ASSOCIATION HYPOXIA INDUCIBLE FACTORS-1 ALPHA (HIF-1A) LEVELS AND EPITHELIAL OVARIAN CANCER (EOC)

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ABSTRACT

Introduction: Ovarian cancer is one of the most common cancers in Indonesia. Delay in diagnosis most due to asymptomatic, drug and unappropriate diagnostic tools. Previous studies have shown Hypoxia Inducible Factor 1 alpha (HIF1a) is a potential hypoxic tumor marker in various solid tumors. This study was conduct to investigate the association of the *HIF-1α* with stage,hystological type of ephitelial ovarian cancer (EOC).

Methods: This study is an observational study with a cross-sectional design. Women diagnosed with epithelial ovarian carcinoma who undergone surgical staging were selected using a consecutive sampling technique. Serum HIF-1α levels were assessed using immunohistochemical methods.

Results: A total of 72 women with epithelial ovarian carcinoma were examined for HIF-1α levels. The was significant relationship between HIF-1α expression and epithelial ovarian cancer stage which positive HIF-1α expression ($p>0.001$). There was no significant association between ovarian cancer histologic stage and HIF-1α levels ($p>0.001$).

Conclusion: We found a significant relationship between HIF-1α expression and epithelial ovarian cancer stage which positive HIF-1α expression was found more in patients with stage III/IV than stage I/II.

Keywords: *Ovarian cancer, HIF-1α, stage, immunohistochemical*

Introduction

Ovarian cancer is leading cause of death in gynecological malignancies. Epithelial ovarian cancer (EOC) is the most common type of ovarian cancer. Despite surgery and aggressive chemotherapy, the prognosis for this woman is poor, with a 5-year survival rate of less than 30%. Women who diagnosed at an early stage, the survival rate is approximately 85–90%. The delay in diagnosis most due to asymptomatic, drug and unappropriate diagnostic tools. [1,2]

Understanding of the mechanism of carcinogenesis and tumor microenvironment (TME) is very important to help find diagnostic tools. Hypoxia is an important event in carcinogenesis because it renders a more aggressive phenotype with increased invasiveness and proliferation, formation of metastases and poorer survival. [3] With the uncontrolled growth of tumor cells and abnormalities in tumor microcirculation hypoxia is an obvious feature of the TME, which is positively associated with tumor growth, angiogenesis, resistance to apoptosis and chemotherapy, and tumor metastasis. [4,5]

Hypoxia-inducible factors (HIFs) constitute a family of transcription factors that are involved in the regulation of the cellular response to hypoxic stress. [6,7] HIF-1 alpha is know as potential tumor marker plays an important role in transcriptional responses including the induction of genes involved in the induction of genes relevant to tumor development, such as angiogenesis, energy/glucose metabolism, cell development, metastasis and apoptosis.[5,8] This study was conducted to investigate the association of the *HIF-1α* with stage,hystological type of ephitelial ovarian cancer (EOC). Women with EOC were recruited in whom pathological diagnosis was confirmed with surgically resected specimen.

METHOD

This study is an analytical observational study with a cross sectional design on women diagnosed with epithelial ovarian carcinoma at the Hasanuddin University Teaching Hospital and Wahidin Sudirohusodo Hospital, Makassar for the period January 2021-November 2021.

Research Sample

The sample of this study were 72 women diagnosed with EOC whose tumors were surgically resected, stage by FIGO classification and pathologically confirmed, were recruited for this study. Clinicopathological characteristics of these cases, such as age, the International Federation of Obstetrics and Gynecology (FIGO) stage, histologic stage, CA-125 and parity.

Laboratory Examination

Serum HIF-1α levels were examined by immunohistochemical method. Determination of the classification of HIF1a expression referred to the study of Birner et al., where HIF1a expression was assessed semiquantitatively on the percentage of stained tumor cells and staining intensity. Expression of HIF-1α was determined by two independent observers by assessing semiquantitatively the percentage of stained tumor cells and the staining intensity. The percentage of positive cells was rated as follows: 2 points, 11–50% positive tumor cells; 3 points, 51–80% positive cells; and 4 points, >81% positive cells. The staining intensity was rated as follows: 1 point, weak intensity; 2 points, moderate intensity; and 3 points, strong intensity. Points for expression and percentage of positive cells were added, and specimens were attributed to four groups according to their overall score: negative, ≤10% of cells stained positive, regardless of intensity; weak expression, 3 points; moderate expression, 4–5 points; and strong expression, 6–7 points.[3]For this study we combined negative and weak expression become “Negative-Weak”, while for moderate and strong become “moderate-strong expression”.

Data analysis

Data analysis were investigated using Microsoft Excel 2013 and SPSS 20. To compare two numerical variables , an unpaired *student t* test was performed if the data were normally distributed and the *Mann-Whitney* test if the data are not normally distributed.

RESULTS

In this study, observations were made 72 patients with demographic distribution and characteristics that can be seen in table l.

Table 1. Characteristics and Distribution of Samples

Variable	Frequency (%)
Age	
30 years	1 (1.4%)
31-40 years old	5 (6.9%)
41-50 years old	27 (37.5%)
>50 years	39 (54.2%)
parity	
Nullipara	15 (20.8%)
Primipara	16 (22.2%)
Multipara	35 (48.6%)
Grande Multipara	6 (8.3%)
Abortion History	
Yes	21 (29.2%)
Not	51 (70.8%)
Ascites	
Yes	47 (65.3%)
Not	25 (34.7%)
Stadium	
1C	7 (9.7%)
2B	7 (9.7%)
2C	2 (2.8%)
3B	2 (2.8%)
3C	47 (65.3%)
4A	2 (2.8%)
4B	5 (6.9%)
CA-125	
35	6 (8.3%)
> 35	66 (91.7%)
Ovarian CA Type	
serious	68 (94.4%)
Endometrioid	3 (4.2%)
Mucinous	1 (1.4%)

HIF1a . expression	
Negative	3 (4.2%)
Weak expression	7 (9.7%)
Moderate expression	32 (44.4%)
Strong expression	30 (41.7%)
Histological Grading	
Low Grade	24 (33.3%)
High Grade	44(61.1%)
Well Differentiated	4 (5.6%)
Total	**72(100%)**

Table 1.

In table 1 showed most age diagnosed with epithelial ovarian cancer is above 50 years with a percentage of 54.2%. Based on parity, ovarian cancer common in multiparous group (48.6%) and serous type was the most common type (94.4%). Data showed negative expression of HIF-1α (4,2%),weak expression (9,7%), moderate (44,4%) and strong expression (41,7%).

Table 2. Analysis of factors associated with HIF1a expression in patients with epithelial-type ovarian carcinoma

Variable	HIF1a . expression				Total		p
	Negative-Weak		Medium-Strong				
	n	%	n	%	n	%	
Age							
30 years	0	0.0	1	100.0	1	100.0	[b] 1000
31-40 years old	1	20.0	4	80.0	5	100.0	
41-50 years old	3	11.1	24	88.9	27	100.0	
>50 years	6	15.4	33	84.6	39	100.0	
parity							
Nullipara	4	26.7	11	73.3	15	100.0	[b] 0.787
Primipara	0	0.0	16	100.0	16	100.0	
Multipara	6	17.1	29	82.9	35	100.0	
Grande Multipara	0	0.0	6	100.0	6	100.0	
Abortion History							
Yes	1	4.8	20	95.2	21	100.0	[a] 0.262
Not	9	17.6	42	82.4	51	100.0	
Ascites							
Yes	7	14.9	40	85.1	47	100.0	[a] 1000
Not	3	12.0	22	88.0	25	100.0	
Stadium							
1-2	5	31.3	11	68.8	16	100.0	[a] **0.037**
3-4	5	8.9	51	91.1	56	100.0	
CA-125							
35	1	16.7	5	83.3	6	100.0	[a] 1000

> 35	9	13.6	57	86.4	66	100.0	
Ovarian CA Type							
serious	9	13.2	59	86.8	68	100.0	
Endometrioid	1	33.3	2	66.7	3	100.0	[b] 1000
Mucinous	0	0.0	1	100.0	1	100.0	
Histological Grading							
Low Grade	2	8.3	22	91.7	24	100.0	
High Grade	8	18.2	36	81.8	44	100.0	[b] 0.379
Well Differentiated	0	0.0	4	100.0	4	100.0	

[a] Fisher-Exact test; [b] Kolmogorov-Smirnov test

Based on table 2, there was no significant difference in HIF-1α expression based on age group, parity, history of abortion, ascites findings, CA-level125, and type CA Ovarian . ($p>0.05$). A significant difference was found in the expression of HIF-1α based on epithelial ovarian cancer stage which positive HIF-1α expression was found more in patients with stage III/IV than stage I/II.

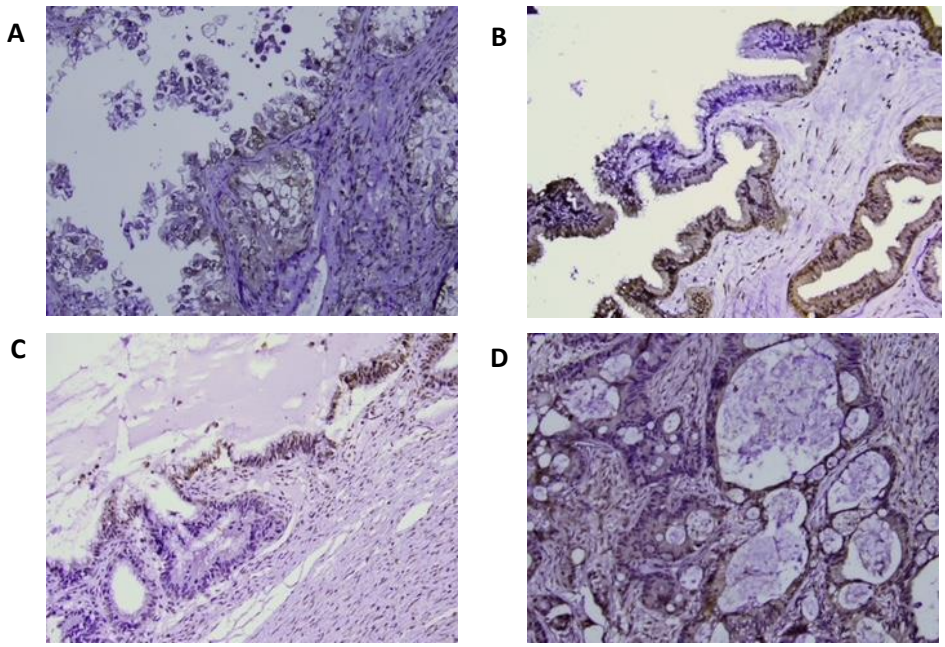

Figure 1 HIF-1α expression levels in ephitelial ovarian cancer. A-B Negative-Weak expression of HIF-1α. C-D Medium-strong expression of HIF-1α

Table 3. Differences in platelet and hemoglobin levels with HIF-1α . expression

Variable	Expression HIF1a (Mean±SD)

	Negative-Weak	Moderate-Strong	p
Hemoglobin	11.74±1.42	10.79±1.88	[a] 0.133
Platelets	347.80±157.10	298.41±163.22	[b] 0.272

[a] Independent-T tests; [b] Mann-Whitney test

Based on table 3, there was no significant difference in hemoglobin levels and HIF-1α expression although weak-negative HIF1a had a higher mean hemoglobin level of 11.74gr/dL than patient listen expression HIF1a medium-strong . ($p>0.05$). There was no difference between rate platelets and expression HIF1a on patient ephitelial ovarian cancer ($p>0.05$).

DISCUSSION

Hypoxia-inducible factor 1 (HIF-1) is a regulator of oxygen homeostasis consisting of two subunits, HIF-1α and HIF-1b. HIF-1 regulates the transcription of a number of genes involved in diverse biological functions including cell proliferation, apoptosis, migration, invasion and angiogenesis. HIF-1α protein overexpression correlates with p53 accumulation and cell proliferation. which plays an important role in the development of cancer cells . [9,10]

Our study showed that there was a relationship between the expression of HIF-1α levels and the stage of epithelial ovarian cancer, whereas moderate-strong positive expression was more common in advanced ovarian cancer. Our research is in line with several studies. [11,12,13] Hypoxic conditions will trigger the release of HIF-1α which promotes the transcription and expression of neuronal pentraxin II (NPTX2). Overexpression of NPTX2 will regulate the release of IL-6 cytokines. When IL-6 binds to the IL-6 receptor (IL-6R) it can activate several signaling pathways, one of which is STAT3. In the nucleus, STAT3 alters the transcription of many genes, including genes involved in proliferation, migration, differentiation, angiogenesis, survival, and chemotherapy resistance. STAT3 activation was found to be more common in advanced ovarian cancer than in early stage ovarian cancer. [14,15]

HIF-1α levels have a positive correlation with VEGF (*Vascular Endothelial Growth Factor*).[16] Increased VEGF expression will promote tumor cell proliferation, and accelerate tumor cell invasion and metastasis. Generally, VEGF is overexpressed in malignant tumors and vascular endothelial cells. VEGF expression level was positively correlated with clinical stage, degree of differentiation, and lymph node metastasis. VEGF expression is increased in the process of tumor

growth and angiogenesis which causes structural and functional abnormalities in tumor blood vessels. [1117]

HIF-1α has a mechanism to stimulate angiogenesis, proliferation, and induce IGFBP3 gene synthesis. The continuous expression of IGFBP3 will prolong the hypoxic phase of the tumor by inhibiting tumor vasculogenesis. When IGFBP3 decreases, cells begin to proliferate and switch to activate HIF-2α especially under hypoxic conditions. HIF-2α plays an important role in cancer aggressiveness by regulating angiogenesis, invasion, and metastasis that accelerates EOC progression. [18]The hypoxia-induced HIF-1A also affects the expression of genes involved in the formation of metastases. Hepatocyte growth factor (HGF) for example is a cytokine that stimulates proliferation and invasion through its receptor, the proto-oncogene c-MET. Invasive cell growth is promoted by HIF-1A-induced c-Met transcription and sensitizes cells to HGF stimulation and stimulates metastasis. [19,20]

Our study showed no association between HIF-1α expression and EOC cancer variables such as CA-125, histologic type, ascites and parity. The tissue samples of this study were examined based on the operating period so that there might be differences in the time of sampling and the time of immunohistochemical examination. HIF-1α increased in the first 24 hours and was subsequently replaced by HIF-2a. However, based on the study conducted by Cheng et al, HIF-1α can also be induced under normoxic conditions by several growth factors, including EGF and IGF-I. [10]

The limitations of our study include not assessing the relationship between HIF-1α levels and the response to several chemotherapy and types of chemotherapy. The relationship between HIF-1α levels and ovarian cancer survival was also not investigated in this study. Further research is needed to determine the relationship between HIF-1α levels and overall survival of epithelial ovarian cancer.

CONCLUSION

We found a significant relationship between HIF-1α expression and the stage of epithelial ovarian cancer which positive HIF-1α expression was more common in patients with stage III/IV than stage I/II. .

ETHICAL PERMISSION

The research has been approved by the biomedical research ethics commission in human medicine at Hasanuddin University . All families of patients who met the inclusion criteria were given a verbal explanation and signed a consent form

FUNDING

No funding sources.

ETHICAL APPROVAL

Not required.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

AUTHOR STATEMENT

Asmuliadi Asis,: Conceptualization, Methodology, Data curation. Johnsen Mailoal*:* Supervision.: Nugraha Utama: Software, Validation.: Ummu Aiman: Writing- Reviewing and Editing, Writing-Original draft preparation. Visualization, Investigation.

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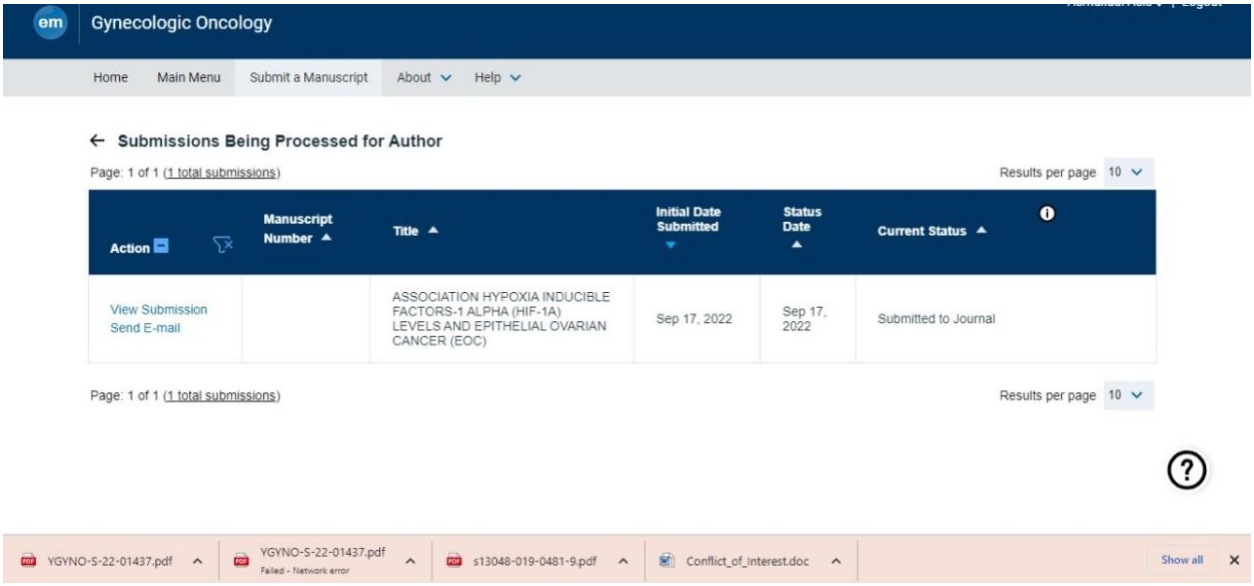
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