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RESEARCH ARTICLE

PREDICTIVE AND PROGNOSTIC VALUES OF HYPOXIA-INDUCIBLE FACTOR (HIF)-1A AND TAU-PROTEIN EXPRESSION IN SEROUS OVARIAN CANCER (SOC) PATIENTS TREATED WITH CHEMOTHERAPY.

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Abstract

Background: Serous ovarian carcinoma (SOC) is considered the most common epithelial ovarian malignancies**.** SOC is still having dismal outcome mainly due to early invasion, spread and resistance to chemotherapeutic agents e.g. platinum based drugs. Recent researches focused on detection of novel therapies and how to overcome chemotherapy resistance in those patients. Hypoxia inducible factor (HIF)-1 α is the transcription factor which is the first detected mediator of cell response to hypoxia in normal and malignant cells.

Tau protein (50–64 kD), which is a gene product that can bind to beta-tubulin. It was isolated from brain tissue and was associated with many neurodegenerative diseases.

Aim of the study to clarify the predictive and prognostic roles of (HIF)-1α and Tau protein expression in malignant tissues of 40 patients with SOC received paclitaxel first-line chemotherapy.

Methods; we have evaluated expression of HIF-1α and Tau protein expression by using immunohistochemistry in malignant tissues of forty patients of SOC. We evaluated the predictive and prognostic roles of expression of both proteins in SOC patients that have received first-line chemotherapy. We have followed patients for about three years to assess overall survival rate (OS) and resistance to chemotherapy.

Results: tissue expression of HIF-1α and Tau-protein in SOC was positively correlated with advanced stage of the tumor (p=0.006& 0.002 respectively), peritoneal implants (p=0.005), higher grade of the tumor(p=0.001& 0.008 respectively), presence of distant metastases (p=0.002& 0.032 respectively), presence of ascites (p=.034& 0.046 respectively) and L.N metastases (p=0.003& <0.001 respectively), chemoresistance (p= 0.03& 0.042 respectively), recurrence of the disease after successive therapy and unfavorable survival rates (p<0.001)

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Conclusion: both HIF-1α and Tau-protein are markers of unfavorable outcome of SOC patients.

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Introduction:-

The 4th commonest type of cancers in females is the epithelial ovarian carcinoma (EOC) **[1],** the serous subtype is the commonest of all types of EOC **[2]** Serous ovarian carcinoma (SOC) is still having dismal outcome mainly due to absence of symptoms in the early stage, late diagnosis, early invasion, spread and resistance to chemotherapeutic agents e.g. platinum based drugs [**3]**. The primary surgical management of SOC is depulking surgery as it has higher rate of invasion and spread to peritoneal cavity via peritoneal fluid **[4**], surgical management is followed by platinum based chemotherapeutic agents like platinum and taxane therapy **[2];** although those agents lead to marked improvement in the management and outcome of SOC patients but the problem is resistance to such chemotherapeutic modalities, the chemotherapy resistance have many mechanisms which become a recent focus of current researches [**5& 6**]. Additionally Recent researches focused on detection of novel therapies and how to overcome chemotherapy resistance in those patients. In addition to its invasive and aggressive nature SOC is a solid rabidly growing tumor which have areas of hypoxia many studies tried to evaluate the role of cancer hypoxia in malignant progression as it is incriminated in progression of many cancers. An easily applied method of assessment of hypoxia is by evaluation of tissue protein expression of Hypoxia inducible factor (HIF)-1 which is a transcription factor that is the primarily detected mediator of cell response to hypoxia [**7**]. Moreover, growing evidences have linked hypoxia related factors expression and oncogenesis [**8**].

Tau protein (50–64 kD), which is a product of a gene that is mapped on chromosome 17 and it shows the ability of combination with beta-tubulin in external and internal surface of the microtubules [**9].** Tau protein was isolated from brain tissue and was associated with many neurodegenerative diseases **[10].**

It is essential to clarify factors that are responsible for resistance to platinum based chemotherapy, SOC progression, recurrence and dismal outcome.

Aim of the study to clarify the predictive and prognostic roles of (HIF)-1α and Tau protein expression in malignant tissues of 40 patients with SOC received paclitaxel first-line chemotherapy.

Patients And Methods:-

Cases included in our prospective cohort study are forty cases of SOC that were surgically treated in Gynecology and Obstetrics department, faculty of medicine Zagazig University. Samples are processed, diagnosed, graded using WHO grading system **[11],** and staged using International Federation of Gynecology and Obstetrics systems and tumor-node-metastasis **[12],** in Pathology department, faculty of medicine Zagazig University. The patients are followed up for 3 years from May 2015 to May 2018 for assessment of response to chemotherapy, recurrence of the disease after successful therapy and for assessment of survival rates in Clinical Oncology and nuclear medicine and Medical Oncology Departments, faculty of medicine Zagazig University. Paraffin blocks from all cases are prepared for immunohistochemical staining for HIF-1 α and Tau protein.

Immunohistochemical staining:

We have used streptavidin-biotin method in immunohistochemistry **[13],** where sections have been incubated with primary mouse monoclonal anti-HIF-1α (Abcam, 1A3) and anti-Tau antibody [E178] (ab32057) antibodies dilution 1:200 (Abcam, Cambridge, MA, USA) at 4 °C overnight. Positive controls used are sections of lung cancer and normal ovarian epithelial tissue were used as positive control for HIF-1 α and Tau protein respectively [**14]**. We have evaluated the degree of HIF-1 α and Tau protein immunoreactivity of HIF-1α and Tau in pathology department, faculty of medicine, Zagazig University.

Evaluation of immunohistochemical expression of HIF-1 α and Tau protein:

HIF-1 α positive cell were evaluated by identification of brown particles in the nucleus of tumor cells and Tau protein positive cells were evaluated by identification of brown particles in the cytoplasm of tumor cells. We considered low expression if <10 % of tumor cells are positively stained and high expression if ≥10 % of tumor cells are positively stained [**15**].

Results:-

Patients' clinicopathological **results;**

We have made the current study on 40 cases of female patients with SOC. Their age ranged from (28-78) years. 27 (61.7%) cases were of high grade and 13 (38.3%) cases were with low grade SOC. Detailed demographic, pathological and follow-up data of patients are found in table 1

Immunohistochemical results of expression of HIF-1α **and Tau-protein in our cases; tables 2& 3; Figures**

- High tissue expression of HIF-1α in SOC was found in 28 cases and it was positively correlated with advanced stage of the tumor (p=0.006), peritoneal implants (p=0.005), higher grade of the tumor (p=0.001), presence of distant metastases (p=0.002), presence of ascites (p=.034) and L.N metastases (p=0.003). there is no significant association was found between its expression and bilaterality of the tumor.
- High tissue expression of Tau-protein in SOC was found in 24 cases and it was positively correlated with positively correlated with advanced stage of the tumor (p=0.002), peritoneal implants (p=0.005), higher grade of the tumor (p=0.008), presence of distant metastases (p=0.032), presence of ascites (p=. 0.046) and L.N metastases (p<0.001). no significant association was found between its expression, bilaterality of the tumor or presence of peritoneal implants.

Follow-up results:-

Tissue expression of HIF-1α in SOC was positively correlated with chemoresistance (p= 0.03), recurrence of the disease after successive therapy and unfavorable survival rates (p<0.001)

Tissue expression of **Tau-protein** in SOC was positively correlated with chemoresistance (p= 0.03), recurrence of the disease after successive therapy and unfavorable survival rates (p<0.001).

Statistical Analysis:-

Program used for statistics is SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA).

Tests used are Mann Whitney U test for non-normally distributed variables**.** Chi-square test Trend of change for comparison between relative frequencies of ordinal data

Overall Survival (OS) and Recurrence Free Survival (RFS) stratification were done according to markers using the method of Kaplan-Meier plot. A p-value <0.05 was significant.

Table 1:-Clinicopathological features, follow-up data and outcome of 40 patients with serous ovarian carcinoma.

Characteristics	All patients (N=40)		Characteristics	All patients (N=40)	
Age (years)			Operation		
Mean ± SD	65.53	±10.83	Radical surgery	10	(25%)
Median (Range)	59	(28 – 78)	Suboptimal	10	(30%)
<40 years	4	(6.7%)	Optimal	20	(45%)
41-59 years	24	(56.7%)			
≥ 60 years	12	(36.7%)			
Positive cytology			Number of cycles	(N=37)	
Absent	29	(65%)	4 cycles	5	(17.5%)
Present	11	(35%)	6 cycles	3	(14%)
			8 cycles	29	(68.4%)
CA125			Response	(N=25)	
≤35U/ml	11	(35%)	NR	10	(22.2%)
>35U/ml	29	(65%)	OAR	15	(77.8%)
Bilaterality			Response after 4-6 cycles	(N=25)	
Unilateral	34	(73.3%)	PD	3	(6.7%)
Bilateral	6	(26.7%)	SD	4	(22.2%)
Implants			PR	15	(64.4%)
Absent	28	(63.3%)	CR	3	(6.7%)
Present	12	(36.7%)			
Ascites			Response after 8 cycles	(N=25)	
Absent	28	(63.3%)	PD	3	(6.7%)

Present	12	(36.7%)		SD	5	(15.6%)
Grade				PR	5	(15.6%)
Low	13	(38.3%)		CR	12	(62.2%)
High	27	(61.7%)				
LN				Follow-up duration (months)		
Node negative	11	(35%)		Mean ± SD	17.01	±9.15
Node positive	29	(65%)		Median (Range)	11	(10 – 36)
M				Recurrence	(N=33)	
M0 (non-metastatic)	30	(76.7%)		Absent	12	(27.9%)
M1 (metastatic)	10	(23.3%)		Present	21	(72.1%)
FIGO Stage				Chemosensitivity	(N=21)	
Stage IA	1	(3.3%)		Chemosensitive	11	(35.5%)
Stage IB	1	(1.7%)		Chemorefractory	10	(64.5%)
Stage IC	2	(3.3%)		Death		
Stage IIA	1	(5%)		Alive	18	(46.7%)
Stage IIB	3	(11.7%)		Died	22	(53.3%)
Stage IIC	3	(10%)				
Stage IIIA	4	(15%)				
Stage IIIB	11	(20%)				
Stage IIIC	4	(6.7%)				
Stage IV	10	(23.3%)				

Table 2:-Relation between clinicopathological features and immunohistochemical staining for Hypoxia-Inducible Factor (HIF)-1α, and TAU protein in 40 patients with serous ovarian carcinoma

Characteristics	All (N=40)		HIF1 α Low (N=12)		High (N=28)		p-value	TAU protein Low (N=16)		High (N=24)		p-value
Age (years)												
Mean ± SD	65.53	±10.83	57.54	±10.20	65.15	±7.68	<0.001*	58.88	±10.06	70.61	±7.75	<0.001•
Median (Range)	57	(25 – 75)	46	(25-65)	69	(46-75)		57	(25-55)	60	(56-75)	
<40 years	4	(6.7%)	4	(100%)	0	(0%)	0.002‡	4	(100%)	0	(0%)	0.006‡
41-59 years	24	(56.7%)	6	(44.1%)	18	(55.9%)		8	(52.9%)	16	(47.1%)	
≥ 60 years	12	(36.7%)	2	(13.6%)	10	(86.4%)		4	(18.2%)	8	(81.8%)	
Positive cytology												
Absent	29	(65%)	11	(53.8%)	18	(46.2%)	0.02‡	14	(61.5%)	15	(38.5%)	0.009‡
Present	11	(35%)	1	(4.8%)	10	(95.2%)		2	(9.5%)	9	(90.5%)	
CA125												
≤35U/ml	11	(35%)	9	(81%)	2	(19%)	0.006‡	7	(81%)	4	(19%)	<0.001‡
>35U/ml	29	(65%)	3	(12.8%)	26	(87.2%)		9	(23.1%)	20	(76.9%)	
Bilaterality												
Unilateral	34	(73.3%)	10	(40.9%)	24	(59.1%)	0.258‡	13	(45.5%)	21	(54.5%)	0.582‡

Bilateral	6	(26.7 %)		2	(25%)		4	(75%)		3	(37.5 %)		3	(62.5 %)	
Implants															
Absent	28	(63.3 %)		10	(52.6 %)		18	(47.4 %)	0.005‡	13	(60.5 %)		15	(39.5 %)	0.061‡
Present	12	(36.7 %)		2	(9.1%)		10	(90.9 %)		3	(13.6 %)		9	(86.4 %)	
Ascites															
Absent	28	(63.3 %)		8	(47.4 %)		20	(52.6 %)	0.034‡	10	(52.6 %)		18	(47.4 %)	0.046‡
Present	12	(36.7 %)		4	(18.2 %)		8	(81.8 %)		6	(27.3 %)		6	(72.7 %)	
Grade															
Low	13	(38.3 %)		8	(78.3 %)		5	(21.7 %)	<0.001 ‡	10	(82.6 %)		3	(17.4 %)	0.008‡
High	27	(61.7 %)		4	(10.8 %)		23	(89.2 %)		6	(18.9 %)		21	(81.1 %)	
LN															
Node negative	11	(35%)		10	(81%)		1	(19%)	0.003‡	7	(81%)		4	(19%)	<0.001 ‡
Node positive	29	(65%)		2	(12.8 %)		27	(87.2 %)		9	(23.1 %)		20	(76.9 %)	
M															
M0 (non-metastatic)	30	(76.7 %)		10	(45.7 %)		20	(54.3 %)	0.002‡	14	(52.2 %)		16	(47.8 %)	0.032‡
M1 (metastatic)	10	(23.3 %)		2	(7.1%)		8	(92.9 %)		2	(14.3 %)		8	(85.7 %)	
FIGO Stage															
Stage IA	1	(3.3%)		1	(100%)		0	(0%)	0.006§	1	(100%)		0	(0%)	0.002§
Stage IB	1	(1.7%)		1	(100%)		0	(0%)		1	(100%)		0	(0%)	
Stage IC	2	(3.3%)		2	(100%)		0	(0%)		2	(100%)		0	(0%)	
Stage IIA	1	(5%)		1	(100%)		0	(0%)		1	(100%)		0	(0%)	
Stage IIB	3	(11.7 %)		2	(71.4 %)		1	(28.6 %)		3	(100%)		0	(0%)	
Stage IIC	3	(10%)		2	(66.7 %)		1	(33.3 %)		1	(66.7 %)		2	(33.3 %)	
Stage IIIA	4	(15%)		1	(11.1 %)		3	(88.9 %)		1	(33.3 %)		3	(66.7 %)	
Stage IIIB	11	(20%)		2	(16.7 %)		9	(83.3 %)		2	(16.7 %)		9	(83.3 %)	
Stage IIIC	4	(6.7%)		0	(0%)		4	(100%)		2	(50%)		2	(50%)	
Stage IV	10	(23.3 %)		0	(0%)		10	(100%)		2	(14.3 %)		8	(85.7 %)	
HIF															
Low	12	(36.7 %)								12	(100%)		0	(0%)	<0.001 ‡
High	28	(63.3 %)								4	(10.5 %)		24	(89.5 %)	
TAU															

Low	16	(43.3 %)		12	(84.6 %)		4	(15.4 %)	<0.001 ‡						
High	24	(56.7 %)		0	(0%)		24	(100%)							

*Independent samples Student's test; • Mann Whitney U test; ‡ Chi-square test; § Chi-square test for trend; p<0.05 is significant.

Table 3:-Relation between immunohistochemical staining for Hypoxia-Inducible Factor (HIF)-1α, and TAU protein in 40 patients with serous ovarian carcinoma

Characteristics	All			HIF α				p-value	Tau				p-value
				Low			High		Low			High	
Operation	(N=40)			(N=12)			(N=28)		(N=16)			(N=24)	
Radical surgery	10	(25%)		13	(59.1%)		2 (5.3%)	<0.001‡	13	(50%)		2 (5.9%)	<0.001‡
Suboptimal	10	(25%)		2	(9.1%)		16 (42.1%)		4	(15.4%)		14 (41.2%)	
Optimal	20	(50%)		7	(31.8%)		20 (52.6%)		9	(34.6%)		18 (52.9%)	
ECOG PS	(N=40)			(N=12)			(N=28)		(N=16)			(N=24)	
ECOG 1	22	(70%)		17	(77.3%)		25 (65.8%)	0.350‡	20	(76.9%)		18 (64.7%)	0.306‡
ECOG 2	18	(30%)		5	(22.7%)		13 (34.2%)		6	(23.1%)		12 (35.3%)	
Number of cycles	(N=37)			(N=17)			(N=20)		(N=13)			(N=24)	
4 cycles	5	(17.5%)		3	(42.1%)		2 (5.3%)	0.002‡	8	(34.8%)		2 (5.9%)	0.010‡
6 cycles	3	(14%)		1	(21.1%)		2 (10.5%)		4	(17.4%)		4 (11.8%)	
8 cycles	29	(68.4%)		13	(36.8%)		16 (84.2%)		11	(47.8%)		28 (82.4%)	
Response	(N=25)			(N=9)			(N=16)		(N=13)			(N=32)	
NR	10	(22.2%)		0	(0%)		10 (27.8%)	0.003‡	0	(0%)		10 (31.3%)	0.042‡
OAR	15	(77.8%)		9	(100%)		6 (77.8%)		13	(100%)		22 (68.8%)	
Response after 4-6	(N=25)			(N=9)			(N=16)		(N=13)			(N=12)	
PD	3	(6.7%)		0	(0%)		3 (8.3%)	0.04‡	0	(0%)		3 (9.4%)	0.557‡
SD	4	(22.2%)		1	(11.1%)		9 (25%)		2	(15.4%)		8 (25%)	
PR	15	(64.4%)		8	(88.9%)		11 (58.3%)		10	(76.9%)		19 (59.4%)	
CR	3	(6.7%)		0	(0%)		3 (8.3%)		1	(7.7%)		2 (6.3%)	
Response after 8	(N=25)			(N=9)			(N=26)		(N=13)			(N=12)	
PD	3	(6.7%)		0	(0%)		3 (8.3%)	0.009‡	0	(0%)		3 (9.4%)	0.036‡
SD	5	(15.6%)		0	(0%)		7 (19.4%)		0	(0%)		7 (21.9%)	
PR	5	(15.6%)		1	(11.1%)		6 (16.7%)		2	(15.4%)		5 (15.6%)	
CR	12	(62.2%)		8	(88.9%)		20 (55.6%)		11	(84.6%)		7 (53.1%)	
Recurrence	(N=33)			(N=21)			(N=22)		(N=14)			(N=12)	
Absent	12	(27.9%)		11	(52.4%)		1 (4.5%)	<0.001‡	12	(50%)		0 (0%)	<0.001‡
Present	21	(72.1%)		10	(47.6%)		21 (95.5%)		12	(50%)		19 (100%)	
Chemosensitivity	(N=21)			(N=10)			(N=21)		(N=12)			(N=12)	
Chemosensitive	10	(35.5%)		6	(60%)		5 (23.8%)	0.04‡	7	(58.3%)		4 (21.1%)	0.046‡
Chemorefractory	11	(64.5%)		4	(40%)		16 (76.2%)		5	(41.7%)		15 (78.9%)	
RFS	(N=23)			(N=21)			(N=22)		(N=20)			(N=12)	
Mean (months) (95%CI)	20.2 months (16.9 – 23.5)			26 months (21.3 – 30.7)			14.6 months (11.3 – 17.9)	0.002†	25.3 months (20.9 – 29.8)			13.9 months (10.5 – 17.3)	<0.001†
1 year RFS	48.8%			71.4%			27.3%		70.8%			21.1%	
2 year RFS	31.4%			52.4%			10.9%		48.7%			10.5%	
3 year RFS	23.2%			52.4%			---		48.7%			---	
Death	(N=40)			(N=22)			(N=28)		(N=22)			(N=22)	
Alive	18	(46.7%)		19	(86.4%)		9 (23.7%)	<0.001‡	22	(84.6%)		6 (17.6%)	<0.001‡
Died	22	(53.3%)		3	(13.6%)		29 (76.3%)		4	(15.4%)		28 (82.4%)	

OS	(N=40)		(N=22)		(N=28)		(N=22)		(N=22)	
Mean (months) (95%CI)	22.3 months (19 – 25.5)		32.6 months (29 – 36.2)		15.8 months (12.5 – 19.1)	<0.001†	32.2 months (28.7 – 35.6)		14.7 months (11.6 – 17.8)	<0.001†
1 year OS	44.9%		86.4%		19.7%		84.6%		15.1%	
2 year OS	44.9%		86.4%		19.7%		84.6%		15.1%	
3 year OS	44.9%		86.4%		19.7%		84.6%		15.1%	

95%CI: 95%Confidence Interval; ‡ Chi-square test; † Log rank test; p<0.05 is significant.

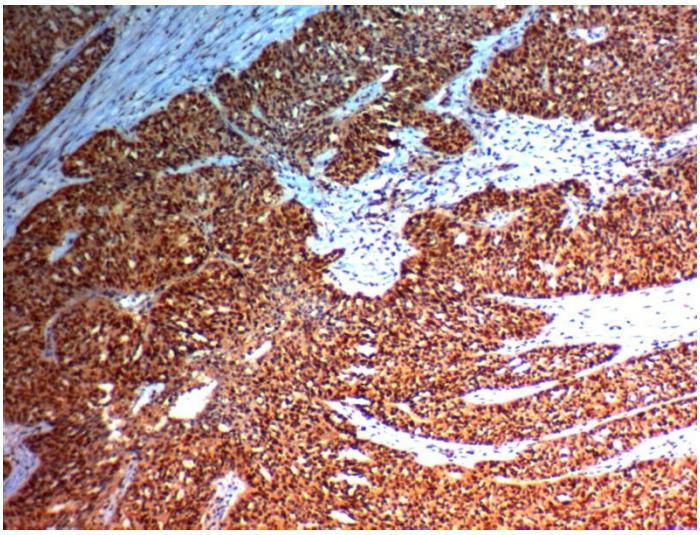

Fig 1 A

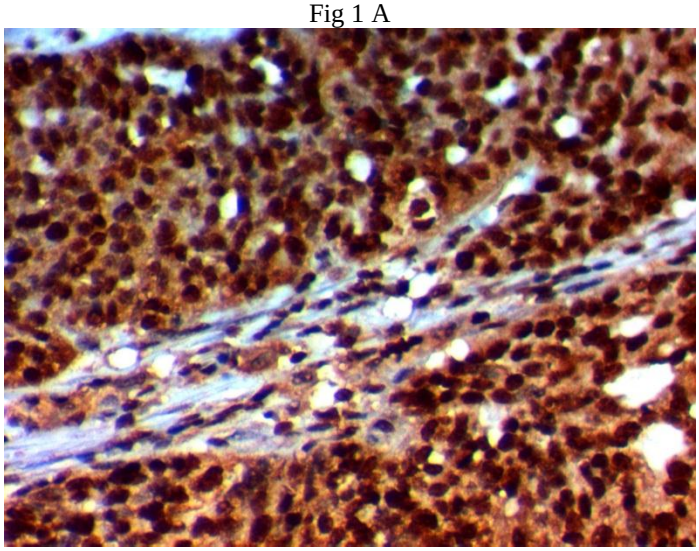

Fig 1 B

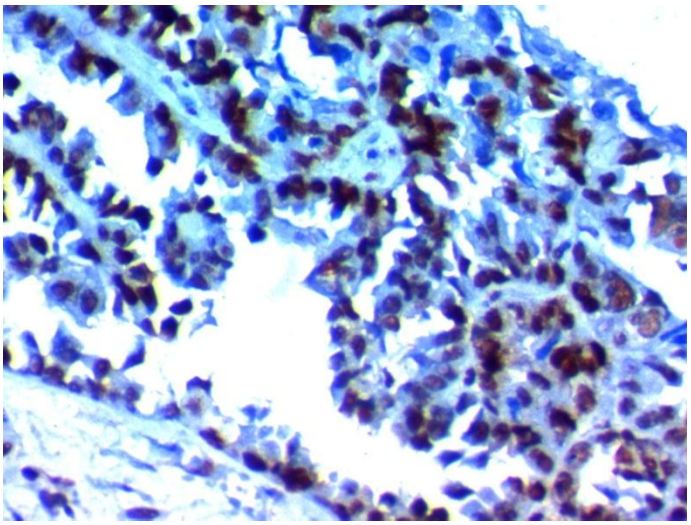

Fig 1 **C**

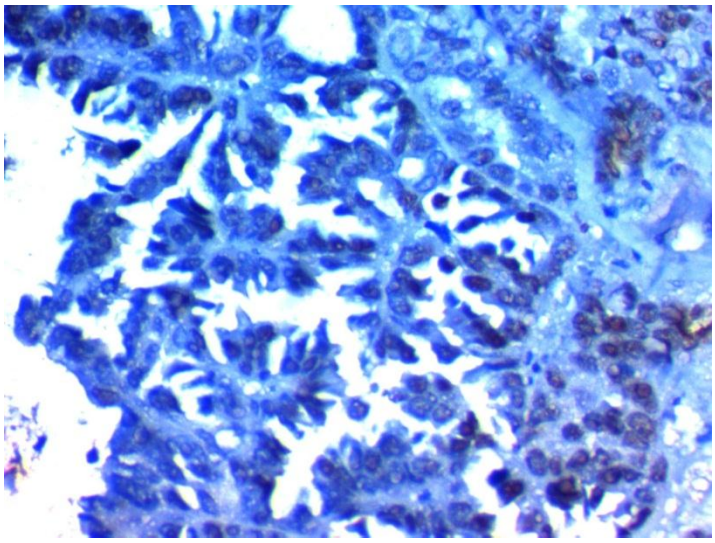

Fig 1 **D**

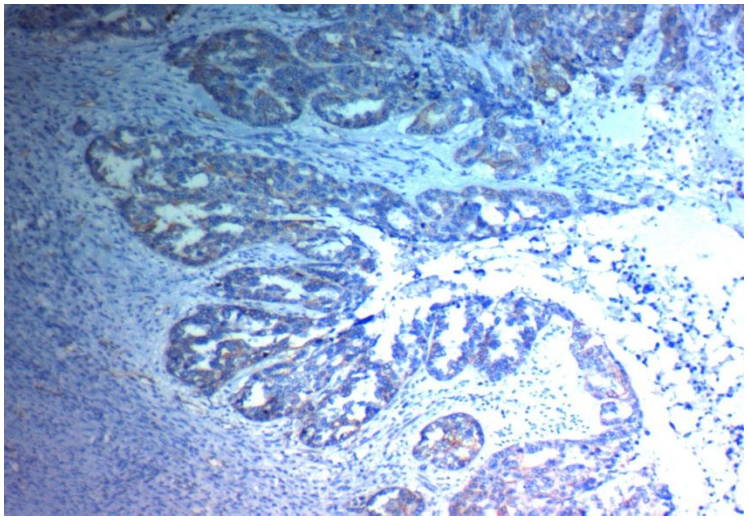

Fig 1 **E**

Figure1:-Immunohistochemical expression of Hypoxia-Inducible Factor (HIF)-1α in serous ovarian carcinoma (SOC): (A) High expression in the nucleus of high grade SOC stage IV x100. (B) High expression in the nucleus of high grade SOC stage IV x400. (C) High expression in the nucleus of high grade SOC stage III x400.) (D) Low expression in the nucleus of low grade SOC stage IIx400 (E) Low expression in the nucleus of low grade SOC stage Ix100

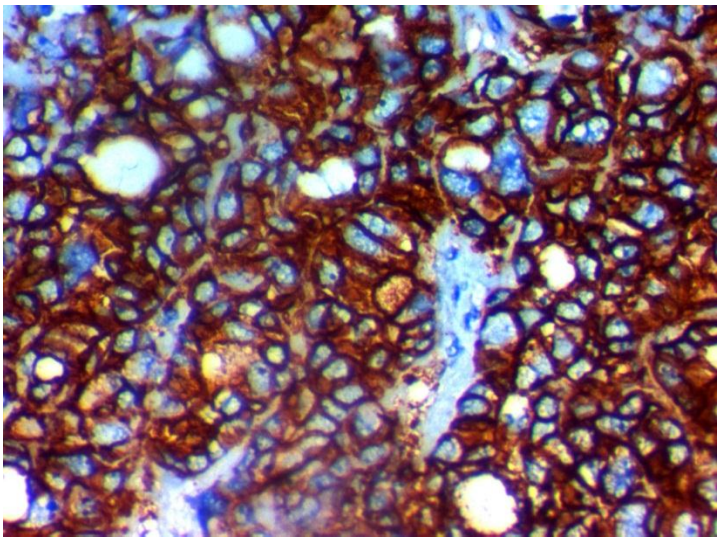

Fig 2 A

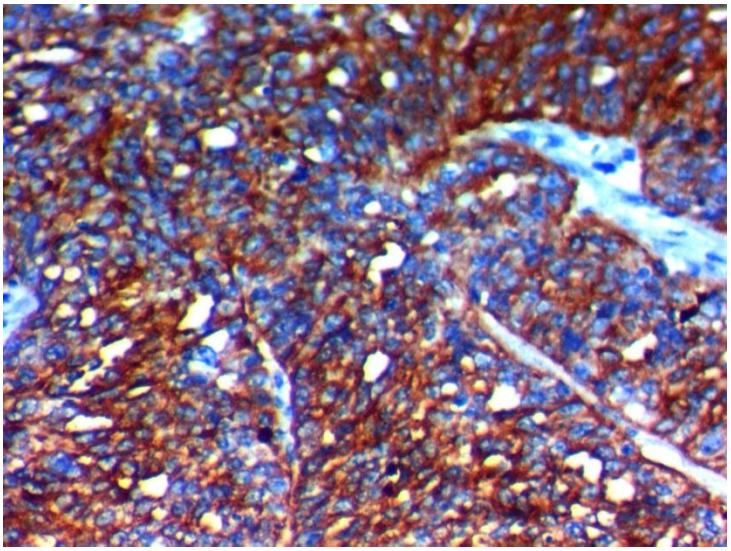

Fig 2 B

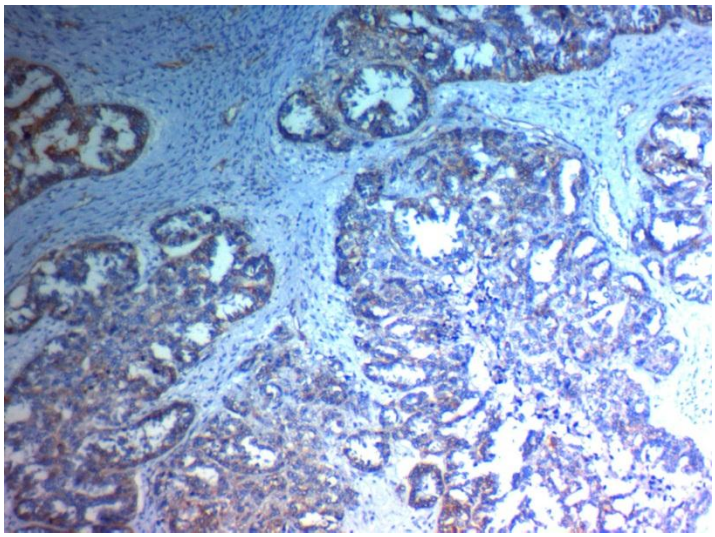

Fig 2 C

Figure2:-Immunohistochemical staining of Tau-protein in serous ovarian carcinoma (SOC): (A) High expression in the cytoplasm of low grade SOC stage IVx400. (B) High expression in the cytoplasm of low grade SOC stage III x400.) (c) low expression in the cytoplasm of low grade SOC stage IIx100

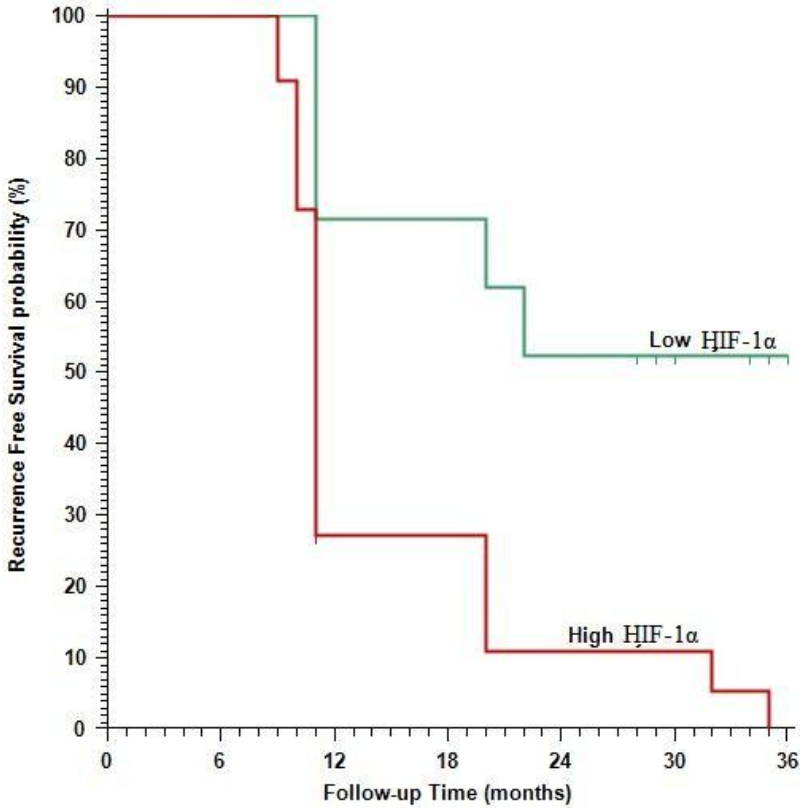

Fig 3 A

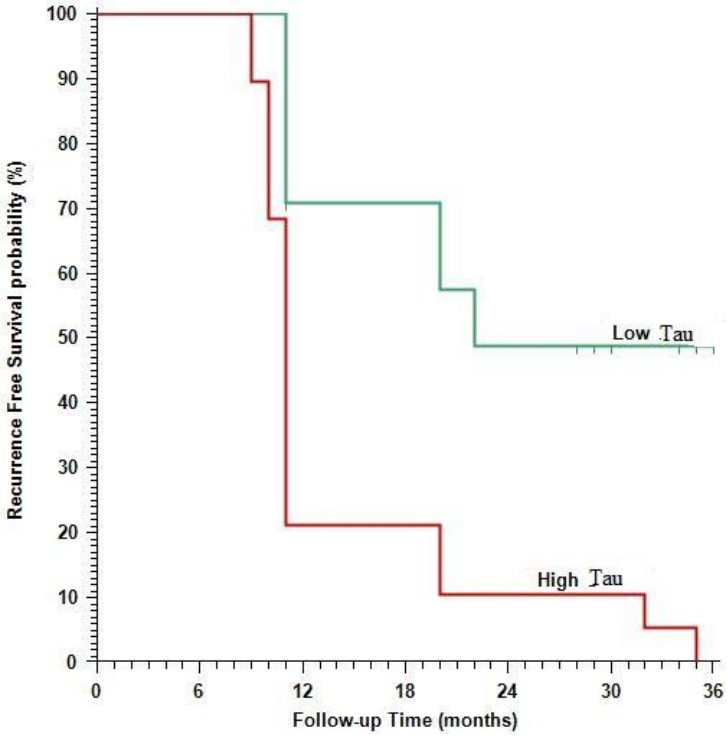
Recurrence Free Survival probability (%)
100
90
80
70
60
50
40
30
20
10
0
0
6
12
18
24
30
36
Follow-up Time (months)
Low Tau
High Tau

Fig 3 B

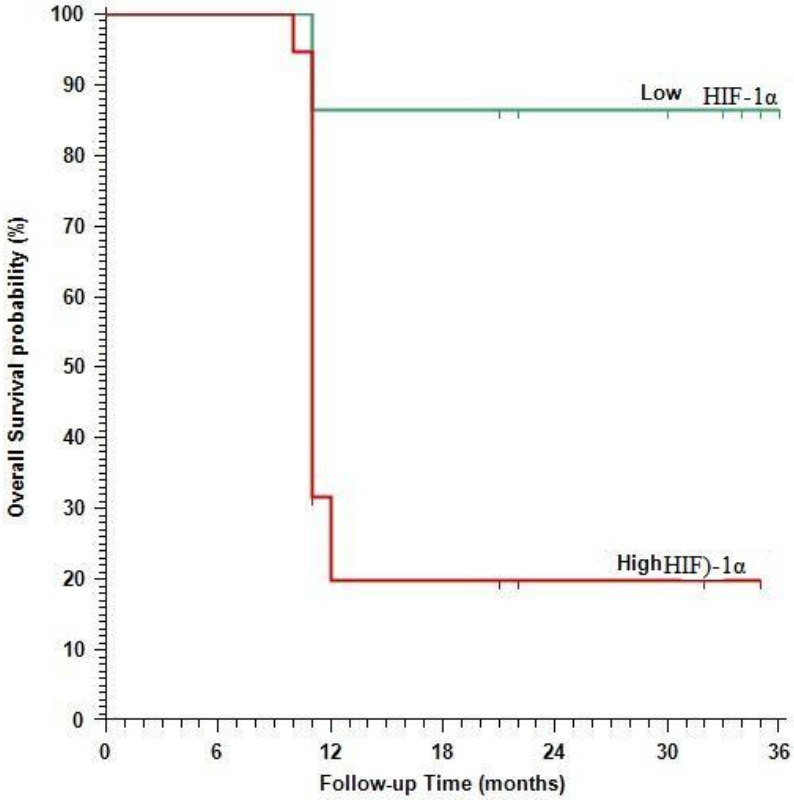
Overall Survival probability (%)
100
90
80
70
60
50
40
30
20
10
0
0
6
12
18
24
30
36
Follow-up Time (months)
Low HIF-1α
High HIF)-1α

Fig 3 C

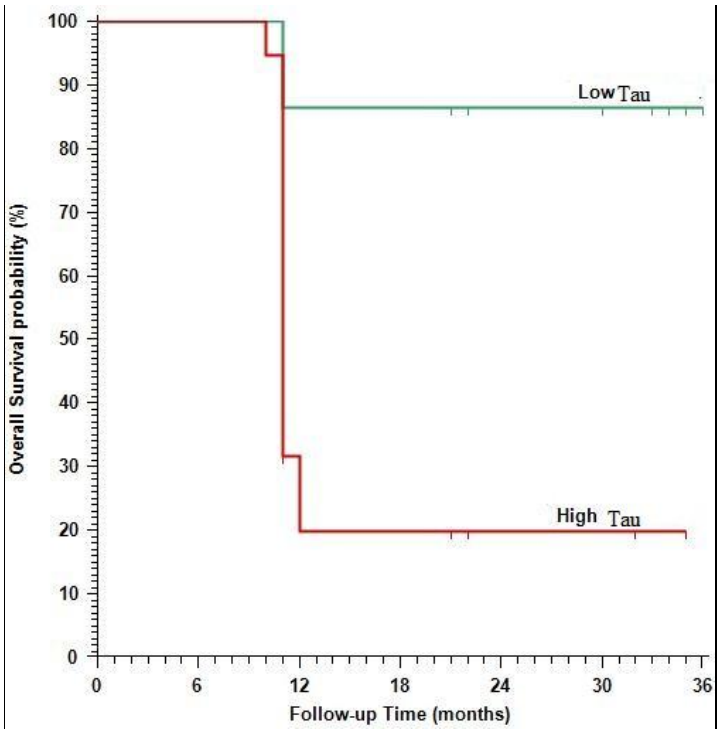

Fig 3 D

Figure 3:-Kaplan Meier Survival curves, of recurrence Free Survival & overall Survival; (A & C) Stratified by Hypoxia-Inducible Factor (HIF)-1α (B & C) Stratified by Tau-protein.

Discussion:-

Many previous studies tried to detect the roles of tissue hypoxia in malignant solid tumors in invasion, progression and resistance to chemotherapy in these tumors mainly SOC as it is a serious and fatal female malignancy [**16]**. We tried to assess severity of hypoxia in such tumor and its negative consequences on patients' outcome by evaluating the expression of the endogenous hypoxia related protein HIF-1α in SOC tissues and correlate its expression with clinical, pathological and prognostic parameters of patients**.** We have found that high expression of HIF-1α is associated with unfavorable pathological criteria e.g. high grade and advanced stage and adverse prognostic parameters e.g. resistance to chemotherapy, tumor recurrence after successive therapy and poor 3 year survival rates.

Similarly, **Osman et al., [15**] found that HIF-1α higher expression was related to large tumor size, multiplicity, bilaterality and advanced stage of the tumor, and **Aces et al., [17], who proved that** HIF-1α was overexpressed in advanced stages of SOC than early stages. Our results were explained by presence of larger areas of hypoxia in larger tumors.

Additionally, **Jie et al., [16] & Jin et al., [18]** have detected the association of HIF-1α higher expression levels with chemoresistance and dismal outcome in SOC patients and explained their results by that HIF-1α leads to induction of epithelial mesenchymal transition in SOC cells which leads to acquiring mesenchymal criteria and dismal outcome. Moreover, **Zheng et al., [19],** stated that HIF-1α is a negative prognostic parameter for hepatocellular carcinoma patients (HCC).

Shimogai R et al., [20], explained the association between higher HIF-1α and poor our come of SOC patients that such type of tumor has many solid areas and papillae which increased areas of hypoxia and hypoxic mediators in the tumor that lead to unfavorable prognostic parameters. So evaluation of HIF-1α expression could expect prognosis of patients with SOC.

In the current study we have proved the association of increased HIF-1α expression and resistance to chemotherapy which clarified the relation between increased hypoxic regions in solid tumors and more dismal outcome but in contrast to our results **Nakai et al.** [21], have proved more response to chemotherapy in patients that have increased HIF-1 α expression, moreover, **Birner et al. 2001**, have found no influence of HIF-1α expression on chemotherapy response in SOC patients. These discrepancies might be due to different evaluation method of HIF-1 expression, different clone of the antibody used different number of patients and follow-up period which yields different results [**22**& **21**], additionally, **Nakai et al**. [21], have used the western blot method for evaluation of HIF-1α expression and they have not considered the cytoplasmic non-functional HIF-1 α so they have overestimated the association between HIF-1α and therapy response.

We have found an association between increased HIF-1α expression and shorter 3 years survival rate similarly, **Nakai et al., [21],** have proved the similar association between increased HIF-1α expression and shorter 5 year survival rate found a shorter 5-year OS has been described in patients with high HIF-expression, while **Shimogai R et al., [20]** have found no association between HIF-1α expression and patients survival.

SOC is a fatal disease with high invasiveness and metastases power in addition to higher incidence of metastases which leads to dismal outcome. So, an essential step of its management is inhibition of its invasion, spread, resistance to chemotherapy and detection of novel therapeutic targets [**23]. Liu et al., [24],** have found the association between HIF- 1α increased expression and cancer progression by increasing invasion and metastases so targeted therapy against HIF- 1α could improve patients' prognosis and decreased chemoresistance especially in a fatal tumor like SOC.

Recent researchers have detected that HIF- 1α inhibitors are able to block malignant progression, and **Zhang et al., [25],** studied the role of digoxin as an HIF- 1α inhibitor which could suppress malignant growth by inhibiting synthesis of HIF-1α. **Zagzag et al. [26],** have clarified that geldanamycin inhibits glioma cells invasion and migration by antagonizing HIF-1α actions.

We have proved the association between Tau-protein overexpression, poor pathological and prognostic parameters in SOC patients in addition to resistance to platinum based chemotherapy and unfavorable survival rates.
Our results are similar to results of **Smoter et al. [15],** who proved that low Tau-protein levels are associated with good response to chemotherapy. The association between Tau-protein expression and chemosensitivity is explained by understanding mechanisms of paclitaxel's action as it is considered competitive to usual action of Tau protein, which means that Paclitaxel could be able to bind beta-tubulin on to inner surface of the microtubules, in the same site of Tau protein binding **[27],** so increased the levels of Tau protein on surfaces of the microtubules might lead to difficult paclitaxel attachment to them, interfere with its work on SOC cells.

Moreover low levels of Tau-protein expression will result in better connection of paclitaxel to the microtubules, more effective chemotherapy action, higher chemosensitivity and better survival rates[14].

The same results were detected in cancer breast in which low levels of Tau-protein is associated with mor complete response to paclitaxel bases chemotherapy, so Tau- protein inhibition will lead to enhancement of paclitaxel activity in such type of cancer [9]. **Tanaka et al. [28],** have proved the same results that breast cancer patients with Tau-protein negativity are more sensitive to paclitaxel than patients with Tau-protein negativity. Which proved our results regarding the predictive role of Tau-protein in SOC, additionally, **Wu et al.[29]** confirmed the predictive and prognostic role of low Tau-protein expression regarding sensitivity to paclitaxel based therapy in gastric cancer.

In cancer cells with low levels of Tau-expression paclitaxel has many roles as it increased the rate of apoptosis in those cells. Paclitaxel combines with tubulin easily in cases of low Tau protein expression levels while higher levels of Tau-protein leads to stabilization of the microtubules protecting SOC cells from the harmful paclitaxel effects, that leads to drug resistance. But as the mechanism of paclitaxel action on cancer cells is complicated so resistance mechanisms to paclitaxel needs further studies [30].

Similarly, Abd elaziz et al., [31] proved the same association of Tau-protein and unfavorable outcome of SOC patients. In contrast to our results, **Steffensen *et al.*, [32], have** reported that levels of Tau-protein expression were not associated with survival **rates.** Different results also were found by **Shao et al., [33],** that increased Tau-protein is a good prognostic marker in breast cancer patients but they did not explained their results but this variable role

might be due to different work of Tau-protein action in different cancer type. These conflicting results about the prognostic roles of Tau-protein expression in SOC needs further studies to prove our results.

As we have studied both HIF-1 α and Tau-protein expression in SOC and we have found significant positive association between expression of them and their combined expression was related to unfavorable criteria, these results point to the association between Tau-protein related chemotherapy resistance and higher levels of hypoxia in solid tumors like SOC. Our study was the first to explain the relation between both HIF-1 α and Tau-protein expression in SOC and patients' prognosis, so further studies are needed to prove our results.

Summary& Conclusion:-

HIF-1α that is a marker of tumor hypoxia is overexpressed in SOC tissues and is related to unfavorable outcome of patients. Tau-protein is related to resistance to chemotherapy in SOC patients that leads to short survival rates. We have found positive association between both markers so targeted therapy against them could improve SOC patients' prognosis.

Recommendations:-

Further prospective studies on larger number of patients are needed to prove our results.

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