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The role of VEGF in predicting progression-free survival (PFS) and overall survival (OS) for ovarian carcinoma patients

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ABSTRACT

Vascular endothelial growth factor (VEGF) plays a pivotal role in highly vascularized tumors such as ovarian cancer. It can be detected in serum and ascitic fluid. This study aimed to determine the difference between VEGF in the blood serum level and ascitic fluid in predicting progression-free survival (PFS) and overall survival (OS) in patients with ovarian cancer. A prospective cohort study was conducted between 2018 and 2021 at Dr Sardjito General Hospital, Yogyakarta, Indonesia. The study included patients who underwent primary surgery for epithelial ovarian cancer. VEGF levels in both serum and ascitic fluid were measured using the human cytokine magnetic 10-plex panel for Luminex (Invitrogen commercial kit), based on the enzyme-linked immunosorbent assay (ELISA). PFS and OS were monitored until the end of the study period. A total of 40 patients were enrolled, with a median follow-up duration of 24 mo. Among participants, 22 patients (55%) experienced disease progression, and 50% survived. The median VEGF levels in serum and ascitic fluid were 720 and 1925 pg/mL, respectively. Receiver operating characteristic (ROC) curve analyses of PFS and OS revealed that VEGF levels in ascitic fluid had better predictive value than serum VEGF levels. The area under the curve (AUC) of ascites vs serum was 0.646 vs 0.567, with sensitivity of 83.3% vs 100% for PFS, whereas the AUC of ascites vs serum was 0.565 vs 0.548, with sensitivity of 50.0% vs 93.8% for OS. However, no statistical significance was observed (p> 0.05). In conclusion, both VEGF ascites and serum failed to predict PFS and OS in ovarian cancer patients.

ABSTRAK

Faktor pertumbuhan endotel vascular- *Vascular endothelial growth factor* (VEGF) sangat penting untuk tumor yang sangat vaskular seperti kanker ovarium. VEGF dapat dideteksi dalam serum dan cairan asites. Tujuan dari penelitian ini adalah untuk menentukan perbedaan antara VEGF dalam kadar serum darah dan cairan asites dalam memprediksi *progression-free survival* (PFS) dan *overall survival* (OS) pada pasien kanker ovarium. Sebuah studi kohort prospektif dilakukan selama periode 2018-2021 di (RSUP Dr. Sardjito, Yogyakarta) Indonesia. Partisipan penelitian adalah pasien dengan operasi primer untuk kanker ovarium epitel. Panel magnetik sitokin manusia 10-plex untuk luminex (kit komersial Invitrogen) digunakan untuk menilai VEGF dalam asites dan serum darah menggunakan uji imunosorben terkait enzim. PFS dan OS diamati pada akhir penelitian. Penelitian ini melibatkan 40 pasien dengan kanker ovarium sebagai subjek. Durasi rata-rata tindak lanjut adalah 24 bulan. Dari peserta, 22 (55%) mengalami bebas perkembangan dan setengah peserta (50%) bertahan hidup. Median VEGF dalam serum dan asites masing-masing adalah 720 pg/mL dan 1925 pg/mL. Hasil nalisis *receiver-operating characteristics* (ROC) dari PFS dan OS menunjukkan kadar VEGF asites memiliki prediksi lebih baik daripada VEGF serum. Luas daerah di bawah kurva (*area under the curve*/AUC) asites vs serum adalah 0,646 vs 0,567, dengan sensitivitas 83,3% vs 100%) untuk PFS, sedangkan AUC asites vs serum adalah 0,565 vs 0,548, dengan sensitivitas 50,0% vs 93,8% untuk OS. Namun, analisi statistik tidak menunjukkan perbedaan yang bermakna (p> 0,05). Dapat disimpulkan, baik asites VEGF dan serum tidak bisa memprediksi PFS dan OS pada pasien kanker ovarium.

Keywords:
VEGF;
progression free survival;
overall survival;
ovarian carcinoma

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INTRODUCTION

Ovarian cancer is the third most common cancer among women in Indonesia.[1] Vascular endothelial growth factor (VEGF) mediated angiogenesis is crucial for the highly vascularized tumors such as ovarian cancer by regulating physiological and pathological angiogenesis.[2,3] VEGF plays a pivotal role in promoting angiogenesis.[4] VEGF is critically involved in the activation of tyrosine kinase signaling pathways. This activation facilitates tumor progression by promoting cellular proliferation, migration, and resistance to apoptotic signals.[5]

VEGF initiates a diverse range of intra- and extracellular signaling cascades that facilitate endothelial cell differentiation, as well as promote cellular survival, proliferation, and migration. Additionally, it contributes to the regulation of vasodilation and the enhancement of vascular permeability.[6] VEGF is linked to tumor progression, vascular remodeling, invasiveness, metastasis, and post-treatment recurrence.[7]

VEGF has been reported as a serological biomarker for clinical diagnosis and prognosis in individuals with ovarian cancer.[8] However, the findings remains inconsistent. VEGF contributes to the intraperitoneal dissemination of ovarian cancer by promoting neovascularization and increasing vascular permeability, which facilitates the growth of intraperitoneal tumors, the development of peritoneal carcinomatosis, and the formation of malignant ascites.[9]

Higher levels of VEGF in the tumor microenvironment or ascitic fluid may lead release of VEGF into the systemic circulation, thereby increasing serum levels due to its capacity to enhance vascular permeability.[10] VEGF levels in both serum and ascitic fluid may serve as potential independent prognostic markers for survival in patients with ovarian cancer.[11] This study aimed to compare VEGF levels in blood serum and ascitic fluid in predicting progression-free survival (PFS) and overall survival (OS) among ovarian cancer patients.

MATERIAL AND METHOD

Patients

A prospective analysis was conducted using clinical records from 40 ovarian cancer patients who underwent primary surgery at Dr Sardjito General Hospital, Yogyakarta, Indonesia between January 2018 and December 2021. A consecutive sampling method was applied in this study. The inclusion criteria were patients with histopathologically confirmed ovarian cancer in any stage who underwent primary surgery and were willing to participate in this study. The exclusion criteria included a history of other malignancies, previous ovarian cancer surgery, prior chemotherapy, pregnancy, or breastfeeding. Biological samples included blood serum and ascitic fluid were collected for this study. VEGF levels in both serum and ascites were measured using the Quantikine ELISA Kit (R&D Systems). The study was approved by the Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital, Yogyakarta (reference number: KE/FK/0749/EC/2018).

Blood serum VEGF measurement

VEGF levels in blood serum were quantified using a Quantikine® ELISA kit (R&D Systems, USA) following the manufacturer's protocol. A total of 100 µL of assay diluent RD1W was added to each well, followed by 100 µL of serum samples, standards, and controls. The plate was incubated for 2 hr at room

temperature, then washed three times using 400 µL of wash buffer per cycle. Subsequently, 200 µL of human VEGF conjugate was added, and the plate was incubated for another 2 hr. After a second wash, 200 µL of substrate solution was added, followed by a 25 min incubation. The reaction was stopped with 50 µL of stop solution, and absorbance was measured spectrophotometrically.

Ascitic fluid VEGF measurement

VEGF levels in ascitic fluid were quantified using a Quantikine® ELISA kit (R&D Systems, USA) following the manufacturer's protocol as VEGF measurement in blood serum. A total of 5 mm of peritoneal fluid was collected during the surgery. The peritoneal cavity was washed with 20 mL of 0.9% NaCl in cases where peritoneal fluid was absent. The sample was delivered at 4°C to the molecular biology lab. The sample was centrifuged at 1200–1400 rpm for 5 min. Part of the supernatant was collected and kept in an Eppendorf freezer. The cellular component was added with freezing media and kept in cryovials. Prior to sample analysis, the temperature was maintained at -80°C.

Statistical analysis

Data were statistically analyzed using the statistical software SPSS 24.0. Descriptive statistics were used to summarize patient characteristics, including means with standard deviations (SD), medians with minimum–maximum ranges, and frequencies with percentages. Receiver operating characteristic (ROC) curve analysis was performed to assess the ability of VEGF levels in serum and ascitic fluid to predict progression-free survival (PFS) and overall survival (OS). The area under the curve (AUC), optimal cut-off values, sensitivity, and specificity were calculated. A p-value of <0.05 was considered statistically significant.

RESULTS

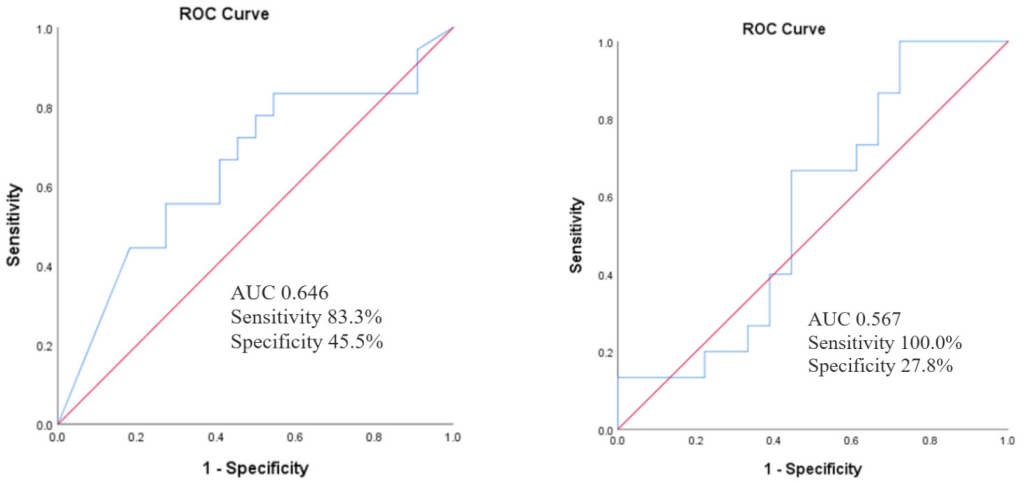
A total of 58 patients who underwent primary surgery were initially selected. However, 18 patients were excluded due to non-ovarian cancer findings based on histopathological examination. The remaining 40 eligible patients were followed up throughout the study, with no cases of loss to follow-up. The characteristics of the patients are presented in TABLE 1. The mean age was 48.35 ± 11.62 yr. The majority of patients had children, histopathological type 2, high-grade tumors, advanced-stage disease, no visible residual tumor on macroscopic examination, preoperative CA-125 levels and ascitic fluid volume above 500 mL, malignant ascites, very low tumor-infiltrating lymphocyte (TIL) levels, and negative lymphovascular space invasion (LVSI). The median preoperative levels of CA-125 and IL-8 were 839.7 and 600.4 pg/mL, respectively. The median follow-up duration was 24 mo. By the end of the study, 50% of the patients were alive, and more than half remained progression-free. The median VEGF levels in serum and ascitic fluid were 720 and 1925 pg/mL, respectively (TABLE 1).

VEGF levels in ascitic fluid showed better predictive performance for PFS compared to serum VEGF, as shown by ROC curve analysis. The AUC for VEGF in ascites was 0.646 pg/mL, whereas for serum VEGF it was 0.567 pg/mL. Ascitic VEGF showed higher sensitivity (83.3% vs. 100%) and specificity (45.5% vs. 27.8%) compared to serum VEGF (FIGURE 1A and B). The cutoff values were 1430.5 pg/mL for ascitic VEGF and 229.5 pg/mL for serum VEGF. However, no statistical significance was observed ($p > 0.05$).

TABLE 1. Characteristics of patients

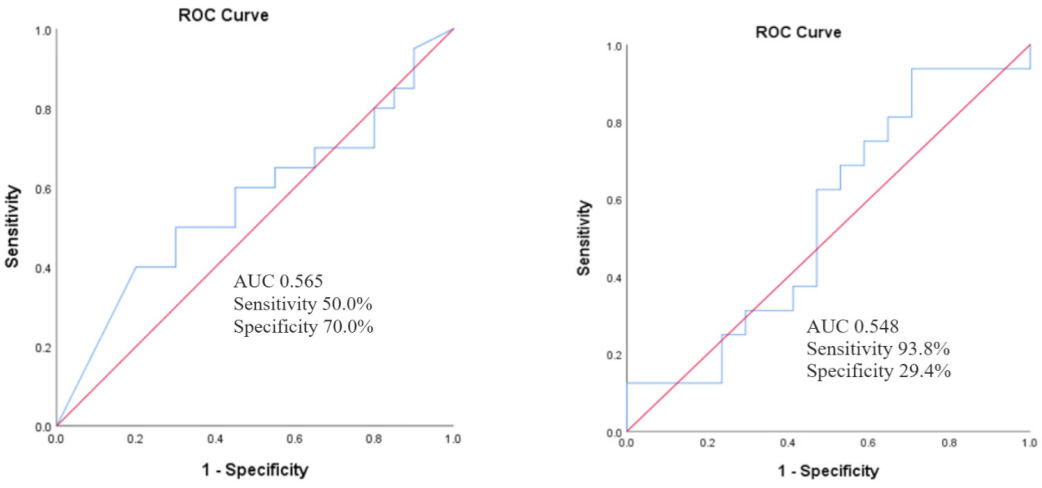
Variable	Mean ± SD/ median (min-max)	n (%)
Age (yr)	48.35 ± 11.62	-
<60	-	34 (85.0)
≥60	-	6 (15.0)
Parity		
Nulipara	-	11 (27.5)
Multipara	-	29 (72.5)
Histopathology type		
1	-	17 (42.5)
2	-	23 (57.5)
Grade		
Low	-	11 (27.5)
High	-	29 (72.5)
Cancer stage		
Early	-	16 (40.0)
Late	-	24 (60.0)
Tumor residual		
R0	-	25 (62.5)
Rx	-	15 (37.5)
Pre-operative Ca-125 (pg/mL)	839.7 (21.9-25000)	-
Pre-operative Ca-125 (mL)		
<500	-	15 (37.5)
≥500	-	25 (62.5)
Ascites (mL)		
<500	-	19 (47.5)
≥500	-	21 (52.5)
Ascites		
Benign	-	12 (30.0)
Malignant	-	28 (70.0)
Total TILs		
Very low	-	22 (55.0)
Low	-	11 (27.5)
High	-	7 (17.5)
LVSI		
Negative	-	28 (70.0)
Positive	-	12 (30.0)
IL-8 ascites (pg/mL)	600.4 (5.5-4499.1)	-
VEGF ascites (pg/mL)	1925 (0-3379)	-
VEGF serum (pg/mL)	720 (18-2182)	-
Progression free survival		
Sensor	-	22 (55.0)
Event	-	18 (45.0)
Overal survial		
Sensor	-	20 (50.0)
Event	-	20 (50.0)

VEGF levels in ascitic fluid also showed superior predictive value compared to serum VEGF for OS. The AUC for VEGF in ascites was 0.565 pg/mL, while the AUC for serum VEGF was 0.548 pg/mL, indicating fair predictive ability. The sensitivity of VEGF in ascites and serum was 50.0% and 93.8%, respectively, whereas the specificity was 70.0% and 29.4% (FIGURE 2A and B). Nonetheless, no statistical significance was observed ($p > 0.05$). The results of this study found no correlation between both VEGF ascites and VEGF serum in predicting both PFS and OS.



A B

FIGURE 1. A) ROC analysis of ascitic VEGF on PFS. B) ROC analysis of serum VEGF serum on PFS



A B

FIGURE 2. A) ROC analysis of VEGF ascites on OS. B) ROC analysis of VEGF serum on OS.

DISCUSSION

Normal functional ovarian epithelium could transform into ascites-producing neoplastic tissue under VEGF overexpression.[12] Ovarian cancer cells exhibit elevated VEGF expression, primarily driven by hypoxic conditions, indicating that these mechanisms play a crucial role in promoting tumor angiogenesis.[13] The expression of VEGF was influenced by hypoxia as well as several growth factors, mediators, and effectors.[14] VEGF promotes angiogenesis, enhances vascular permeability, stimulates proliferation, survival, and migration of cancer cells, thus leading to ovarian cancer progression.[15,16] The elevation of VEGF levels is linked to lower survival in ovarian cancer.[17]

The cut-off of ascitic VEGF and serum VEGF in PFS in this study were 1430.5 and 229.5 pg/mL, and in OS were 2672.5 and 263.5 pg/mL, respectively. These findings showed that ascitic VEGF had lower sensitivity but higher specificity than serum VEGF. VEGF concentration in cancer patients was found to be highest in cyst fluids, followed by ascites, and lowest in serum.[18] In accordance with these studies, it was found that ascitic VEGF had a higher AUC in both predicting PFS and OS in our study participants. VEGF levels in ascites are higher than in the serum due to the role of VEGF in promoting neovascularization, enhancing vascular permeability, and facilitating intra-abdominal tumor growth and spread, which collectively contribute to the development of malignant ascites and the progression of the disease. Serum VEGF level could be affected by other factors such as inflammatory conditions, therefore, it might be a less valid predictor.[18] Serum VEGF level not only represents VEGF from tumor but also from platelets and leukocytes.[19] Therefore, ascitic VEGF is more specific than VEGF serum, while serum VEGF is more sensitive than ascitic VEGF. This result aligned with a preexisting study; it was found that ascitic VEGF had higher specificity (45.5% vs 27.8%) and lower sensitivity (83.3% vs 100.0%) to serum VEGF for PFS. Similar findings were also found in this study for OS, where ascitic VEGF had higher specificity (70.0% vs 29.4%), but lower sensitivity (50.0% vs 93.8%) to serum VEGF.

This study found that ascitic VEGF and serum VEGF failed to predict PFS and OS in ovarian cancer patients ($p>0.05$). This might be due to disease progression and residual disease. Almost half of patients in this study presented in the early stage of cancer, and the majority of patients had no macroscopically seen residual disease after surgery. A study conducted by Manher *et al.*,[20] found that no significant correlation between VEGF and OS in ovarian cancer until disease progression. Trifanescu *et al.*,[17] also concluded that for patients in early-stage cancer or who had no residual disease, VEGF did not always have a prognostic value. Regarding PFS, Harloziňska *et al.*,[18] found no significant value of VEGF as a predictor of survival in ovarian cancer patients. González-Palomareset *et al.*,[21] evaluated no significant association between VEGF level and PFS was found. Similar to these studies, Gadducci *et al.*,[22] found elevated serum VEGF and normal VEGF had no statistically significant difference in ovarian cancer patients' survival. Previous study also concluded that VEGF had significant association with cancer stage, tumour differentiation, tumour size, residual disease, lymph node invasion, and ascites.[23] Ovarian cancer patient's survival could be influenced by several factors including age, body mass index, and cancer stage.[24,25] Hence, it might provide a potential explanation for the inability of VEGF levels in ascites and serum to serve as predictive markers for PFS and OS among ovarian cancer patients.

Study limitation

This prospective cohort study was a single-centre study with small participants. In contrast with the previous study, our results showed no statistical significance was observed from ascitic and serum VEGF in predicting PFS and OS. Therefore, both ascitic and serum VEGF failed to serve as predictors for PFS and OS in ovarian cancer patients in our study. Further research are recommended to conduct multicenter studies with a larger pool of samples, and a broader representation of ovarian cancer stages to enhance the generalizability and statistical power of the findings.

CONCLUSION

In conclusion, ascitic VEGF is more selective and less sensitive than serum VEGF. However, ascites and serum VEGF failed to predict PFS and OS in ovarian cancer.

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REFERENCES

1. Gondhowiardjo S, Christina N, Ganapati NPD, Hawariy S, Radityamurti F, Jayalie VF, *et al*. Five-year cancer epidemiology at the national referral hospital: hospital-based cancer registry data in Indonesia. JCO Global Oncol 2021; 7:190-203. https://doi.org/10.1200/GO.20.00155
2. Ramakrishnan S, Subramanian IV, Yokoyama Y, Geller M. Angiogenesis in normal and neoplastic ovaries. Angiogenesis 2005; 8(2):169-82. https://doi.org/10.1007/s10456-005-9001-1
3. Chung AS, Ferrara N. Developmental and pathological angiogenesis. Annu Rev Cell Dev Biol 2010; 27:563-84. https://doi.org/10.1146/annurev-cellbio-092910-154002
4. Ansari MJ, Bokov D, Markov A, Jalil AT, Shalaby MN, Suksatan W, *et al*. Cancer combination therapies by angiogenesis inhibitors; a comprehensive review. Cell Commun Signal 2022; 20(1):49. https://doi.org/10.1186/s12964-022-00838-y
5. Konner J, Dupont J. Use of soluble recombinant decoy receptor vascular endothelial growth factor trap (VEGF trap) to inhibit vascular endothelial growth factor activity. Clin Colorectal Cancer 2004; 4(Suppl 2):S81-5. https://doi.org/10.3816/ccc.2004.s.013
6. Melincovici CS, Bosca AB, Susman S, Marginean M, Mihu C, Istrate M, *et al*. Vascular endothelial growth factor (VEGF) - key factor in normal and pathological angiogenesis. Rom J Morphol Embryol, 2018; 59(2):455-67.
7. Apte RS, Chen DS, Ferrara N. VEGF in signaling and disease: beyond discovery and development. Cell 2019; 176(6):1248-64. https://doi.org/10.1016/j.cell.2019.01.021
8. Hefler LA, Zeillinger R, Grimm C, Sood AK, Cheng WF, Gadducci A, *et al*. Preoperative serum vascular endothelial growth factor as a prognostic parameter in ovarian cancer. Gynecol Oncol 2006; 103(2):512-7. https://doi.org/10.1016/j.ygyno.2006.03.058
9. Pourgholami MH, Cai ZY, Lu Y, Wang L, Morris DL. Albendazole: a potent inhibitor of vascular endothelial growth factor and malignant ascites formation in OVCAR-3 tumor-

bearing nude mice. Clin Can Res 2006; 12(6):1928-35. https://doi.org/10.1158/1078-0432.CCR-05-1181
10. Rudlowski C, Pickart AK, Fuhljahn C, Friepoertner T, Schlehe B, Biesterfeld S, *et al*. Prognostic significance of vascular endothelial growth factor VEGF expression in ovarian cancer patients: a long-term follow-up. Int J Gynecol Cancer 2006; 16(Suppl 1):183-89. https://doi.org/10.1111/j.1525-1438.2006.00307.x
11. Liang B, Guo Z, Li Y, Liu C. Elevated VEGF concentrations in ascites and serum predict adverse prognosis in ovarian cancer. Scand J Clin Lab Invest 2013; 73(4):309-14. https://doi.org/10.3109/00365513.2013.773593
12. Schumacher JJ, Dings RP, Cosin J, Subramanian IV, Auersperg N, Ramakrishnan S. Modulation of angiogenic phenotype alters tumorigenicity in rat ovarian epithelial cells. Cancer Res 2007; 67(8):3683-90. https://doi.org/10.1158/0008-5472.CAN-06-3608
13. Shen GH, Ghazizadeh M, Kawanami O, Shimizu H, Jin E, Araki T, *et al*. Prognostic significance of vascular endothelial growth factor expression in human ovarian carcinoma. Brit J Cancer 2000; 83(2):196-203. https://doi.org/10.1054/bjoc.2000.1228
14. Horiuchi A, Imai T, Shimizu M, Oka K, Wang C, Nikaido T, *et al*. Hypoxia-induced changes in the expression of VEGF, HIF-1 alpha and cell cycle-related molecules in ovarian cancer cells. Anticancer Res 2002; 22(5):2697-702.
15. Weis S, Cui J, Barnes L, Cheresh D. Endothelial barrier disruption by VEGF-mediated Src activity potentiates tumor cell extravasation and metastasis. J Cell Biol 2004; 167(2):223-9. https://doi.org/10.1083/jcb.200408130
16. Naora H, Montell DJ. Ovarian cancer metastasis: integrating insights from disparate model organisms. Nat Rev Cancer 2005; 5(5):355-66. https://doi.org/10.1038/nrc1611
17. Trifanescu OG, Gales LN, Tanase BC, Marinescu SA, Trifanescu FA, Gruia LM, *et al*. Prognostic role of vascular endothelial growth factor and correlation with oxidative stress markers in locally advanced and metastatic ovarian cancer patients. Diagnostics 2023; 13(1):166. https://doi.org/10.3390/diagnostics13010166
18. Harloziňska A, Sedlaczek P, Kulpa J, Grybos M, Worjik E, Dalen VA, *et al*. Vascular endothelial growth factor (VEGF) concentration in sera and tumor effusions from patients with ovarian carcinoma. Anticancer Res 2004; 24(2 C):1149-57.
19. Dvorak HF. Vascular permeability factor/vascular endothelial growth factor: A critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. J Clin Oncol 2002; 20(21):4368-80. https://doi.org/10.1200/JCO.2002.10.088
20. Mahner S, Woelber L, Eulenburg C, Schwarz J, Carney C, Jeanicke F, *et al*. TIMP-1 and VEGF-165 serum concentration during first-line therapy of ovarian cancer patients. BMC Cancer 2010; 10:139. https://doi.org/10.1186/1471-2407-10-139
21. González-Palomares B, Coronado Martín PJ, Maestro de Las Casas ML, Veganzones de Castro S, Rafael Fernández S, Vidaurreta Lázaro M, *et al*. Vascular endothelial growth factor (VEGF) polymorphisms and serum VEGF levels in women with epithelial ovarian cancer, benign tumors, and healthy ovaries. Int J Gynecol Cancer 2017; 27(6):1088-95. https://doi.org/10.1097/IGC.0000000000001006
22. Gadducci A, Ferdeghini M, Fanucchi

A, Annicchiarico C, Ciampi B, Prontera C, *et al*. Serum preoperative vascular endothelial growth factor (VEGF) in epithelial ovarian cancer: relationship with prognostic variables and clinical outcome. Anticancer Res 1999; 19(2B):1401-5.
23. Bandiera E, Franceschini R, Specchia C, Bignotti E, Trevisiol C, Gion M, *et al*. Prognostic significance of vascular endothelial growth factor serum determination in women with ovarian cancer. ISRN Obstet Gynecol 2012; 2012:245756. https://doi.org/10.5402/2012/245756
24. Okunade KS, Adejimi AA, Ohazurike EO, Salako O, Osunwusi B, Adenekan MA, *et al*. Predictors of survival outcomes after primary treatment of epithelial ovarian cancer in Lagos, Nigeria. JCO Glob Oncol 2021; 7:89-98. https://doi.org/10.1200/GO.20.00450
25. Wimberger P, Lehmann N, Kimmig R, Burger A, Meier W, Hoppenau B, *et al*. Impact of age on outcome in patients with advanced ovarian cancer treated within a prospectively randomized phase III study of the Arbeitsgemeinschaft Gynaekologische Onkologie Ovarian Cancer Study Group (AGO-OVAR). Gynecol Oncol 2006; 100(2):300-7. https://doi.org/10.1016/j.ygyno.2005.08.029