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Impact of Ascites and Plasma Levels of VEGF-A in Epithelial Ovarian Cancers

Bhaskari J[1] **and Lakshmi Krishnamoorthy**[1,2*]

[1]*Department of Biochemistry, Kidwai Memorial Institute of Oncology, Bangalore, Karnataka, India*

[2]*Department of Biochemistry, Sri Shankara Cancer Hospital and Research centre, Bangalore, Karnataka, India*

[*]**Corresponding author:** Lakshmi Krishnamoorthy, Department of Biochemistry, Kidwai Memorial Institute of Oncology, Bangalore, Karnataka, India, Tel: 080-26094072; E-mail: vkrishlakshmi@gmail.com

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Abstract

In a study analysing the plasma and ascitic fluid levels of VEGF-A in 220 epithelial ovarian cancer patients, we found that the patients presenting with markedly elevated levels of VEGF-A in their ascitis fluid came back with disease recurrence within 2 years post primary line of treatment. VEGF-A levels in the ascites of patients was a better marker for prognosis and aggressive disease compared to plasma VEGF-A levels. Although, plasma VEGF-A levels did not associate with recurrence, it did associate with other clinico-pathological factors studied as the stage, grade, CA-125 levels and the serous histopathology. VEGF-A plasma levels did not correlate with increased production of ascites but increased VEGF-A levels in the ascites correlated with aggressive disease, recurrence and mortality. The results of our study show that while the measurement of plasma VEGF-A levels may be a biomarker for malignant disease, levels of VEGF-A in ascitic fluid was a strong predictive marker for poor prognosis and rapid recurrence.

Keywords: VEGF-A; Ovarian carcinomas; Plasma levels

Introduction

Epithelial ovarian carcinomas are one of the most dreaded malignancies that eventually lead to recurrence and mortality when diagnosed in late stages of the disease. These cancers are notorious for their complex tissue heterogeneity and tumorigenesis. They are inconspicuous to diagnosis at early stages and present with unapparent symptoms at late-stage. There are no clear biomarkers for EOCs and hence these silent killers manage to maintain a disappointing 5 year survival of 27% in patients who are diagnosed with late stage disease. The 5 year survival is encouraging at 93% in patients who are diagnosed at early stage of the disease. Thus, the disease aggression and response to treatment is highly dependent on the stage of the disease at the time of diagnosis. Some of the telling tales of EOCs is the presence of ascites and rising CA-125 levels in the serum of the patient. Other symptoms such as bloating and pelvic pain are often confusing and fail to catch the clinician's attention

Even the increase in CA-125 levels is considered highly non-specific in recent times and is used to assess response to treatment. CA-125 levels are also observed in both healthy and malignant cells of mesothelial (pleural, pericardial, peritoneal, endometrial) and non-mesothelial origin (amniotic membrane, tracheobronchial and cervical epithelium) [1]. Hence a raised CA125 level is not a specific biomarker for malignancies per se although they are found in greater frequencies in malignancies. Hence tackling EOCs with inefficient biomarkers has lended to its impregnable status of being an obscure assassin in the current times.

The need for a suitable biomarker is thus very necessary as also the need for a suitable prognostic marker. The importance of ascites production is grave in the diagnosis of EOCs. Ascites production is often relative to the increased vascular permeability and one of the foremost robust angiogenic molecules that contribute to pathogenic vasculature in carcinogenesis is the Vascular Endothelial Growth Factor – A (VEGF-A).

The increased expression of VEGF-A has been the centre of many studies in the past decade. VEGF-A expression has been proved to be upregulated in several carcinomas including non-small cell lung cancer, breast cancer, gastric cancer, colo-rectal cancer and prostate cancer [2-5]. The serum VEGF-A levels have been studied in epithelial ovarian cancers and has been found to correlate with poor prognosis. VEGF-A plasma levels has not been explored and the levels of VEGF-A in ascites have not been enquired into to define it as a plausible biomarker or prognostic marker.

Here, in this study, we have investigated into a case cohort of 220 epithelial ovarian tumour patients and measured their plasma VEGF-A levels; as well as the corresponding ascites VEGF-A levels wherever the patient has presented with ascites.

Materials and Methods

Sample collection

A total of 220 patients who were diagnosed with primary epithelial ovarian cancer at the Kidwai Memorial Institute of Oncology, Bangalore, India, were taken into the study.

The initial institutional diagnosis of epithelial ovarian cancer was confirmed by review of pathologic slides by senior pathologists. Of the 220 samples collected, 178 (80.9%) were malignant cases, 23 (10.5%) were borderline or Low Malignant Potential (LMP) tumours and 19 (8.6%) were benign cases. Of these, number of pre-mensus samples stood at 63 (28.6%) and post-mensus samples at 157 (71.4%). 15 healthy control subjects, matched for age and menopausal status, were recruited for the study. The average age of case cohort was 51 ± 12 years and 48 ± 17 years for controls. 58.2% cases had high stage (FIGO) disease and 57.2% were high grade. The histopathogical

subtypes were observed to be at 60.9%, 18.2%, 2.7%, 3.2%, 15% for serous, mucinous, clear cell, endometrioid and poorly differentiated epithelial cell carcinomas. 49.2% cases exhibited bilateral affliction. 77.7% cases had high CA-125 levels and 11.8% had moderately high levels of CA-125. 70.5% cases presented with ascites. 39% cases had residual disease and 23% cases had disease recurrence within 2 years following de-bulking surgery.

5mL of blood was collected from all subjects in heparin vacutainers. The plasma was separated immediately by centrifugation and the separated plasma samples were stored at -80°C until further use for VEGF-A measurement through ELISA. 5mL ascites samples were collected from patients, centrifuged and stored in -80°C until further use. All samples were collected prior to treatment.

The tumors were graded according to WHO criteria and staged according to the Federation of Gynecology and Obstetrics (FIGO) classification. Information on cancer diagnosis, FIGO stage, histological grade and subtype, pre operative CA-125 levels, residual disease, recurrence and follow-up were abstracted from the patient's medical charts.

Study approval was given by the Institutional Review Board and the Medical Ethics Committee of Kidwai Memorial Institute of Oncology and written informed consent was obtained from all participants.

Estimation of VEGF in plasma and ascites

Plasma and ascites VEGF-A levels were measured by solid phase Enzyme Linked Immuno Sorbent Assay using Quantikine® ELISA for Human VEGF-A (R&D Systems, Minneapolis USA) following manufacturer's protocol. Samples that exhibited high values were diluted and re-analysed to have concentrations that could be read within the standard curve range. Ascites samples required multiple-fold dilution with diluent (provided in assay kit) before ascertaining the VEGF-A levels. The minimum detectable concentration of VEGF-A was less than 9.0 pg/mL. All samples were analysed in duplicate and the average of the values were taken to establish the concentration of VEGF-A in the sample.

Statistical analysis

Chi square test was applied to examine the significance of VEGF-A levels in plasma and ascites. Fischer's exact test was used to study the correlation between the disease status and other clinicopathological factors with the VEGF-A levels. All tests were carried out two-sided. The association of plasma and ascitic fluid VEGF-A levels with disease recurrence was assessed using logistic regression analysis. P-value of <0.05 was considered statistically significant. The statistical analysis was done using SPSS version 21.

Results

VEGF-A plasma concentration was found to be upregulated in the case cohort substantially and a receiver operating curve analysis presented a cut off of 50 pg/mL for malignancies against benign growth and controls. The average value of VEGF-A in controls was found to be at 33 pg/mL while the reference range for the controls was established at levels upto 50 pg/mL. The mean value for plasma VEGF-A levels in the benign, borderline and malignant subset of samples stood at 151.2 pg/mL, 92 pg/mL and 326 pg/mL respectively. The malignant subset showed a 6.5 fold increase in plasma VEGF-A levels compared to controls and a 3 fold increase when compared to benign and borderline cases. The mean secreted levels of VEGF-A in the case cohort as a whole was 288pg/mL. Figure 1 shows the distribution of VEGF levels across the case cohort and its sub-sets. Table 1 depicts the demography of patients recruited, the percentage number of cases showing significantly higher levels of VEGF-A and the mean plasma VEGF-A levels under each subset.

Sl No:	Clinico-pathological parameters	Total no of cases, (n=220)	% cases with elevated plasma VEGF-A levels	Mean plasma VEGF-A level (pg/mL)	P value
1.	Mensus				0.0565
	Pre	63 (28.6%)	68.3% (43/63)	166.5	
	Post	157 (71.4%)	80.3% (126/157)	172.03	
2.	Stage				<0.001
	I and II	73 (33.2%)	61.6% (45/73)	95.5	
	III and IV	128 (58.2%)	85.9% (110/128)	390.7	
3.	Grade				0.0026
	Low grade	75 (34.1%)	62.7% (47/75)	97.7	
	High grade	126 (57.3%)	81.8% (103/126)	378.9	
4.	Histopathology				<0.001
	Serous	134 (60.9%)	87.3% (117/134)	206	
	Mucinous	40 (18.2%)	50% (20/40)	96	
	Clear cell	6 (2.7%)	33.3% (2/6)	90.5	

	Endometrioid	7 (3.2%)	2.9% (2/7)	38	
	Poorly differentiated	33 (15%)	84.9% (28/33)	184.2	
5.	CA-125 levels				<0.001
	0<35	23 (10.5%)	52.2% (12/23)	77.8	
	35-110	26 (11.8%)	53.9% (14/26)	164	
	110-1000	98 (44.6%)	78.6% (77/98)	367	
	>1000	73 (33.2%)	90.4% (66/73)	310	
6.	Ascites presence				0.4689
	Yes	155 (70.5%)	75.5% (117/155)	350	
	No	65 (29.5%)	80% (52/65)	179	
7.	Recurrence in <2 yrs				0.0248
	Yes	49 (22.3%)	78% (38/49)	242	
	No	118 (53.6%)	59.3% (70/118)	110	

Table 1: Correlation between clinico-pathological parameters and plasma VEGF-A levels in the case cohort.

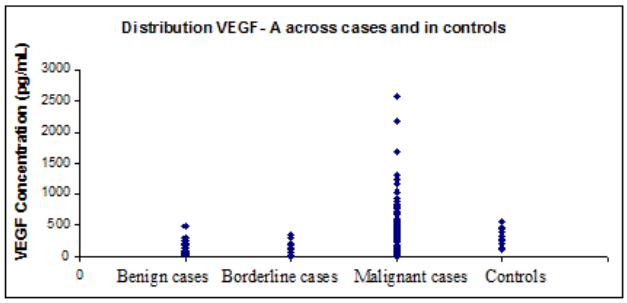

Figure 1: Distribution of VEGF-A across case subsets.

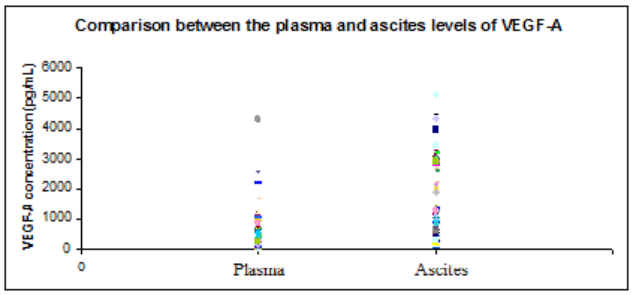

Figure 2: Distribution of VEGF-A is plasma and ascites samples.

The levels of VEGF-A did not vary much with age or mensus status and no significant correlation was observed. The plasma VEGF-A levels correlated significantly with high stage and high grade (p value<0.001 and 0.0026). The plasma levels also associated significantly with the serous subtype (p value <0.001). Increased plasma levels of VEGF-A did not associate with the presence of ascites, however an association was observed with disease recurrence.

Among the cases who presented with ascites, the average levels of VEGF-A in ascites was found to be 361 pg/mL, 528 pg/mL and 2136 pg/mL amongst the benign, borderline and malignant subsets respectively. Figure 2 gives a comparison between the plasma and ascites levels of VEGF-A. Ascitic fluid levels of VEGF-A significantly associated with recurrence as well. The subset of cases who presented with recurrence within a span of two years showed a mean VEGF-A level of 2383 pg/mL in their ascites while the mean level of VEGF-A in the case cohort who did not have disease recurrence within two years was 757 pg/mL. There was a significant association between the ascitic fluid levels of VEGF-A and disease recurrence.

Discussion

Massive ascites is a distinctive and consistent feature in patients with advanced ovarian cancer and the occurrence of ascites in ovarian cancer is significantly higher than in other types of carcinomas [6,7]. Hence, understanding the pathophysiology of ascites in EOC's is necessary to make vivid the role played by them in disease aggression.

In a study conducted by Huang et al. tumour spread in EOCs was accompanied by ascites. Increased ascites production causes abdominal distension, nausea, cachexia, anorexia, asphyxia and poor prognosis in patients with advanced stage ovarian cancer [8]. Puls et al. have reported that ascites development correlated significantly with a decreased 5-year survival rate amongst women with stage III or IV EOC. They have reported a 5% survival rate in the subset with ascites compared to 45% in the subset without ascites [9].

The effect of VEGF-A on vascular permeability is believed to be crucial for malignant ascites formation. One of the common causes of increased vascular permeability is the up-regulated expression of VEGF-A, although in our study there was no significant correlation

between increased VEGF-A plasma levels and presence of ascites. On the other hand, highly elevated levels of VEGF-A was observed in the ascites of the malignant subset of cases who came back with recurrent disease confirming a strong correlation between increased VEGF-A in ascitic fluid and recurrence.. A significant correlation was observed between increased plasma levels of VEGF-A and stage, grade and serous subtypes of EOCs.

The results of our study hence suggest that elevated levels of plasma VEGF-A expression significantly associated with malignancy and elevated levels of VEGF-A in ascites associated significantly with recurrence in host patients and hence with poorer prognosis and progression free survival.

This is a significant finding since the non-malignant subset of cases (with a mean ascitic fluid VEGF-A level of 361 pg/mL) did not express markedly high levels of VEGF-A in their ascites but the malignant subset of cases (with a mean ascitic fluid VEGF-A level of 2136 pg/mL) exhibited highly elevated levels of VEGF-A in their ascites. In general, it was observed that patients exhibiting levels of VEGF-A higher than ~900-1000 pg/mL in their ascites were marked for aggressive disease and earlier recurrence (recurrence within 2 years from debulking surgery and primary line of treatment).

Measurement of VEGF-A levels in the ascites of patients is hence a promising biomarker for aggressive EOC than CA-125 or plasma VEGF-A levels. Verheul et al. in their study, observed that malignant ascites and pleural effusion in cancer patients contained high levels of active VEGF-A and hypothesized that blocking VEGF-A expression may benefit patients with recurrent ascites or pleural effusion. They also observed that elevated VEGF-A levels in ascites indicated increased proliferation [10].

This gives rise to the hypothesis that although elevated VEGF-A in plasma is observed in late stage and high grade malignancies, it is the elevation of VEGF-A in ascites that contributes towards aggressive proliferation, recurrence and poor prognosis. In patients with high concentrations of VEGF-A in their ascites, this growth factor is responsible for poor prognosis and recurrence and hence these patients may benefit from anti-VEGF therapy.

Acknowledgements

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