11.387^*$	0.003*
Solid	26	4 (15.4)	22 (84.6)		
Cystic and solid	44	11 (25.0)	33 (75.0)		
Laterality, n (%)					
Unilateral	46	16 (34.8)	30 (65.2)	$\chi^2 = 1.565$	0.211
Bilateral	40	9 (22.5)	31 (77.5)		
Histopathologic types, n (%)					
HGSC	26	6 (23.1)	20 (76.9)	$\chi^2 = 3.754$	MCp = 0.448
LGSC	12	6 (50.0)	6 (50.0)		
Endometrioid	16	5 (31.2)	11 (68.8)		
Clear cell carcinoma	12	2 (16.7)	10 (83.3)		
Mucinous carcinoma	20	6 (30.0)	14 (70.0)		
Tumour grade, n (%)					
Low	22	11 (50.0)	11 (50.0)	$\chi^2 = 6.281^*$	0.012*
High	64	14 (21.9)	50 (78.1)		
Stage, n (%)					
I	22	11 (50.0)	11 (50.0)	$\chi^2 = 6.948^*$	0.031*
II	20	3 (15.0)	17 (85.0)		
III	44	11 (50.0)	33 (75.0)		
Omental deposits, n (%)					
Negative	36	15 (41.7)	21 (58.3)	$\chi^2 = 4.765^*$	0.029*
Positive	50	10 (20.0)	40 (80.0)		
Capsular invasion, n (%)					
Negative	22	10 (52.6)	9 (47.4)	$\chi^2 = 6.566$	0.010*
Positive	64	15 (22.4)	52 (77.6)		
Vascular invasion, n (%)					
Negative	24	11 (45.8)	13 (54.2)	$\chi^2 = 4.537^*$	0.033*
Positive	62	14 (22.6)	48 (77.4)		
Disease-free survival, n (%)					
No	63	22 (34.9)	41 (65.1)	$\chi^2 = 3.911^*$	0.048*
Recurrence	23	3 (13.0)	20 (87.0)		
Overall survival, n (%)					
No	75	24 (32.0)	51 (68.0)	$\chi^2 = 2.442^*$	FEp = 0.164
Death	11	1 (9.1)	10 (90.9)		

BNIP3 – Bcl-2/adenovirus E1B 19-kDa interacting protein 3, FE – Fisher exact test, HGSC – high grade serous carcinoma, LGSC – low grade serous carcinoma, MC – Monte Carlo test, SD – standard deviation, U – Mann-Whitney U test

p-value for comparison between the studied categories

* Statistically significant at $p \leq 0.05$

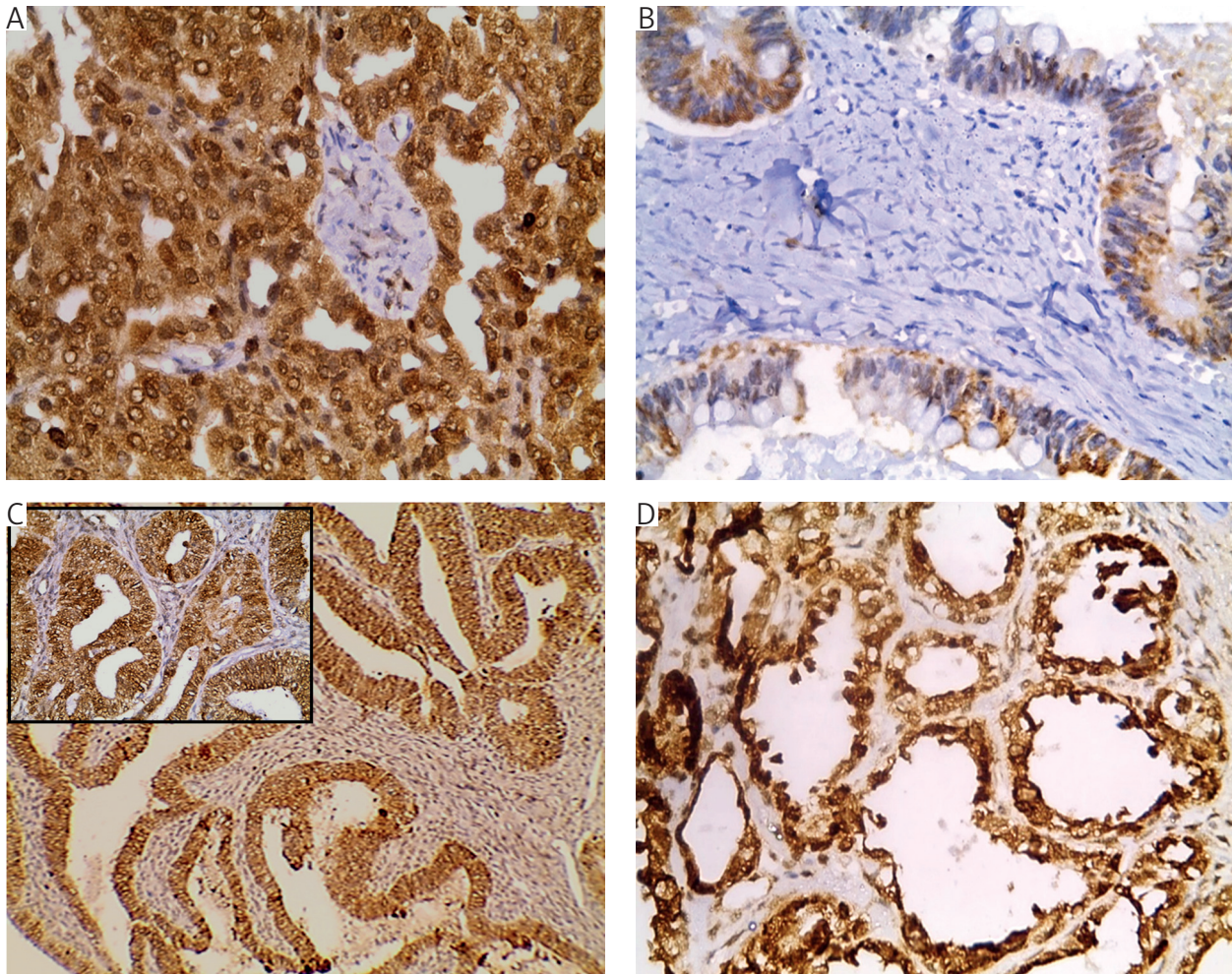


Figure 3. Hypoxia-inducible factor 1- α (HIF1- α) immunohistochemical expression in malignant surface epithelial ovarian tumor specimens. **A)** High-grade serous carcinoma showing high HIF1- α expression (400 \times). **B)** Mucinous carcinoma showing low HIF1- α expression (400 \times). **C)** Endometrioid carcinoma showing high HIF1- α expression (200 \times , inset 400 \times). **D)** Clear cell carcinoma showing high HIF1- α expression (400 \times)

Jia *et al.* [21] reported that BNIP3 could affect ovarian cancer cells proliferation and migration. In addition, Bristow *et al.* [22] stated that the truncated BNIP3; lacking the trans-membrane domain, abolished BNIP3-induced mitochondria-dependent apoptosis in ovarian cancer cells.

Lee *et al.* [23] stated that BNIP3 expression in tumors was tissue specific; being highly expressed in breast, lung, cervical cancers and glioma. In contrast, BNIP3 was epi-genetically silenced in colorectal, gastric, pancreatic, cancers, leukemia, and lymphoma.

Hypoxia is associated with poor prognosis in many solid tumors. Some cancer cells will survive hypoxia through growth factor autocrine production, increased capacity for invasion, resistance to chemotherapy, and induction of autophagy [24]. Hypoxia-induced autophagy maintains cell survival by reactive oxygen species generation which could remove damaged mitochondria through a process called “mitophagy” and provides cells with adenosine triphosphate and nutrients that are deficient in hypoxic environments [25].

In this study, high HIF1- α expression was associated with a high tumor grade, stage III tumors, as well as the presence of omental deposits, capsular and vascular invasion. High HIF1- α expression was associated with poor OS and DFS with a significant difference.

In agreement with our results, Alabiad *et al.* [15] stated that high HIF1- α expression was associated with high grade and stage, and presence of distant and nodal metastases in serous ovarian carcinoma. This found that high HIF1- α expression was related to poor chemotherapeutic response and decreased 3-year recurrence-free and OS rates. So, they stated that HIF1- α is a poor prognostic marker in serous ovarian carcinoma patients.

In our study, the relation between BNIP3 and HIF1- α immunohistochemical expression was statistically significant as the majority of cases with high HIF1- α expression showed also high BNIP3 expression.

Mellor *et al.* [26] explained the relation between BNIP3 and HIF1- α as autophagy could be regulated

Table III. Relation between hypoxia-inducible factor 1- α expression and clinicopathologic parameters of the studied cases ($N = 86$)

PARAMETERS	HIF1- α EXPRESSION		TEST OF SIGNIFICANCE	P-VALUE
	Low ($N = 22$)	HIGH ($N = 64$)		
Age [years]				
Mean \pm SD	52.91 \pm 11.48	54.88 \pm 10.60	$t = 0.735$	0.465
Median (minimum–maximum)	55.0 (35.0–74.0)	54.50 (29.0–73.0)		
Size [cm]				
Mean \pm SD	12.27 \pm 4.17	10.25 \pm 4.93	$U = 490.0^*$	0.033*
Median (minimum–maximum)	12.0 (6.0–18.0)	10.0 (4.0–25.0)		
Gross, n (%)				
Cystic	16	8 (50.0)	$\chi^2 = 6.620^*$	0.037*
Solid	26	4 (15.4)		
Cystic and solid	44	10 (22.7)		
Laterality, n (%)				
Unilateral	46	14 (30.4)	$\chi^2 = 1.224$	0.269
Bilateral	40	8 (20.0)		
Histopathologic types, n (%)				
HGSC	26	4 (15.4)	$\chi^2 = 5.500$	$^{MC}p = 0.238$
LGSC	12	6 (50.0)		
Endometrioid	16	4 (25.0)		
Clear cell carcinoma	12	2 (16.7)		
Mucinous carcinoma	20	6 (30.0)		
Tumour grade, n (%)				
Low	22	10 (45.5)	$\chi^2 = 6.133^*$	0.013*
High	64	12 (18.8)		
Stage, n (%)				
I	22	10 (45.5)	$\chi^2 = 6.730^*$	0.035*
II	20	5 (25.0)		
III	44	7 (15.9)		
Omental deposits, n (%)				
Negative	36	15 (41.7)	$\chi^2 = 8.410^*$	0.004*
Positive	50	7 (14.0)		
Capsular invasion, n (%)				
Negative	22	9 (47.4)	$\chi^2 = 6.081^*$	$^{FE}p = 0.019^*$
Positive	64	13 (19.4)		
Vascular invasion, n (%)				
Negative	24	11 (45.8)	$\chi^2 = 7.172^*$	0.007*
Positive	62	11 (17.7)		
Disease-free survival, n (%)				
No	63	19 (30.2)	$\chi^2 = 2.593$	0.107
Recurrence	23	3 (13.0)		
Overall survival, n (%)				
No	75	21 (28.0)	$\chi^2 = 1.802$	$^{FE}p = 0.276$
Death	11	1 (9.1)		

FE – Fisher exact test, HGSC – high grade serous carcinoma, HIF1- α – hypoxia-inducible factor 1- α , LGSC – low grade serous carcinoma, MC – Monte Carlo test, SD – standard deviation, U – Mann-Whitney U test
 p -value for comparison between the studied categories
 * Statistically significant at $p \leq 0.05$

Table IV. Relation between Bcl-2/adenovirus E1B 19-kDa interacting protein 3 expression and hypoxia-inducible factor 1- α expression in the studied cases (N = 86)

PARAMETERS	TOTAL	BNIP3 EXPRESSION		TEST OF SIGNIFICANCE	P-VALUE
		LOW (N = 25)	HIGH (N = 61)		
HIF1- α expression, n (%)					
Low	22 (25.6)	19 (22.1)	3 (3.5)	47.064*	< 0.001*
High	64 (74.4)	6 (7.0)	58 (67.4)		
Total, n (%)	86 (100.0)	25 (29.1)	61 (70.9)		

BNIP3 – Bcl-2/adenovirus E1B 19-kDa interacting protein 3, HIF1- α – hypoxia-inducible factor 1- α

p-value for relation between BNIP3 and HIF1- α

* Statistically significant at $p \leq 0.05$

Table V. Overall survival with Bcl-2/adenovirus E1B 19-kDa interacting protein 3 immunohistochemical expression

PARAMETERS	RESTRICTED MEAN SURVIVAL TIME (AT TIME POINT 24 MONTHS)	95% CI	MEDIAN [MONTHS]	END OF STUDY (%)	P-VALUE
BNIP3 expression					0.021*
Low	23.857	23.58–24.13	–	95.2	
High	22.875	22.16–23.58	–	67.4	

BNIP3 – Bcl-2/adenovirus E1B 19-kDa interacting protein 3, CI – confidence interval, HIF1- α – hypoxia-inducible factor 1- α

Table VI. Disease-free survival with Bcl-2/adenovirus E1B 19-kDa interacting protein 3 immunohistochemical expression

PARAMETERS	RESTRICTED MEAN SURVIVAL TIME (AT TIME POINT 24 MONTHS)	95% CI	MEDIAN [MONTHS]	END OF STUDY (%)	P-VALUE
BNIP3 expression					0.002*
Low	23.161	22.23–24.10	–	85.9	
High	20.654	19.35–21.96	23.0	32.2	

BNIP3 – Bcl-2/adenovirus E1B 19-kDa interacting protein 3, CI – confidence interval

by the hypoxia-inducible protein, BNIP3, and that HIF1- α could induce BNIP3 under hypoxic conditions in many tumors. In turn, this could promote tumor aggressiveness, therapy resistance, and worsen patients' OS.

Similarly, Chen *et al.* [27] studied BNIP3 and HIF1- α in salivary gland adenoid cystic carcinoma. They found that high BNIP3 and HIF1- α were associated with high histological grade and stage. In their study, BNIP3 expression was correlated with HIF1- α expression. Their data indicated that hypoxia-induced autophagy evaluated by BNIP3 and HIF1- α expression may play a role in tumorigenesis and could be a target for gene therapy.

Sun *et al.* [28] reported that hypoxia-induced BNIP3 reprogrammed tumor cell metabolism in uveal melanoma cells and promoted their survival and metastasis. They explained the relation between tumor hypoxia and BNIP3 – induced autophagy as in response to hypoxia as BNIP3-mediated autophagy alleviated mitochondrial dysfunction and enhanced mitochondrial oxidative phosphorylation with simultaneous reduction in the production of mitochondrial

reactive oxygen species. This, in turn, impaired HIF1- α protein stability and inhibited glycolysis. Their observations demonstrated a novel mechanism by which BNIP3 promoted malignant progression by mediating hypoxia-induced autophagy suggesting that BNIP3 could be an important therapeutic target to prevent metastasis in cancer patients.

This study is limited by the small sample size, relatively short follow-up period, and the lack of available data on BRCA mutations and homologous recombination deficiency status. However, these limitations are inherent in the exploratory nature of the study and do not preclude meaningful interpretation of the observed histopathological findings.

Conclusions

High BNIP3 and HIF1- α expression is significantly associated with adverse clinicopathological features in terms of higher tumor grade, stage III tumors, presence of omental deposits as well as capsular and vascular invasion. Also, high expression of BNIP3 and HIF1- α is related to shortened OS and DFS in

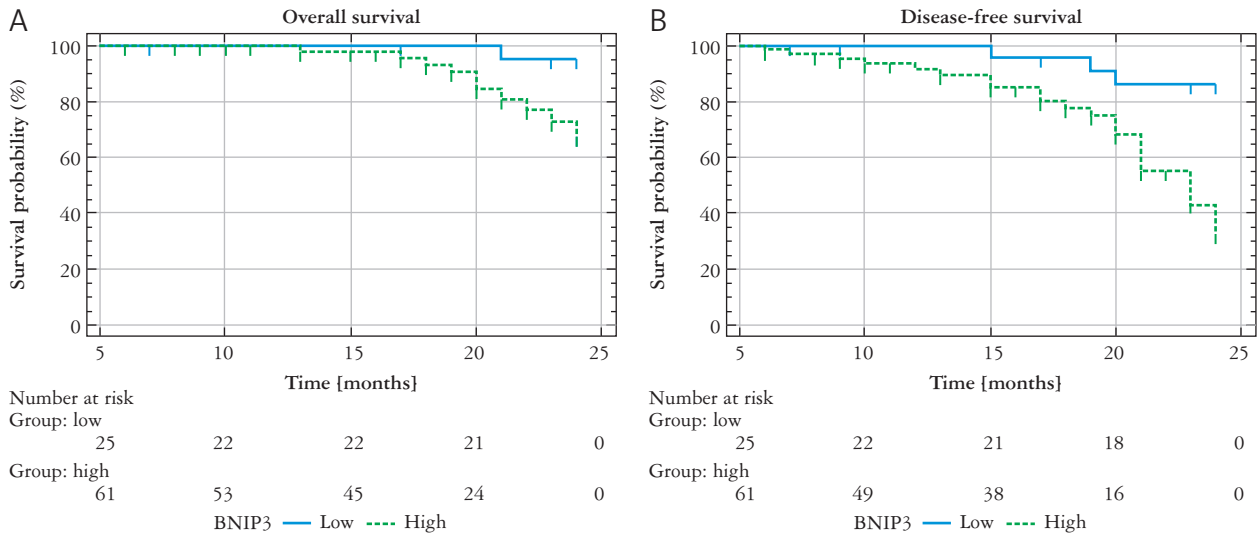


Figure 4. Kaplan-Meier curves for overall survival (A) and disease-free survival (B) in relation to Bcl-2/adenovirus E1B 19-kDa interacting protein 3 expression

BNIP3 – Bcl-2/adenovirus E1B 19-kDa interacting protein 3

Table VII. Overall survival with hypoxia-inducible factor 1- α immunohistochemical expression

PARAMETERS	RESTRICTED MEAN SURVIVAL TIME (AT TIME POINT 24 MONTHS)	95% CI	MEDIAN {MONTHS}	END OF STUDY (%)	P-VALUE
HIF1- α expression					
Low	23.850	23.56–24.14	–	95.0	0.029*
High	22.913	22.22–23.60	–	68.2	

CI – confidence interval, HIF1- α – hypoxia-inducible factor 1- α

Table VIII. Disease-free survival with hypoxia-inducible factor 1- α immunohistochemical expression

PARAMETERS	RESTRICTED MEAN SURVIVAL TIME (AT TIME POINT 24 MONTHS)	95% CI	MEDIAN {MONTHS}	END OF STUDY (%)	P-VALUE
HIF1- α expression					0.005*
Low	23.100	22.09–24.11	–	85.0	
High	20.785	19.51–22.05	23.0	34.1	

CI – confidence interval, HIF1- α – hypoxia-inducible factor 1- α

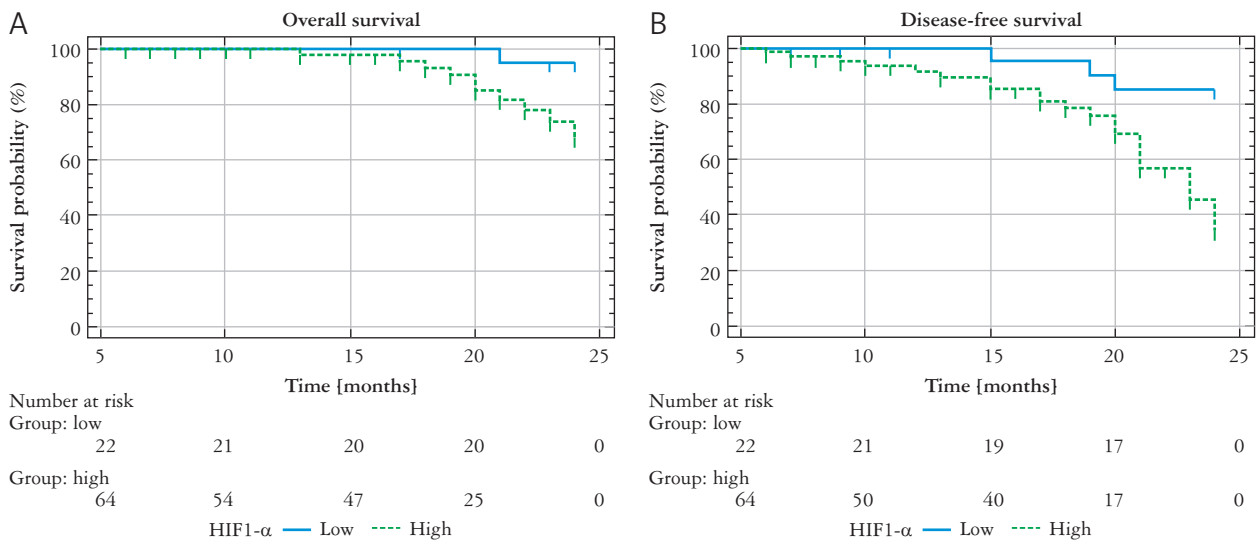


Figure 5. Kaplan-Meier curves for overall survival (A) and disease-free survival (B) in relation to hypoxia-inducible factor 1- α expression

HIF1- α – hypoxia-inducible factor 1- α

Table IX. Univariate and multivariate COX regression analysis for the parameters affecting overall survival

PARAMETERS	UNIVARIATE		#MULTIVARIATE	
	P-VALUE	HR (LL – UL 95% CI)	P-VALUE	HR (LL – UL 95% CI)
Histopathologic types				
HGSC	0.008*	6.093 (1.608–23.090)	0.002*	8.912 (2.245–35.377)
Tumour grade (high)	0.560	1.579 (0.340–7.320)		
Stage (III)	0.017*	12.204 (1.553–95.903)	0.045*	8.729 (1.051–72.476)
Omental deposits	0.025*	10.481 (1.338–82.078)	0.055	7.784 (0.959–63.194)
BNIP3 expression (high)	0.051	7.794 (0.988–61.50)		
HIF1- α expression (high)	0.062	7.183 (0.909–56.779)		

BNIP3 – Bcl-2/adenovirus E1B 19-kDa interacting protein 3, CI – confidence interval, HGSC – high grade serous carcinoma, HIF1- α – hypoxia-inducible factor 1- α , HR – hazard ratio, LL – lower limit, UL – upper limit
 # All variables with $p < 0.05$ was included in the multivariate
 * Statistically significant at $p \leq 0.05$

Table X. Univariate and multivariate COX regression analysis for the parameters affecting disease-free survival

PARAMETERS	UNIVARIATE		#MULTIVARIATE	
	P-VALUE	HR (LL – UL 95% CI)	P-VALUE	HR (LL – UL 95% CI)
Age [years]	0.031*	1.045 (1.004–1.088)	0.035*	1.063 (1.004–1.125)
Histopathologic types				
HGSC	0.002*	3.861 (1.672–8.913)	0.009*	3.392 (1.354–8.499)
Tumour grade (high)	0.307	1.757 (0.595–5.185)		
Stage (III)	0.149	1.858 (0.800–4.314)		
Capsular invasion	0.030*	9.236 (1.240–68.777)	0.074	9.577 (0.804–114.143)
BNIP3 expression (high)	0.006*	5.779 (1.654–20.187)	0.568	1.715 (0.270–10.906)
HIF1- α expression (high)	0.010*	5.156 (1.470–18.078)	0.563	1.667 (0.295–9.407)

BNIP3 – Bcl-2/adenovirus E1B 19-kDa interacting protein 3, CI – confidence interval, HGSC – high grade serous carcinoma, HIF1- α – hypoxia-inducible factor 1- α , HR – hazard ratio, LL – lower limit, UL – upper limit
 # All variables with $p < 0.05$ was included in the multivariate
 * Statistically significant at $p \leq 0.05$

malignant surface epithelial ovarian tumor patients. Yet, BNIP3 and HIF1- α expressions show unadjusted survival associations, and are not demonstrated as independent prognostic factors in this cohort.

As mechanisms that regulate hypoxia-induced autophagy in EOC remain unclear, further studies are required to clarify the role of BNIP3 and HIF1- α in ovarian cancer progression and investigate the possibility of their targeting in treatment of ovarian carcinoma.

Disclosures

1. Institutional review board statement: This study was approved by the Ethics Committee of the Faculty of Medicine, Tanta University (approval decision no: 36264PR1326/8/25, dated: 12.08.2025).
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

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