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RESEARCH PAPER

Preoperative Serum Vascular Endothelial Growth Factor as a Predictor of Malignant Ovarian Tumor

Marzina Faruq[1*], Shirin Akhter Begum[2], Mehriban Amatullah[3], Tasfia Mahmud[4], Gopa Kundu[5], Reeta Rani Sarker[6], Samina Sultana[7]

[1]Department of Obst. & Gynae, 100 bed Upzila Health Complex, Kashiani, Gopalgonj, [2]Department of Gynaecological Oncology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, [3]Department of Obstetrics and Gynaecology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, [4]Sirajul Islam Medical College, Dhaka, Bangladesh, [5]Department of Obs & Gynae, Upazila Health Complex, Kamar Khanda, Sirajganj, Bangladesh, [6]Department. of Obs & Gynae, OSD, DGHS, Dhaka, attached to: Colonel Malek Medical College & Hospital, Manikgonj, [7]Department of Obst. & Gynae, Dhaka Medical College, Dhaka, Bangladesh

Abstract

Background: Effective biomarkers for accurate characterization of newly diagnosed ovarian tumors and adnexal masses are of paramount importance. The availability of an ideal serum marker is highly recommended. Vascular endothelial growth factor (VEGF) may be considered as a predictor of ovarian cancer.

Objective: This study explored the efficacy of preoperative serum VEGF for detection of malignant ovarian tumor.

Methods: This cross sectional analytical study was conducted among 86 patients who were enrolled for this study according to final histopathology report and who did not receive any kind of treatment. Serum levels of VEGF-A were determined by using ELISA method. ROC curve was plotted to achieve the best cutoff value of serum VEGF. The Mann-Whitney test was used to compare the VEGF distribution across sub-groups of patients.

Result: A statistically significant difference in the levels of serum VEGF level was observed between benign and malignant ovarian tumor patients. Ovarian cancer patients had a higher preoperative median S. VEGF level of 753.8 pg/ml than that of benign ovarian masses (median 241.8 pg/ml; *p* value 0.001). The ability of serum VEGF to differentiate malignancy from benign masses at a cut-off value of 547.85 pg/ml gave a sensitivity of 90.1%, a specificity of 93.5%.

Conclusion: This study revealed that preoperative serum VEGF may be used as a feasible vascular marker and predictor of malignant ovarian tumors.

Key words: Ovarian tumor, ovarian cancer, Serum Vascular Endothelial Growth Factor.

Introduction

Ovarian cancer is the 4th most common cancer of female reproductive organs around the world.[1] Globally the incidence of ovarian cancer is 6.6 per lac and mortality is 4.2 per lac.[2] In Bangladesh, ovarian cancer is the 3rd most common cancer occurring in female reproductive organs.[2] Ovarian cancer has relatively asymptomatic nature at its early stage. Rapid progression of disease, the high rate of recurrence and poor prognosis make this cancer the most lethal gynecological malignancy.[3] The need for the development of reliable serum biomarkers for early detection of ovarian cancer, which are both sensitive and specific, remains a long awaited priority. Currently, there is no recommended effective early detection test for ovarian cancer in the general population. Most ovarian cancers are developed from three categories of cells: epithelial cells, germ cells and sex cord stromal cells. Among them, epithelial ovarian cancer accounts for 90% cases.[4] So commonly accepted and frequently used tumor marker is CA 125.[4] However, CA-125 is not exclusively expressed on ovarian tumor cells but also by a number of other cell types including the pleura, peritoneum and mullerian epithelium. Several factors undermine the significance of CA-125 as a biomarker because of the absence of its expression in about 20% of ovarian cancer and elevated expression in some benign and physiological conditions (liver cirrhosis, endometriosis, peritonitis, menstruation and pregnancy).[4]

Angiogenesis is the process of new vessel formation and hallmark of tumor progression. Solid tumors cannot

***Correspondence:** Marzina Faruq, Department of Obst. & Gynae, 100 bed Upzila Health Complex, Kashiani, Gopalgonj.*
Email: tonudmc57@gmail.com.
ORCID ID: 0009-0005-9189

grow larger than 2-3 mm diameter without inducing their own blood supply.[5] In cancer, angiogenesis is the mechanism where growth of new blood vessels occurs from existing ones to supply cancerous growth. The development and the progression of the tumor and its metastasis are the result of an efficient vascular response. The beginning of this process is known as "angiogenic switch", through which tumors acquire the ability to grow and disseminate beyond their primary site.[6] The switch consists of different steps like perivascular detachment & vessel dilation, angiogenic sprouting, new vessel creation & development and recruitment of perivascular cells.[6] New blood vessels will support tumor growth, specifically feeding hypoxic and necrotic areas of the tumor to provide it with essential nutrients and oxygen.[6] During tumor angiogenesis, the angiogenesis process is uncontrolled and up regulated as a result of a predominance of pro angiogenic factors within the tumor micro-environment along with down regulation of anti-angiogenic influences.[6] The hypoxic micro environment activates the angiogenic network promoting the sprouting of new blood vessels into the tumor and inducing the expansion of the tumor.[6] This implies that quantifying the extent of angiogenesis could serve as an indicator of tumor behavior and prognosis. Angiogenic growth factors and their corresponding receptors which are released and activated by cancer cells are the main responsible of the start of the angiogenic process. One of the primary and widely distributed mediators of angiogenesis is vascular Endothelial Growth Factor (VEGF).[6] There are several angiogenic factors and yet Vascular Endothelial Growth Factor (VEGF-A) has been accepted as the single most robust molecule in the process of angiogenesis.[7] VEGF is a secreted protein which promotes angiogenesis in tumors. VEGF family includes five secreted proteins (VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor. In terms of endothelial biology and tumor angiogenesis, VEGF-A (hereafter referred as VEGF) – in particular VEGF-A165 – is considered to be the most physiologically relevant form.[8]

There are many studies performed to establish the relationship between circulating VEGF levels and tumor behaviors of ovarian cancer patients. In different studies researchers employed different cut off values for serum VEGF to differentiate malignant ovarian tumor from benign ones. These boundary values, used to define high and low expression levels need to be standardized. Present study done with the motive to uncover the epidemiological features of VEGF and its predictive value in ovarian cancer. The result of the present study may be helpful to establish serum VEGF as a possible tumor marker for ovarian cancer. In the current study, VEGF and CA-125 levels were assessed in preoperative sera of patients with all types of ovarian tumors with a view to estimate diagnostic and predictive impact on ovarian cancer. Therefore investigations have been done to identify a definite cut off value, sensitivity, specificity, Positive and negative predictive value of serum VEGF and serum CA 125 in patients with ovarian tumors. Here levels of serum VEGF and CA-125 were measured against the histopathology result. Histopathology report has been considered as gold standard for measurement of these two biomarkers. The purpose of this study was to better understand the role of serum VEGF as a biomarker for the early detection of ovarian cancer.

Materials and Methods

This was a cross sectional analytical study was done in Department of Gynecological oncology, Bangabandhu Sheikh Mujib Medical University (BSMMU) and National Institute of Cancer and Research Hospital, Dhaka over one year period from March 2020 to February 2021.

The study population included all patients admitted in the department of Gynecological oncology of Bangabandhu Sheikh Mujib Medical University (BSMMU) and National Institute of Cancer and Research Hospital (NICRH), Dhaka who were diagnosed as primary ovarian tumor or adnexal masses and maintained inclusion and exclusion criteria of this study.

Women with newly diagnosed cases of primary ovarian tumor and adnexal masses who were admitted in department of gynecological oncology, non pregnant were included in this study after their consent of participation. Women with ovarian tumor and adnexal masses who were already on treatment or received treatment for ovarian tumor, Metastatic or recurrent malignant ovarian tumor, Diagnosed cases of malignancy other than ovarian cancer, PID (pelvic inflammatory disease), Endometriosis, pregnancy, Associated medical disorders – e.g. vascular diseases were excluded. Convenience purposive sampling was done.

Sample size determination and Sampling technique:

$$N = \frac{n}{p}$$

Where, $$n = Z^2 X \frac{pq}{d^2}$$

Z	Confidence interval normal distribution value, i.e. for 95% CI, Z	= 1.96
p	Sensitivity of VEGF	= 0.9
q	(1- p)	= 0.1
d	Acceptable standard error	= 0.05
P	Prevalence of malignant ovarian tumor	= 0.8

Then,

N = 172.8

= 173

According to this formula the required sample was 173. But due to time constrain and available resources 86 samples were taken who were admitted in the department of Gynecological oncology of BSMMU and NICRH, Dhaka and met eligibility criteria of the study.

This study was conducted in Department of Gynecological oncology, BSMMU and NICRH, Dhaka. Approval from Institutional Review Board and ethical committee was taken from both institutions and from BCPS. The study subjects were enrolled according to the inclusion and exclusion criteria. The purpose and procedure of the study was discussed with the patients. Written informed consent was taken from those who agreed to participate in the study. Detailed history and Clinical examination included general examination, systemic examination and routine gynecological examination was done. Based on the clinical and investigative findings, a provisional diagnosis was made and the patient was managed according to standard protocol. Before surgical procedure, 3ml of blood was collected from each patient, immediately centrifuged for 10 minutes and serum was kept frozen at -70 degree Celsius until measurement. Then S. VEGF was measured by ELISA (Enzyme linked immunosorbent assay) using VEGF- A Human ELISA EIA- 4826 kit in the department of Microbiology and immunology of BSMMU using ELISA READER Machine, DAS, ITALY. For each patient separate data collection sheet was used. Data were collected from the patients on variables of interest using the predesigned structured questionnaire by interview, observation, clinical examination, investigations and from the history sheet of the patients. The final data analysis was done for 86 patients, who underwent laparoscopy or laparotomy and tissue was obtained for histopathological diagnosis. Depending on the values of S. VEGF and S. CA-125, ROC curves were plotted and cut-off values were determined. All cases were categorized as Benign and malignant ovarian tumors according to cut-off values. Finally, values were correlated with the histopathology reports. Predictive values of both serum markers obtained and compared. A structured pre-tested questionnaire developed for data collection which included all the variables of interests.

Patient's blood sample was drawn from ante cubital vein by venipuncture with the subject sitting comfortably in a chair or on hospital bed. The patient's blood was obtained 24- 48 hours preoperatively and immediately centrifuged for 10 minutes. The serum was frozen at -70 degree C until examination. For the measurement of VEGF in serum, an Enzyme-linked immunosorbent assay kit (VEGF-A human ELISA EIA-4826) was used in Department of Microbiology and immunology, BSMMU. The assay recognized both natural and recombinant Hu VEGF -165.

Microsoft Office Access 2013 was used to transcript and to store all the data. SPSS 23 was used for data editing and cleaning.

Results

Table I shows the socio demographic characteristics of study population. It was observed that more than half of the patients 17 belonged to age ≤40 years in benign ovarian tumors and 30 patients of malignant ovarian tumors belong to age ≥40 years. The mean age was 38.13±17.47 years in benign ovarian tumor group and 41.36±16.33 years in malignant ovarian tumor group.

Table II shows that the median Serum VEGF of benign ovarian tumor was 241.8 pg/ml and median value of CA- 125 was 44.5 u/ml. The median serum VEGF of malignant ovarian tumor was 753.8 pg/ml and median CA-125 was 155 u/ml. The difference was statistically significant ($p<0.05$) between two groups using both biomarkers.

CA-125 for prediction of malignant ovarian tumor, true positive 42 cases, false positive 15 case, false negative 13 cases and true negative 16 cases identified by histopathology. S. VEGF for prediction of malignant ovarian tumor, true positive 50 cases, false positive 2 case, false negative 5 cases and true negative 29 cases identified by histopathology.

Table IV shows the accuracy or validity of Serum VEGF and CA-125 to differentiate malignant and benign ovarian tumor patients.

Receiver-operator characteristic (ROC) curve constructed using S. VEGF values of the patients' malignant ovarian tumors with a best combination of sensitivity and specificity which gave S. VEGF a cut-off value of 547.850 pg/ml with 90.1% sensitivity, 93.5% specificity as the value for identifying the malignant ovarian tumor. Receiver-operator characteristic (ROC) curve was constructed using CA-125 values of the patients' malignant ovarian tumor which gave CA-125 a cut-off value of 51.50 u/ml with 76.4% sensitivity and 51.6% specificity as the value for identifying malignant ovarian tumor. (Figure 1, 2).

Table I: Socio demographic characteristics of study population (n=86)

Characteristics	Benign ovarian tumor (n=31)	Malignant ovarian tumor (n=55)
Age (in years)		
≤20	7	9
21 – 30	6	8
31 – 40	4	8
41 – 50	6	16
51 – 60	5	9
61 – 70	3	2
> 70	0	3
Mean±SD	38.13±17.47	41.36±16.33
Range(min-max)	13-70	13-75
Education		
No education	3	9
Primary incomplete	2	10
Primary complete	5	11
Secondary incomplete	7	10
Secondary complete	10	13
Degree/Honors and above	4	2
Occupation		
House wife	16	39
Gov. service	2	1
Private service	4	2
Others	9	13
Average monthly income (Taka)		
<10000	4	3
1001-30000	10	17
30001- 50000	12	25
>50000	5	10
Personal habit		
Non smoker	31	55
Menstrual history		
Premenopausal	20	31
Postmenopausal	11	24
Parity		
Nulliparous	0	5
P (1- 4)	17	29
Multiparous	4	9
Co morbidities		
Hypertension	7	18
Diabetes mellitus	5	13
Thyroid disorders	1	11

Table II: Estimation of preoperative S. VEGF and CA-125 levels according to histopathology of ovarian tumors (n=86)

Group	No.	S. VEGF (pg/ml) Median	*p* value	Ca-125(u/ml) Median	*p* value
Benign ovarian tumor	31	241.8		44.5	
Malignant ovarian tumor	55		0.001[s]		0.001[s]
		753.8		155	

s= significant
p value reached from Mann-Whitney Test

Table III: Comparison of VEGF level and CA-125 level with histopathology (n=86)

	Histopathology	
CA- 125	Positive (Malignant ovarian tumor)	Negative (Benign ovarian tumor)
(≥51.50 u/ml) (n= 57)	42(True positive)	15(False positive)
(<51.50 u/ml) (n= 29)	13(False negative)	16(True negative)
S. VEGF		
(≥547.85 pg/ml) (n= 52)	50(True positive)	2(False positive)
(<547.85 pg/ml) (n= 34)	5(False negative)	29(True negative)

Table IV: Accuracy of S. VEGF and CA-125 level to differentiate malignant and benign ovarian tumor patients (n=86)

Validity Test	CA-125	VEGF
Sensitivity	76.4	90.1
Specificity	51.6	93.5
Accuracy	67.4	91.8
PPV	73.7	96.2
NPV	55.2	85.3

Receiver-operator characteristic (ROC) curve was constructed using S. VEGF values of the patients' malignant ovarian tumors with a best combination of sensitivity and specificity which gave S. VEGF a cut-off value of 547.850 pg/ml with 90.1% sensitivity, 93.5% specificity as the value for identifying the malignant ovarian tumor. Receiver-operator characteristic (ROC) curve was constructed using CA-125 values of the patients' malignant ovarian tumor which gave CA-125 a cut-off value of 51.50 u/ml with 76.4% sensitivity and 51.6% specificity as the value for identifying malignant ovarian tumor.

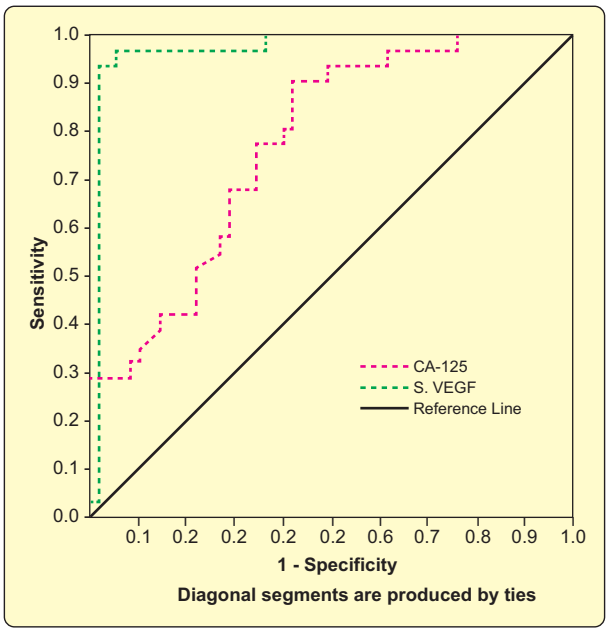

Figure 1: Receiver-operator characteristic (ROC) curve of CA-125 and S. VEGF for prediction of malignant ovarian tumor.

Discussion

In this study, mean age of benign cases was 38.13±17.47 years and that of malignant cases was

41.36±16.33 years which are consistent with the previous study where mean age of benign cases was 37.0 ± 2.4 years and malignant cases 58.9 ± 6.3 years.[9] As with most cancers, the risk of developing ovarian cancer increases with older age. Similarly, in this study more than half of women with ovarian cancer belong to age e" 40 years. The relationship between age and the outcome of ovarian cancer is uncertain. Although many researchers have pointed out that the younger age of ovarian cancer is associated with the improved outcome.

In the current study, out of 86 patients 31(36.04%) cases were benign and rest 55(63.95%) were malignant ovarian tumors. Among malignant ovarian tumors 43(78.18%) were epithelial carcinoma of ovary, 8(14.54%) were Germ cell tumors of ovary, 4(7.27%) were Sex-cord stromal tumors of ovary. In a study final ovarian pathology revealed 73 malignant ovarian masses and 77 were benign. Among malignant ovarian masses there were 63 epithelial carcinoma of ovary, Germ cell tumors were 7, sex cord stromal tumors were 2 and secondary ovarian tumor was 1.[9] Tahmina S. found that among the 96 adnexal masses 10(10.4%) cases were malignant, 33 cases were benign neoplastic, 1 was borderline and 52 cases were benign like tubo-ovarian masses, ectopic pregnancy, endometriotic cyst.[10] That study was done on non-neoplastic and neoplastic ovarian (benign and malignant) masses. But present study included only neoplastic masses.

Both the serum VEGF and CA-125 concentration were significantly lower in the benign group than the malignant group in present study. Previous study found that median value of plasma level of VEGF (168.48 pg/ml) and CA-125 (133.30 u/ml) in ovarian cancer patients were statistically higher compared to the healthy subjects (39.31 pg/ml and 9.94 u/ml; p < 0.001).[11] Other study found the higher level of serum VEGF in malignant ovarian tumor (765.51 pg/ml) compared to benign ovarian tumors (57.93 pg/ml; p <0.05).[12] Another study found that median value of serum VEGF in malignant ovarian tumors was (426.8 pg/ml) higher than control group (186.9 pg/ml; p< 0.001).[13] Robati et al found in their study that mean preoperative serum VEGF was significantly higher (47.84 vs. 20.49, p value <0.001) in cancer arm compared to benign arm. Similarly, the mean preoperative CA-125 serum level was higher (143.38 vs. 28.61, p value <0.001) in study arm than that of control arm.[14] So present study is similar to the previous studies.

Reasons for varying serum VEGF levels may be due to the differences in storage of specimens or assay techniques. The mean value of serum VEGF was higher, may be because of the maximum number (64%) of malignant masses in the study population.

In current study we compared status of serum VEGF with one of the established tumor markers for malignant ovarian tumor, i.e. CA-125. ROC curves were plotted for serum VEGF and CA-125 for prediction of malignant adnexal masses and ovarian tumors. The present study augmented the importance of VEGF as a predictor for malignancy which are close to the result of previous study.[15] They found sensitivity and specificity of VEGF-A 100% and 100%, respectively at a cut-off value of 496.8 pg/ml.[15] Another study found that the ability of VEGF to differentiate malignancy from benign masses at a cut-off VEGF level of 280 pg/ml gave a sensitivity of 76%, a specificity of 70%, a positive predictive value of 89% and a negative predictive value of 52%.[9] According to the diagnostic specificity of serum VEGF, the study showed sensitivity of VEGF and CA-125 (48% vs. 67%), specificity of VEGF and CA-125 (94% vs. 92%), PPV of VEGF and CA-125 (94% vs. 94%) and their NPV was (47% vs. 51%) at a cut-off value of 187.45 pg/ml for serum VEGF and 28.89 u/ml for CA-125, respectively.[11] In opposition to present observations, the study of Cooper et al., to assess the clinical relevance of serum VEGF levels in distinguishing patients with ovarian cancer from those with benign adnexal masses, found that at a cut-off value of 246 pg/ml for VEGF and 35 u/ml for CA-125 sensitivity was (74% vs. 90%), specificity was (71% vs. 71%), PPV (88% vs. 93%) and NPV was (48% vs. 63%), respectively.[16] So the current study did not agree with Lawicki et al. and Cooper et al.[11,16] The difference may be due to the recruitment of only epithelial ovarian tumors in previous studies and the present study included all epithelial, germ cell and sex cord stromal cell types of ovarian tumors in the present study.

The result of this study reveals that preoperative measurement of serum levels of VEGF may be a predictive biomarker due to its better sensitivity, specificity and accuracy. Thus this marker may facilitate detection of malignant ovarian tumor which might improve life expectancy of ovarian cancer patients.

Conclusion

Thus in conclusion, this study revealed that serum VEGF has better sensitivity and specificity for identifying malignant ovarian tumors. Accuracy of S. VEGF to detect malignant ovarian tumor is 91.8%. So, it suggests a promising future role of VEGF as a predictor in ovarian cancer.

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