Al-Esraa University College Journal for Medical Sciences

Volume 5 | Issue 7 Article 8

September 2024

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Yasir Naeem Khlaif
Medical Laboratory Techniques Department, College of Health and Medical Techniques, Middle Technical University, Baghdad, Iraq, edc0100@mtu.edu.iq

Mayada Noori Iqbal
Medical Laboratory Techniques Department, College of Health and Medical Techniques, Middle Technical University, Baghdad, Iraq

Hind Jaber Hassoon
Medical Laboratory Techniques Department, College of Health and Medical Techniques, Middle Technical University, Baghdad, Iraq

Recommended Citation

Khlaif, Yasir Naeem; Iqbal, Mayada Noori; and Hassoon, Hind Jaber (2024) "Estimation of the Immunological Markers (CTLA4 and VEGF) of the Adjuvant Ovarian Cancer in Iraqi Women Patients," *Al-Esraa University College Journal for Medical Sciences*: Vol. 5: Iss. 7, Article 8.
DOI: https://doi.org/10.70080/2790-7937.1007

AL-ESRAA UNIVERSITY COLLEGE JOURNAL OF MEDICAL SCIENCES 2024;5(7):62–69

RESEARCH ARTICLE

Estimation of the Immunological Markers (CTLA4 and VEGF) of the Adjuvant Ovarian Cancer in Iraqi Women Patients

Yasir Naeem Khlaif *, Mayada Noori Iqbal, Hind Jaber Hassoon

Medical Laboratory Techniques Department, College of Health and Medical Techniques, Middle Technical University, Baghdad, Iraq

ABSTRACT

Background: The most frequent cause of death for females with gynecological cancer diagnoses is ovarian cancer (OC). Furthermore, generally speaking, it ranks as the 5th most frequent cause of death for females. The majority of cases had advanced diagnoses, which worsens the disease's prognosis. **Objectives:** The aims of the study is to Estimate the immunological markers "cytotoxic T lymphocyte associated antigen 4" (CTLA4) and "Vascular Endothelial Growth Factor" (VEGF) in Adjuvant women (OC). **Materials and methods:** 90 Adjuvant women OC were included in this study (30 of them were taking (1-6) dose of biotherapy Bevacizumab (Avastin) Group 1 (G1), 30 of them were taking (>6) dose of avastin Group 2 (G2), and the last 30 taking chemotherapy Group 3 (G3) in addition to 40were healthy used as control group. The "Quantitative measurement by Enzyme-linked immunosorbent assay" (ELISA) of human were used to estimate the immunological marker (CTLA4 and VEGF). **Results:** High significance difference at ($P = 0.001$) between the means age and occupation of OC studied groups while no significant difference at (P value = 0.4 and 0.3) between the means of residency and body mass index (BMI) in studied groups. However, the significant difference at (P value = 0.04) between the mean of family history in studied groups. High significant difference between studied groups and the control group for CTLA4 and VEGF at (P value < 0.001) also in Receiver operative characteristic Curve (ROC) The presence of CTLA4 and VEGF were highly significant difference of all studied groups (G1, G2 and G3). with elevated of sensitivity and specificity of CTLA4 (80 and 86.67 in G1, 76.6 and 86.6 in G2 and 80 and 86.67 in G3 respectively), Also higher sensitivity and specificity of VEGF (60 and 93.9 in G1, 80 and 100 in G2, and 73.3 and 100 in G3 respectively), also the correlation between levels of CTLA4 with VEGF were highly significance in G1 and G2, but no significant correlations in G3 at ($P = 0.0001$, 0.0003 and 0.088 respectively). **Conclusion:** The studied parameters may play an important role for the evaluation of therapeutic response on adjuvant OC women patients.

Keywords: Ovarian cancer, CTLA4, VEGF, Biotherapy, Avastin, Chemotherapy

Received 20 June 2024; accepted 20 July 2024.
Available online 3 September 2024

* Corresponding author.
E-mail address: edc0100@mtu.edu.iq (Y. Naeem Khlaif).

https://doi.org/10.70080/2790-7937.1007

1. Introduction

The most frequent cause of death for females with gynecological cancer diagnoses is ovarian cancer (OC). Furthermore, generally speaking, it ranks as the 5th most frequent cause of death for females. The majority of cases had advanced diagnoses, which worsens the disease's prognosis [1]. In ovarian cancer (OC), "cytotoxic T lymphocyte associated antigen 4" (CTLA4) is a protein receptor an immunological checkpoint that is necessary to regulate immune responses and T-cell activation trans membrane protein that is crucial for immune system modulation because it inhibits T-cell activation [2]."Vascular endothelial growth factor" (VEGF) "is a signal molecule that stimulates blood vessel growth". The new blood vessels production from preexisting vasculature, which is called (angiogenesis), and the formation of the embryonic circulatory system, which is called vasculogenesis, depend on this subfamily of growth factors. VEGF is essential for the production of new blood vessels during fetal growth, after damage, and after exercise [3]. Tumor debulking surgery and chemotherapy are the standard therapy for advanced (OC). While many different kinds of chemotherapy regimens have been tried to treat advanced (OC), carboplatin plus paclitaxel is now the most effective and standard first-line therapy [4]. By preventing angiogenesis and boosting the effects of chemotherapy, bevacizumab, also known as asvastin, is a vital component of the therapy of (OC). Research indicates that when bevacizumab is used in conjunction with chemotherapy, like paclitaxel, patients with recurrent platinum-sensitive (OC) may have longer progression-free survival times and better responses to therapy [5, 6]. Blockading CTLA4 enhances the infiltration of T cells and suppresses the regrowth of cells of cancer between chemotherapy treatments this strategy has the potential to address tumor-induced immune tolerance and enhance the overall effectiveness of chemotherapy [7].

Avastin is a monoclonal antibody that targets VEGF in an attempt to prevent it from binding to receptors through the inhibition of VEGF, this activity may limit the formation of blood vessels in tumors, hence impeding the progression and spread of the tumor. Research demonstrates that Avastin is effective in treating several malignancies by inhibiting VEGF, which reduces the blood supply to the tumor and slows tumor growth and metastasis [8].

2. Materials and methods

2.1. Patients and samples

Adjuvant (OC) women were taken 5 ml in gel tube to get clear serum. The patients were attending the rapid treatment taking unit to receive treatment were at Al-Amal National Hospital for Oncology, Al-Nahrain Center for Cancer Diagnosis and Treatment Laboratory in Al-Harithiya, Al-Yarmouk Teaching Hospital and Fallujah Teaching Hospital. in Iraq during the period from January 2024–May 2024, and 40 as healthy control group.90 adjuvant (OC) patients were divided into three groups 30 taking (1–6) dose of biotherapy Bevacizumab (Avastin) group 1 (G1), 30 taking $>$ 6 dose of avastin group 2 (G2) and last 30 taking chemotherapy group 3 (G3) and 40 as control group. Quantitative measurement of human (CTLA4 and VEGF). This was achieved by "Enzyme-linked immunosorbent assay" (ELISA).

2.2. Evaluations of CTLA4 and VEGF serum level

The "cytotoxic T lymphocyte associated antigen 4" (CTLA4) and "Vascular Endothelial Growth Factor" (VEGF) "solid-phase sandwich enzyme-linked immunosorbent assay"

Table 1. Demographical characteristic of the studied groups.

Parameters		Patients	Control	Sign.
Age	Mean ±SD	54.888 ±14.1544	42.8000 ± 11.716	"T-test = 4.2 p-value = 0.001"** HS
Residency	Urban	82 (91)	38 (95)	Chi-square= 0.59
No. (%)	Rural	8 (9)	2 (5)	p-value = 0.4 NS
Family History	Yes	9 (10)	0 (0)	Chi-square= 4.29
No. (%)	No	81 (90)	40 (100)	p-value = 0.04* S
Occupation	Employed	11 (12)	15 (37.5)	Chi-square= 11.01
No. (%)	Unemployed	79 (88)	25 (62.5)	p-value = 0.001** HS
BMI	Normal Weight	24 (26.7)	2 (5.0)	T-test = -.941
No. (%)	Over Weight	25 (27.8)	26 (65.0)	p-value = 0.3 NS
	Obese	41 (45.5)	12 (30.0)	
	Mean ± SD	29.5389 ± 6.2284	31.1333±11.9948	

(ELISA). catalogue number (SL0594Hu and SL1811Hu respectively) www.sunlongbiotech.com.

2.3. Ethical approval

The ethical committees of the Middle Technical University College of Health and Medical Techniques gave their approval for the study.

2.4. Statistical analyses

The Analysis of Statistical 26 program in SPSS was applied to detect the effect of different parameters. "One-way ANOVA and T-test" was applied to significance difference between means. "Chi-square" test was applied to significance difference in percentage 0.05 and 0.01 probability and computer program "MedCalc" in Windows for applications in laboratory medicine. Receiver operative characteristic Curve (ROC) and Person's correlation coefficients [9, 10].

3. Results

3.1. Demographical characteristic of the study groups

The study of (OC) were detected high significant difference at (P value = 0.00) between the means of age and occupation in studied groups. while not significant difference at (P value = 0.4 and 0.3) between the means of residency and body mass index (BMI) respectively in studied groups. However, the significant difference at (P value = 0.04) between the mean of family history in studied groups. shown in Table 1.

3.2. Distribution of CTLA4 and VEGF in study groups

The study of (OC) were detected high significance between the study groups and healthy control group in CTLA4 and VEGF at (p value = 0.0001) was shown in Table 2.

3.3. Receiver operative characteristic Curve among studies groups.

The results presented in Table 3 for the patients Groups (G1, G2, and G3) illustrate that the concentrations markers CTLA4 were (0.873, 95 % CI 0.740-0.954, 0.859, 95 %

Table 2. Comparison between patients studied groups and control.

Groups			CTLA4	VEGF
Patients	G1	Mean ± SD	231.440[b]± 109.119	221.393[b]±57.837
		SE	19.92236	10.5596
	G2	Mean ±SD	254.5567[b]±98.752	192.990[b]±68.160
		SE	18.0296	12.444
	G3	Mean ±SD	255.7667[b]±110.108	228.060[b]±150.59
		SE	20.102	27.494
Control		Mean ±SD	399.5600[a]±105.2752	297.0133[a]±62.9140
		SE	27.1819	16.2443
P-VALUE			0.0001**	0.0001**

Table 3. ROC of concerning study groups and biomarkers.

Groups	Markers	Area under curve	Asymptotic sig.	"Asymptotic 95% confidence interval" "Lower bound"	"Asymptotic 95% confidence interval" "Upper bound"	The best Cut off	Sensitivity (%)	Specificity (%)
G1	CTLA4	.873	.0001	.740	.954	≤280	80	86.67
	VEGF	.820	.0001	.677	.918	≤234.2	60	93.3
G2	CTLA4	. 859	.0001	.723	.945	≤293.3	76.6	86.6
	VEGF	. 906	.0001	.781	.972	≤215.8	80	100
G3	CTLA4	.871	.000	.765	.977	≤290	80	86.67
	VEGF	.818	.000	. 674	.917	≤202.6	73.3	100

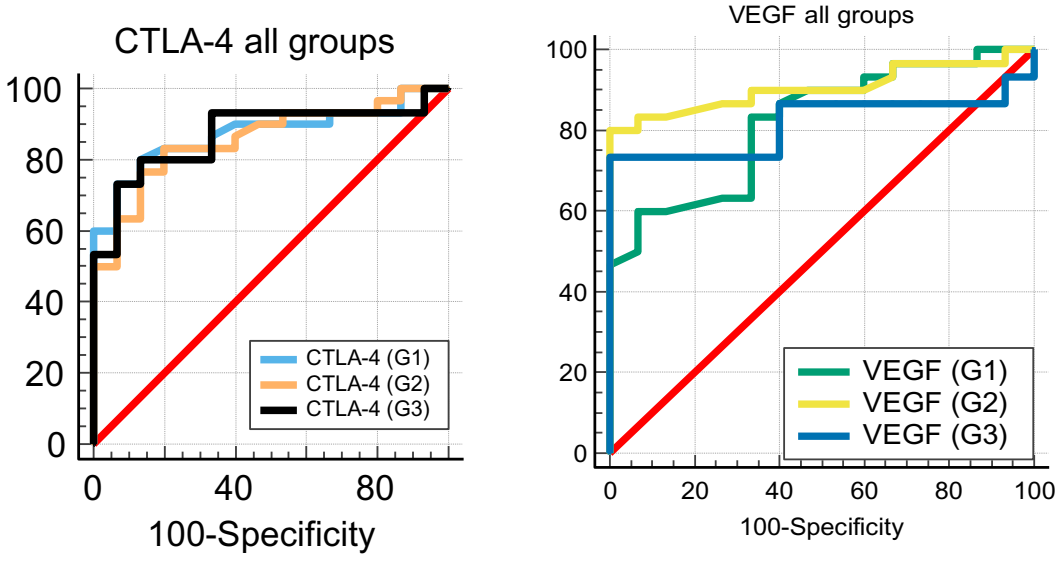

Fig. 1. ROC curve (A, B) of studied marker (CTLA4 and VEGF).

CI 0.723-0.945 and 0.871, 95 % CI 0.765-0.977) and for VEGF were (0.820, 95 % CI 0.677-0.918, 0.906, 95 % CI 0.781-0.972 and 0.818, 95 % CI 0.674-0.917), respectively. Furthermore, applying ROC curve for analyzing data in the studied groups. Fig. 1 represent ROC curve concerning the CTLA4 and VEGF markers for the studied groups.

Table 4. Person's correlation coefficients of biomarkers in OC patients.

Groups		Variable(s)	CTLA4
G1	VEGF	"Pearson Correlation"	0. 562**
		"Sig. (2-tailed)"	0.0001
G2	VEGF	"Pearson Correlation"	0.516**
		"Sig. (2-tailed)"	0.0003
G3	VEGF	"Pearson Correlation"	0.257**
		"Sig. (2-tailed)"	0.088

**High significant difference; *Significant difference.

The level of samples of CTLA4 for each groups showed high significant difference in G1,G2 and G3 at ($p = 0.0001$, 0.0001 and 0.000 respectively). The level of samples of VEGF for each groups showed high significant difference at ($p = 0.0001$, 0.0001 and 0.000 respectively) effectively distinguishing OC patients. with elevated of the sensitivity and specificity of CTLA4 (80 and 86.67 in G1, 76.6 and 86.6 in G2 and 80 and 86.67 in G3 respectively), Also higher sensitivity and specificity of VEGF (60 and 93.9 G1, 80 and 100 G2, and 73.3 and 100 G3 respectively).

3.4. Person's correlation coefficients between CTLA4 and VEGF in ovarian cancer patients

The results represented in Table 4 showed that both CTLA4 and VEGF were had highly significant correlation between levels of CTLA4 with VEGF in G1 and G2 at (p value = 0.0001 and 0.0003 respectively), but not significant correlation observed in G3 at (P value = 0.088).

4. Discussion

The significant difference in the OC is more prevalent at an older age compared to the health control group. This could be due to various factors, including biological changes, risk factors that accumulate with age, or screening practices that identify the disease more often in older individuals. This study similar to other studies [11, 12]. The no significant difference shown that residency (urban vs. rural) does not appear to be a distinguishing factor for ovarian cancer in this study. Both groups have a similar distribution of urban and rural residents, suggesting that environmental or lifestyle factors related to residency might not be a major effect on the incident of ovarian cancer in this sample This study agree to other studies [13, 14]. This significant difference underscores the importance of family history as a risk factor for (OC). Genetic predisposition plays a critical role, and having a family history of OC increases the likelihood of developing the disease. This finding aligns with other research that highlights genetic factors in the etiology of OC This study similar to other studies [12, 15]. The association between the study groups and control group was high significance ($P < 0.01$) for age groups where in patient group older age (≥ 60) more significant for ovarian cancer. In contrast younger age in control group is healthy. Matsas and Huang showed the high significant of age when found the risk of (OC) increases with age at (p-values ≤ 0.05) [16, 17] that agree with current study. Brezis supported current study when found the median overall survival (mOS) for elderly patients with epithelial ovarian cancer (EOC) was significantly lower compared to the control cohort, with values of 41.26 months for the elderly and 69.78 months for the control group at (p value $<$ 0.0001) [18]. Overall, The efficiency of the body's defenses often declines with age in

those who are older than 60. Older persons are more vulnerable to infections, illnesses, and some types of cancer due to immune-senescence, a decrease in immunological function. A low immune system in elderly patients is one of the causes leading to ovarian cancer [19, 20]. Ilic agree with current finding when found not significance difference among the three BMI groups at ($p = 0.3$) in (OC) [21]. while Beeghly-Fadiel disagree with the current study where found significant differences between BMI and ovarian cancer "each 5-unit increase in mean peri diagnosis" [22]. Huang supported the present study in their studies they discovered a highly significant difference at (P value = 0.01) between CTLA4 and the usage of Avastin, which was highly significant difference [23]. Abodunrin and Silberstein agree with the present study which found in meta-analysis showed a significant difference at (p value = 0.02) indicating a significant benefit with Avastin [24]. In the present study high sensitivity and specificity of CTLA4 in all groups with high significant difference at p value < 0.01 among OC patients, resemble to the study presented by James, which showed results similar to these findings [25]. Świderska agree with current study, that founded the serum CTLA4 concentration was high, the sensitivity was (70.3%) and the specificity was (90.7%) at ($p = 0.000004$) [26]. Maryam Agree with current finding, in G1 and G3 of VEGF, which found Sensitivity was 61.3% and specificity was 82.2% [27]. Faruq supported current result in G3 of VEGF the patients malignance (OC) with a best combination of specificity and sensitivity which gave with (93.5%) specificity, (90.1%) sensitivity as the value for identifying the malignance (OC) [28]. Trifanescu disagree with current finding which found VEGF to predict recurrence with 30% specificity [29]. Obermair disagree with present study, which found (ROC) curves shown the (VEGF) does not represent a beneficial tool for early diagnosis of (OC), sensitivity of (54%) and a specificity of (77%) [30].

Jlassi agree with The current study, which showed correlation coefficients with CTLA4 expression at p value = < 0.01 [31]. Liu also showed patients a higher expression in CTLA4 and were positively correlated similar to the current study [32]. Egiz agree with present study which showed VEGF expression was correlated with broad metastasis in OC [33]. Ding showed similar results to current study in G1 and G2 of VEGF which found In OC case group, the high serum VEGF-A levels correlated significance at ($p = 0.008$) [34]. Raspollini agree with present study in G3 of VEGF which found VEGF were not correlated with responsiveness to chemotherapy [35].

5. Conclusion

The study, showed the relationship between the immunological markers of the CTLA4 and VEGF and patient responses to Avastin and chemotherapy in (OC). Through analysis of studied groups and control group, we reached several significant conclusions. Firstly, The high significance difference of age, occupation, and family history as risk factors for ovarian cancer, Conversely, the lack of significant findings for residency and BMI suggests that these factors alone may not be sufficient indicators. Secondly, high significant difference in CTLA4 and VEGF between studied groups and control group. Thirdly, CTLA4 and VEGF high Sensitivity and Specificity in ROC and positive correlation between them in current study.

6. Recommendations

We recommended a study of immunotherapy (anti-CTLA4) and effect on patients on different dose, study mutation causes (OC) and follow up for patients after and before dose.

Acknowledgements

My profound gratitude goes out to all those who made contributions to the study. also I want to thank the organizations and institutions that provided funding for this study.

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