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Original article

Significance of expression of PD-L1, VEGF and CD8+ T cells in ovarian serous carcinoma: an Immunohistochemical study

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Abstract:

Background: Ovarian cancer is a lethal gynecological malignancy worldwide. The immune system plays a key role in preventing tumor development. Angiogenesis and immune evasion are vital for ovarian cancer progression and metastasis. **Aim**: This work aimed to evaluate the expression of PD-L1, VEGF and CD8 +T cells in ovarian serous carcinomas and correlate them with clinic-pathological variables.–**Material and methods**: An Immunohistochemical study of PD-L1, VEGF and CD8+ T cells was performed on 50 cases of ovarian serous carcinomas.-**Results**: PD-L1 & VEGF were over-expressed with statistical significance , in higher grade (P <.01), advanced stage , case with lymph node metastasis and distant metastasis(P <.05) .Higher CD8+ T cells in epithelium and stroma was related to advanced stage, only intraepithelial CD8+ T cells was directly correlated with tumor grade & lymph node metastasis & stage (P <.05). Positive correlation between PD-L1 & VEGF and Intraepithelial CD8+T cells was detected (r=.048)–**Conclusion**: Neoplastic cells in ovarian serous carcinomas- expressing PD-L1 and VEGF along with intraepithelial CD8+T cells- might expect poor prognosis. An association between PD-L1 and VEGF could predict their role in immune evasion. Administration of immunotherapy (anti PD-L1) with antiangiogenic agents might increase antitumor activity. To enhance our knowledge about the immunological environment of epithelial ovarian tumors, larger-scale studies are advised.

Keywords: PD-L1, VEGF and CD8+T cells.

Introduction:

Ovarian cancer represents the ninth[th] cancer in order of frequency among females all over the world, and it is the Fourth most common cause of cancer-related deaths in women **(1).** In EGYPT, ovarian cancer represents 4.1% of malignant tumors affecting females **(2).**

The majority of ovarian cancer arises from surface epithelium. Epithelial ovarian cancer (EOC) cannot be confined to a single entity owing to its diverse neoplastic subtypes with distinctive clinical, genetic and pathological characteristics **(3).** This ovarian cancer is classified into two types; I and II, having different prognostic and predictive value. Type I, representing approximately 30% of cancers, is slow-growing, typically and genetically stable comprising low-grade serous, endometrioid, clear-cell, and mucinous carcinomas. Type II represents 70% and is more aggressive, genetically unstable and includes high-grade serous carcinomas **(4).**

The silent nature of early-stage disease and lack of screening possibilities contribute to the delay of diagnosis. Another main factor worsening prognosis is the frequent development of chemo resistance **(5).** Therefore, there is a need for novel, more targeted therapies for those patients.

The immune system; play a key role in suspension of tumor development **(6).** Epithelial ovarian cancer (EOC) has a distinctive relationship with the immune system, due to its location in the peritoneal cavity, and the characteristic features of the tumor microenvironment **(7).**

The tumor microenvironment (TME) in ovarian cancer is a complicated system comprised of immune cells (B and T lymphocytes), fibroblasts & multiple chemokines and cytokines **(8).** The complex and dynamic interactions between such factors, and the tumor cells, exerts either antitumor or protumor effect **(9).**

The tumor microenvironment (TME) displays a complex medium of organized physiological processes that contribute to the progression and metastasis of tumors, including tumor angiogenesis and immune suppression **(10).**

The immune evasion of ovarian cancer cells is promoted by both PD-L1 and PD-L2. Programmed cell death-ligand 1 (PD-L1) is an immune checkpoint molecule expressed on tumor cells and tumor-infiltrating immune cells, act as a major coinhibitory factor , by binding to its receptor, programmed death 1 (PD-1) on lymphocytes, involved in the suppression of cancer immunity **(11).** Many studies showed that PD-L1 played tumor-intrinsic roles in cancer progression and chemoresistance via participating in some oncogenic pathways **(12).**

The angiogenic mediator that has been extensively studied and markedly expressed on ovarian cancer cells is the vascular endothelial growth factor (VEGF) **(13).** The VEGF family is composed of five members that promote proliferation, survival and migration of endothelial cells and is essential for blood vessel formation **(7).** The VEGF has been reported to exert not only

tumor angiogenesis but also immunosuppressive activity **(14).**

The CD4 + and CD8 + T cells, natural killer T lymphocytes, dendritic cells, CD3 + T-lymphocytes and CD20 + B-lymphocytes- are involved in the antitumor immune response. These cells directly participate in the presentation of tumor antigens and/or the attack of tumor cells **(15).**

The recent emergence of immunotherapy, specifically immune checkpoint inhibitors, had increased interest towards novel therapeutic interventions **(16).** This insight into the role of angiogenesis led to the establishment of several treatment methods targeting the VEGF pathway.

The aim of this work is to evaluate the expression of VEGF, PD-L1 and CD8+ T cells- in ovarian serous carcinomas and correlate their expression with different clinico pathological variables to clarify their actions hoping to find new prognostic and therapeutic options of ovarian serous carcinomas.

Material and methods:

Study Groups:

This is a retrospective study, performed on formalin fixed paraffin embedded biopsy specimens of selected 50 cases of serous ovarian carcinoma categorized as; 20 cases of low grade serous ovarian carcinoma and 30 cases of high grade. Cases were collected from files and archives of Pathology department & Early Cancer Detection Unit and department of Obstetrics and Gynecology, Faculty of Medicine, Benha University, during the period from January 2018 to December 2022. The study was approved by the Ethical Committee of faculty of medicine, Benha University (M.S .6.4.2023).

The control cases were 10 cases of benign ovarian cysts. The specimens were 20 oophorectomies and 30 were hysterectomy with bilateral salpingo-opherectomy, omentectomy & peritoneal washing with cytology.

Inclusion criteria: availability of clinicopathological data retrieved from the patients' files included age, laterality, presence or absence of ascites & peritoneal implants, histopathological grade, lymph node metastasis (N), distant metastasis (M), and the FIGO 2009 staging system.

Exclusion criteria: Serous ovarian carcinoma cases with history of chemotherapy and cases with no available paraffin blocks or clinicopathological data- were excluded from the current study.

A-Histopathological examination:

Formalin fixed /Paraffin embedded blocks were cut at 5 μm thickness and stained using hematoxylin and eosin stain. Two observers reviewed the microscopic sections from all the cases, unaware of their diagnosis.

The ovarian serous carcinoma cases were graded into low and high grades **(17).** Patients were staged using the International Federation of Gynaecology and Obstetrics (FIGO) 2009 staging system and were further divided into early stage (I–II) and advanced stage (III–IV) for the purpose of statistical analysis **(18).**

***B-Immunohistochemical Procedure*:**

For immunohistochemical analysis, streptavidin-biotin technique was used following the manufacturer's instructions (***Neomarker, LABVISION, USA*, CA 94538-7310***)*. The sections were stained with 0.02% diaminobenzidine (DAB) solution- as chromogen. Finally, sections were counterstained with hematoxylin then dehydrated and mounted. Negative controls were performed by omitting the primary antibody; positive controls were added as shown in Table (1)**.**

***Immunohistochemical assessment: of PD-L1, VEGF and CD8+ T cells expression within the studied cases*:**

The percentages of positive cells of **PD-L1** and **VEGF** were scored as mentioned by previous study **[(18)].** As regard **CD8**, each slide was screened for a hotspot of CD8+ TILs at ×20 power, within each hotspot; one high power field (HPF) at × 400 magnifications was evaluated to ensure valid equally comparable areas and scored following previous study **[(19)].**

Statistical analysis:

Results were analyzed using IBM SPSS Statistics for Windows, Version 22.0 **(SPSS Inc., Chicago, IL, USA**. *P* >.05 is non-significant, *P* <.05 is significant and *P* ≤ .01 is highly significant.

Results:

***Clinicopathological features of the studied cases*:**

The age of studied cases ranged from 39 to 80 years old with the mean age 41±11.7 years. The clinicopathological variables are listed in Table (2).

***Immunohistochemical expression of PD-L1, VEGF and CD8+ T cells and their relation with the clinicopathological features in the studied cases*:**

PD-L1 was positively showed cytoplasmic and membranous staining in tumor cells in 50% of the cancer cases in comparison to control ones. PD-L1 expression was higher among patients with higher grade (*P* <.01) as shown in Figure (1: A, B), advanced stage and cases with positive lymph node metastasis (*P* <.05). There was no statistically significant correlation between PD-L1 and other clinicopathological parameters in the studied cases (*P* >.05) as illustrated in Table (2)**.**

As shown in Table (2), among 50 cases of ovarian serous carcinoma, 54% stained positively for VEGF. Over-expression of VEGF in the cytoplasm of tumor cells was closely related to higher grade (*P* <.01) as shown in Figure (1: C& D), advanced stage (*P* <.01), presence of peritoneal implants (*P* <.05) ,positive lymph node metastasis (*P* <.05) and distant metastasis (*P* <.05) with statistically significant positive correlation.

CD8+ was detected as brown staining in the cytoplasm of tumor infiltrating lymphocytes. Assessments of CD8+ T cell infiltration revealed their presence in the epithelial and stromal regions of tumors Figure (2**:** C). Tumors with advanced stage have a relatively higher number of CD8+ T cells, irrespective of their location (*P* <.05). Moreover, there is a statistically significant positive correlation between the

intraepithelial CD8+ T cells and histological grade Figure (2: A, B) & lymph node metastasis and tumor stage (P <.05) as shown in Table (2).

Correlation between PD-L1, VEGF, intraepithelial CD8+ T cells and stromal CD8+ T cells

PD-L1, VEGF and intraepithelial CD8+T cells were identified to be closely related by Spearman correlation, as there was a significant positive correlation between PD-L1 and VEGF in ovarian serous carcinoma cases (r=.048). Additionally, intraepithelial CD8+T cells was closely related to PD-L1 and VEGF in the studied cases (r=0.022 and r= 0.000) respectively. But, there was insignificant statistical correlation between stromal CD8+T cells and either VEGF, PD-L1 or intraepithelial CD8+T cells (r=0.275, r=0.580 and r=0.422) respectively as illustrated in Table (3)

Table (1): Data for using PD-L1, VEGF and CD8 antibodies

Antibody	Type	Cat.No.	Dilution	Positive control	incubation	Antigen retrieval
PD-L1	**Rabbit Polyclonal antibody**	**Thermo Fisher Scientific , Cat. No. PA5-28115**	**1:100**	**Lung Placenta**	**15 minutes**	**Citrate buffer PH 6.0**
VEGF	**Mouse Monoclonal antibody**	**Thermo Fisher Scientific, Cat. No. , MA1-16629**	**1:100**	**angiosarcoma**	**30 minutes**	**Citrate buffer PH 6.0**
CD 8	**Mouse Monoclonal antibody**	**Thermo Fisher Scientific, Cat. No 14-0081-82**	**1:50**	**Spleen and thymus**	**30 minutes**	**Citrate buffer PH 7.2**

PDL1: Programmed Death Ligand 1, VEGF: Vascular Endothelial Growth Factor

Table (2): Relation between the immunohistochemical expression of PD-L1, VEGF, intraepithelial CD8+ T cells & stromal CD8+ T cells and clinicopathological features of the studied cases:

Clinicopathological features		PD-L1			VEGF			Intraepithelial CD8+T cells			Stromal CD8+T cells			Total
		-ve	+ve	P	-ve	+ve	P	Low	High	P	Low	High	P	
Grade	Low	16 (80%)	4 (20%)	** <0.01	14 (70%)	6 (30%)	** <0.01	12 (60%)	8 (40%)	* <0.05	14 (70%)	6 (30%)	>0.05	20 (40%)
	High	9 (30%)	21 (70%)		9 (30%)	21 (70%)		10 (33%)	20 (67%)		13 (43%)	17 (57%)		30 (60%)
Laterality	Uni-lateral	15 (48%)	16 (52%)	>0.05	13 (42%)	18 (58%)	>0.05	13 (42%)	18 (58%)	>0.05	16 (52%)	15 (48%)	>0.05	31 (62%)
	Bi-lateral	10 (53%)	9 (47%)		10 (53%)	9 (47%)		9 (47%)	10 (53%)		11 (58%)	8 (42%)		19 (38%)
Ascites	No	10 (53%)	9 (47%)	>0.05	10 (53%)	9 (47%)	>0.05	11 (58%)	8 (42%)	>0.05	11 (58%)	8 (42%)	>0.05	19 (38%)
	Yes	15 (48%)	16 (52%)		13 (42%)	18 (58%)		11 (35%)	20 (65%)		16 (52%)	15 (48%)		31 (62%)
Peritoneal implants	No	19 (59%)	13 (41%)	>0.05	19 (59%)	13 (41%)	* <0.05	16 (50%)	16 (50%)	>0.05	17 (53%)	15 (47%)	>0.05	32 (64%)
	Yes	6 (33%)	12 (67%)		4 (22%)	14 (78%)		6 (33%)	12 (67%)		7 (39%)	11 (61%)		18 (36%)
LN metastasis (N)	N0	20 (63%)	12 (37%)	* <0.05	18 (56%)	14 (44%)	* <0.05	18 (56%)	14 (44%)	* <0.05	19 (59%)	13 (41%)		32 (64%)
	N1	5 (28%)	13 (72%)		5 (28%)	13 (72%)		4 (22%)	14 (78%)		8 (44%)	10 (56%)	>0.05	18 (36%)
Distant metastasis (M)	M0	19 (56%)	15 (44%)	>0.05	19 (56%)	15 (44%)	* <0.05	14 (41%)	20 (59%)	>0.05	19 (56%)	15 (44%)		34 (68%)
	M1	6 (37%)	10 (63%)		4 (25%)	12 (75%)		8 (50%)	8 (50%)		5 (31%)	11 (69%)	>0.05	16 (32%)
FIGO stage	Stage I+II	14 (67%)	7 (33%)	* <0.05	18 (86%)	3 (14%)	** <0.01	13 (62%)	8 (38%)	* <0.05	14 (67%)	7 (33%)	* <0.05	21 (42%)
	Stage III-IV	11 (38%)	18 (62%)		5 (17%)	24 (83%)		9 (31%)	20 (69%)		10 (35%)	19 (65%)		29 (58%)
Total		25 (50%)	25 (50%)	-	23 (46%)	27 (54%)	-	24 (48%)	26 (52%)	-	24 (48%)	26 (52%)	-	50

PDL1: Programmed Death Ligand 1, VEGF: Vascular Endothelial Growth Factor, LN: Lymph node

*Significant , **Highly significant

Table (3): Correlation between PD-L1, VEGF, intraepithelial CD8+ T cells and stromal CD8+ T cells expressions in the studied cases:

Markers	NO	Expression	PD-L1		VEGF		Intraepithelial CD8+ T cells		Stromal CD8+ T cells	
PD-L1	**25**	**-ve**	**-ve**	**+ve**	**-ve**	**+ve**	**Low**	**High**	**Low**	**High**
					15 (60%)	**10 (40%)**	**15 (60%)**	**10 (40%)**	**11 (44%)**	**14 (56%)**
	25	**+ve**			**8 (32%)**	**17 (68%)**	**7 (28%)**	**18 (72%)**	**13 (52%)**	**12 (48%)**
Spearman p					**r=0.048***		**r=0.022***		**r=0.580**	
VEGF	**23**	**-ve**	**15 (65%)**	**8 (35%)**			**16 (70%)**	**7 (30%)**	**13 (56%)**	**10 (44%)**
	27	**+ve**	**10 (37%)**	**17 (63%)**			**6 (22%)**	**21 (78%)**	**11 (41%)**	**16 (59%)**
Spearman p			**r=0.048***		**-**		**r=0.000****		**r=0.275**	
Intraepithelial CD8+ T cells	**22**	**Low**	**15 (68%)**	**7 (32%)**	**16 (73%**	**6 (27%)**			**12 (55%)**	**10 (45%)**
	28	**High**	**10 (36%)**	**18 (64%)**	**7 (25%)**	**21 (75 %)**			**12 (43%)**	**16 (57%)**
Spearman p			**r=022***		**r=0.000****		**-**		**r=0.422**	
Stromal CD8+ T cells	**24**	**Low**	**11 (46%)**	**13 (54%)**	**13 (54%)**	**11 (46%)**	**12 (50%)**	**12 (50%)**		
	26	**High**	**14 (54%)**	**12 (46%)**	**10 (38%)**	**16 (62%)**	**10 (44%)**	**16 (56%)**		
Spearman p			**r=0.580**		**r=0.275**		**r=0.422**		**-**	
Total	**50**		**25**	**25**	**23**	**27**	**22**	**28**	**24**	**26**

PDL1 :Programmed Death Ligand 1 ,VEGF :Vascular Endothelial Growth Factor , *Significant , **Highly significant

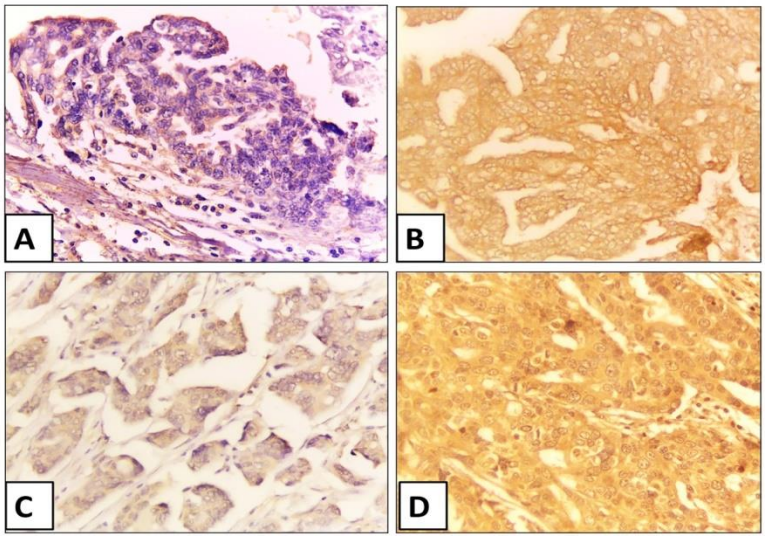

Figure (1): PD-L1 and VEGF immunohistochemical expression in ovarian serous carcinoma. **A:** Low grade serous carcinoma showing weak focal cytoplasmic expression of PD-L1 (IHC x 400)., **B:** High grade serous carcinoma showing strong diffuse membranous and cytoplasmic expression of PD-L1 (IHC × 400)., **C**: Low grade serous carcinoma showing weak focal cytoplasmic expression of VEGF (IHCx400). **D:** High grade serous carcinoma showing strong diffuse cytoplasmic expression of VEGF (IHC × 400).

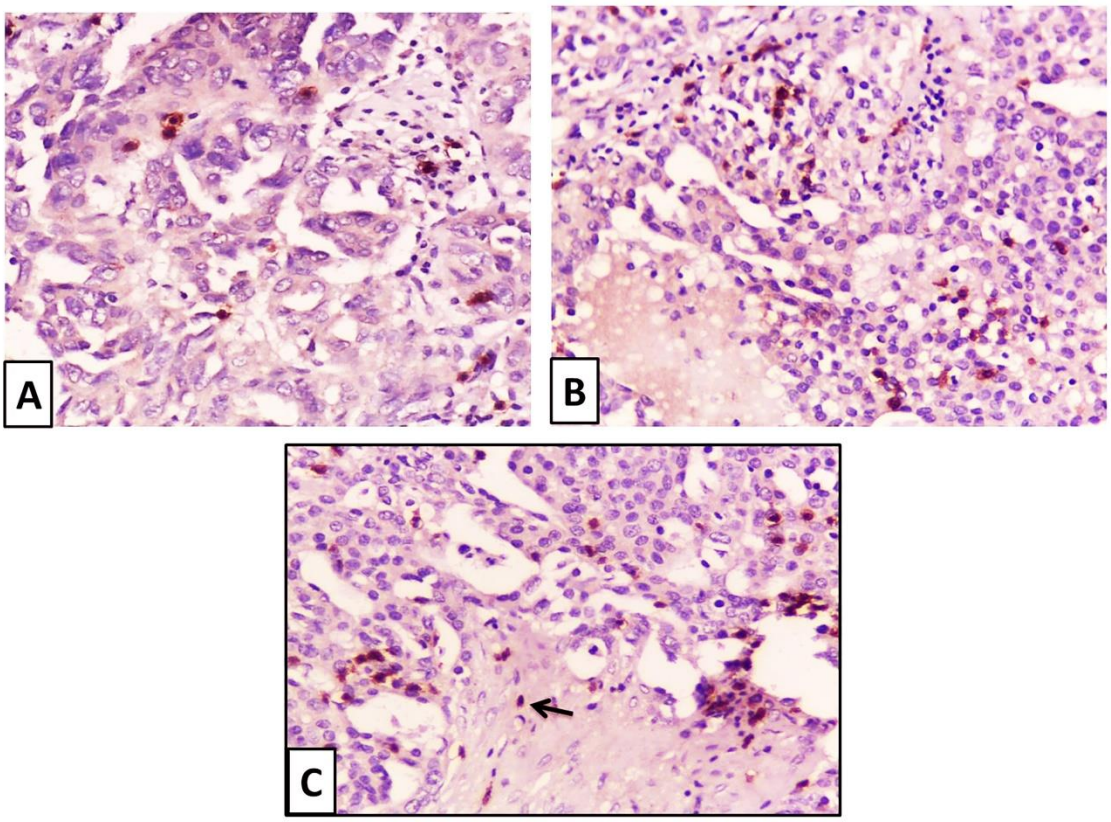

Figure 2: CD8 + T lymphocytes in both epithelial and stromal component in ovarian serous **carcinoma. A:** Low grade serous carcinoma showing low cytoplasmic expression of intraepithelial CD8+T cells (A, IHCx400). **B:** High grade serous carcinoma showing high expression of intraepithelial CD8 in tumor infiltrating lymphocytes (IHCx400) .**C**: high grade serous carcinoma showing both intraepithelial and stromal CD8 + in TILs (arrow) (IHCx400)

Discussion:

Ovarian cancer is considered one of the most aggressive gynecological malignancies worldwide. The immune system plays a key role in suspension of tumor development [(20)]**.** In the current study, PD-L1 was expressed in tumor cells in 50% of cases of serous ovarian carcinoma in relation to benign cases. Previous investigation was in agreement with ours [(21)] explaining the overexpression of PD-L1 by the fact that T-cell receptor sensitization and interferon gamma (IFN-γ) secretion within the tumor microenvironment, leads to expression of PD-1 receptor and its corresponding ligand, PD-L1, on the surfaces of the tumor cell and antigen-presenting cell.

There was a statistically significant direct correlation between PD-L1 expression and tumor grade (P <.01), lymph node metastasis and tumor stage (P <.05). PD-L1 expression was highly associated with more

aggressive cancers, those results were concluded by previous analysis [9,17, ,21].

PD-L1, a co-inhibitory factor that is expressed on many types of cancer cells, by binding to its receptor, PD-1 on lymphocytes, PD-L1 induces T cell energy and their elimination via apoptosis or by transmitting signal that inhibits lymphocyte activation to promote tumor cell growth, proliferation and metastasis [17].

Concomitant with the result of our study, PD-L1 might play an important role in ovarian cancer progression; it could act as a predictor for tumor aggressiveness and hence poor prognosis.

Previous study was in contrast to ours [22] found that PD-L1 expression was not correlated with tumor grade and clinical stage. This contradiction is probably due to the lack of approved system of immunohistochemical evaluation, diverse cut off values, and variegated expression patterns of PD-L1 across distinct cell types, such as stroma cells, epithelium, and macrophages.

VEGF plays a pivotal role in tumor angiogenesis by promoting proliferation, survival and migration of endothelial cells [23].

In this study, there was a significant statistical correlation between VEGF expression and tumor grade, stage (P <.01), the presence of peritoneal implants, lymph node metastasis and distant metastasis (P <.05). Previous studies [18, 24] were in agreement with our finding which found that upregulated VEGF in ovarian cancer is an indicative of higher stage, grade and exacerbated tumour progression. These results suggest that over-expression of VEGF is associated with more aggressive ovarian cancers.

VEGF modulates immunosuppression directly and indirectly via its impact on cells responsible for mediating innate and adaptive immune response. Tumor-associated macrophages was reprogrammed from the M1 subtype with anticancer activity to a pro-humoral M2 phenotype, inhibition of dendritic cell maturation which affect the presentation of antigens and activation of cytotoxic CD8+ cells, with excessive proliferation of atypical endothelial cells exhibiting an immunosuppressive phenotype [10] .

Tumor angiogenesis has been supposed to induce tumor progression and metastasis, thus antiangiogenic therapy can play a major role in reversing the adverse effects of VEGF both in angiogenesis and immune suppression and it can be regarded as a treatment option for patients with ovarian cancer.

In the current study, there was a statistically significant positive correlation between VEGF and PD-L1 (r= 0.048) agreeing with previous study [7], which found a synergistic action between anti VEGF and anti PDL1. In the same line, previous study [25] found that VEGF induced angiopoietin 2 (ANGPT2) can upregulate PDL1 expression on M2 polarized macrophages. PD-L1 directly interacts with vascular endothelial growth factor receptor-2 (VEGFR2) and then activates the FAK/AKT pathway, which further induces angiogenesis and tumor progression [21].

PD-L1 up regulation can be motivated by cytokines induced by tumor-infiltrating immune cells, including interferon (IFN), tumor necrosis factor (TNF alpha), interleukin (IL-4), and vascular endothelial growth factor (VEGF) [26].

The biological synergy observed between angiogenesis and tumor-related immune responses has encouraged many studies into the efficacy of combining antiangiogenic therapy and immunotherapy in a range of solid tumors, including melanoma [27] and renal cell carcinoma [28]. This suggests that the concurrent administration of immunotherapy comprising of cancer vaccine, adoptive cell therapy, a PD-1 or PD-L1 inhibitor, along with antiangiogenic agents might have more pronounced antitumor efficacy in patients with ovarian serous carcinoma compared to the administration of any of these therapeutic modalities alone.

Tumor-infiltrating lymphocyte (TILs) that may be present in the TME include CD4+T-helper cells CD8+cytotoxic T-lymphocytes. CD8+ T cells would normally have antitumor activity [29].

Regarding the expression of intraepithelial CD8+ T cells in the current work, there was a significant correlation between intraepithelial CD8+ T cells expression and tumor grade, lymph node metastasis and tumor stage (P <.05). These results were agreeing with previous scientific researches [9,30] which found that intra-tumoral CD8 + T lymphocyte infiltration was associated with advanced stage, high grade, and metastasis in the ovarian cancer. Therefore, it was advocated to adversely affect the antitumor immune response.

This suggests that the infiltrated T cells could promote the development of ovarian cancer, and provide another mechanism of immune evasion mediated by T cells. This supports the hypothesis of previous study [31] concluding that tumour-infiltrating lymphocytes may promote the migration and invasion of ovarian carcinomas though metastasis-related genes.

In contrast to ours, previous study [32] stated that the infiltration of CD8+ cytotoxic T cells was associated with a better prognosis in EOC patients.

So, there is a controversy over the role of CD8 in the prognosis of ovarian cancer. Ovarian cancer tumor microenvironment is highly immunosuppression protecting the tumor from the body's defensive immune cells, and facilitating tumor progression [7]. The CD8 +T cells would normally have an antitumor activity. However, their functions are suppressed by the up regulation of immune checkpoint molecules such as PD-1 that negatively affect the function of the CD8 + T lymphocytes, greatly attenuating their cytotoxicity towards the tumor cells.

As regard the expression of stromal CD8, there was a significant correlation between stromal CD8 expression and the tumor stage (P <.05). This is consistent with previous study [9] which found that higher stage cancers could have a relatively higher number of CD8+ T cells, irrespective of their location. Additionally, one study [20] found that the presence of stromal CD8 + T

lymphocytes was more common in late stage patients than in the early stage and that there was no relationship between the proliferation of stromal CD8 T-lymphocytes and other clinicopathological variables.

As regard correlation between PD-L1, VEGF, intraepithelial CD8+ T Cells and stromal CD8+ T cells- there was a significant statistical positive correlation between intraepithelial CD8 +T cells , VEGF and PDL1 (r= 0.000 and r=0.022 ,respectively). This is in agreement with previous study [(9)], which found that expression of VEGF was enhanced by cancer cells following co-culturing with CD8+ T cells via up regulation of IL-8 and IL-10. VEGF modulates the innate and adaptive immune response through its interaction with immune cells, in turn; immunosuppressive immune cells can produce proangiogenic factors and promote angiogenesis, creating a positive feedback loop [(31)].

Also, previous investigation [(17)] found that infiltration of T cells promotes the metastasis of ovarian cancer cells via the modulation of metastasis related genes and PD-L1 expression. Moreover, A previous study [(33)] revealed a positive relationship between PD-L1 expression and the presence of tumor-infiltrating regulatory T cells and/or lymphocytes with the CD8+ phenotype.

On the contrary, previous study [(34)] found an inverse correlation observed between CD8+ T lymphocyte levels and PD-L1 expression on cancer cells. This discrepancy is probably due to lack of approved system of immunohistochemical evaluation and altered cut off values.

Concerning the stromal CD8+ T cells, there was insignificant statistical correlation between stromal CD8+ T cells and other markers: PD-L1, VEGF and intraepithelial CD8+ T cells (r=0.580, r=0.275 and r=0.422, respectively).

Notably, the CD8+ T cells that localize in the epithelium of ovarian cancer, rather than in the stroma, have been identified and serve as a prognostic factor. Parallel with this, a previous study [(32)] found that a stronger correlation has been reported when tumour infiltrating lymphocytes (TILs), especially CD8+, are located within the neoplastic epithelium rather than stromal TILs.

Thus, to improve our current knowledge about the immunological environment of epithelial ovarian tumors, specific immune cells need to be further investigated in large scale study especially with the development of synthetic biology techniques called adoptive cell therapy which is a promising strategy for the treatment of cancer, which utilizes the cells of the immune system to eliminate cancer [(35)].

Conclusions:

This study concluded that PD-L1, VEGF and intraepithelial CD8+ T cells- were suggested to predict tumor aggressiveness and hence poor prognosis in serous ovarian carcinoma. An association was noticed between PD-L1 and VEGF in tumor cells predicting their role in immune evasion.

Hence, administration of immunotherapy (anti PD-L1) in combination with antiangiogenic agents (anti VEGF) could be more effective strategies that may increase antitumor activity.

Recommendation:

Further studies using different molecular methods on PD-L1 and VEGF- are recommended to explore more about the mechanisms by which they may contribute to the progression of ovarian serous carcinoma. Additionally, a large scale study on the prognostic role of CD8+T cells in ovarian serous carcinoma- is recommended.

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