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A Study Of Expression Of Epidermal Growth Factor Receptor (EGFR) And Vascular Endothelial Growth Factor (VEGF) In Epithelial Ovarian Neoplasms.

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ABSTRACT

Ovarian carcinoma is the 6th most common carcinoma among women in the world and forms 1.7 to 8.7% of female cancers in India. It is the most common cause of gynecological cancer death in women. Surface epithelial ovarian carcinoma accounts for 90 to 95% of ovarian malignancies. Among various prognostic indicators, EGFR a 170 kDa glycoprotein maintained its independent prognostic value and brings about increased DNA synthesis, cell proliferation, and differentiation. With the availability of EGFR inhibitors, the selection of patients for targeted therapy becomes more important. VEGF is a dimeric glycoprotein functioning as a tumor angiogenesis factor. Bevacizumab – Anti VEGF, antibody shows promise in the treatment of ovarian cancer. To study the expression of EGFR (Epidermal Growth Factor Receptor) and VEGF(Vascular Endothelial Growth Factor) in epithelial ovarian neoplasms, which could thence be, used as therapeutic targets in the future. This study was conducted in the Department Of Pathology, Karpagam Faculty of Medical Sciences & Research, Coimbatore, Tamil Nadu, India from December 2021 to December 2023.30 cases of paraffin sections of ovarian specimens diagnosed as borderline and malignant epithelial ovarian neoplasms were subjected to staining with Immunohistochemical markers-EGFR and VEGF.Out of 4 borderline ovarian neoplasms, 50% showed positivity for EGFR while 75% of them showed positivity for VEGF. Among malignancies, 80.76% of them showed EGFR positivity while 84.02% showed VEGF positivity. With Immunohistochemical analysis, the percentage of EGFR and VEGF expression showed a significant increase in malignant tumors compared to borderline tumors. Even among malignancies, EGFR and VEGF showed a significant correlation with tumor grade and FIGO stage. High-grade and advanced-stage tumors showed EGFR and VEGF overexpression compared to low-grade and early-stage carcinomas.

Keywords: Surface epithelial ovarian carcinoma, EGFR, VEGF, Immunohistochemical Analysis, Bevacizumab.

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INTRODUCTION

Ovarian carcinoma is the 6th most common carcinoma among women in the world and it ranks fifth in cancer deaths among women. Surface epithelial ovarian carcinoma accounts for 90 to 95% of ovarian malignancies.[1] Surface epithelial tumors, statistically the most important group of neoplasms are derived from surface coelomic or germinal epithelium that is continuous with the mesothelium that covers the peritoneal cavity, sharing with it a common origin and many morphological features.[2]

The ovarian surface epithelium involved in metaplastic or neoplastic conditions often undergoes 'Mullerian differentiation' and may produce any of the adult structures formed by the Mullerian ducts including tubal, endometrial, and endocervical mucosa, singly or in combination. It has also been noted that many of the surface epithelial tumors arise from the invaginated portion of the epithelium that forms surface epithelial glands and cysts. [3] Another proposed origin of some ovarian epithelial tumors (especially serous type) is the epithelium of the tubal fimbriae and fimbriae are the most common sites of early serous carcinoma in women with BRCA mutations. Type 1 tumors are slow-growing, generally confined to the ovary at the time of diagnosis, and developing from well-established precursor lesions. Type 2 tumors are rapidly growing, highly aggressive neoplasms for which well-defined precursor lesions have not been identified. More than 75% of them have TP53 mutations.[4] Ovarian carcinoma is comparatively asymptomatic in the early stage and is aptly called a "silent killer disease".70% of patients present in stages III and IV underscoring the need for early biomarkers since the survival rates vary significantly with the stage at diagnosis.[5] The long-used CA-125 is raised in only 50% of early-stage ovarian Cancers. It is also highly non-specific. The need of the hour is other complimentary biomarkers in early diagnosis and prognostication. Two of these novel biomarkers are EGFR (Epidermal Growth Factor Receptor) and VEGF (Vascular Endothelial Growth Factor). A multivariate Cox analysis regression model showed that high serum VEGF expression in stage I patients is correlated with an 8-fold increase in cancer mortality.[6] Compared to benign ovarian lesions, early-stage ovarian cancer patients showed raised levels of VEGF. Hence when used in combination with CA-125, the sensitivity was increased up to 96% and specificity up to 77%. Higher levels of EGFR and VEGF are associated with metastases, the development of ascites, and poorer prognosis.[7]

MATERIALS AND METHODS

This retrospective study was conducted in the Department of Pathology, Karpagam Faculty of Medical Sciences & Research, Coimbatore, Tamil Nadu, India from December 2021 to December 2023. Out of the total 9313 cases of histopathological specimens received, 171 were ovarian neoplasms, out of which 92 were surface epithelial ovarian neoplasms. Out of these 92 surface epithelial ovarian neoplasms,62 were benign, 4 were borderline and 26 were malignant. Case details especially age, complaints, procedure done, grade, and stage of tumors were obtained from pathology registers. Hematoxylin and Eosin sections of the paraffin tissue blocks were reviewed. Out of the 92 surface epithelial ovarian neoplasms, 26 ovarian malignancies and 4 borderline tumors were selected and their corresponding paraffin tissue blocks were obtained for immunohistochemical analysis of EGFR and VEGF. Hence IHC slides were analyzed for the presence of the reaction, and cellular localization of the staining – EGFR shows membrane and/or cytoplasmic staining. VEGF also shows cytoplasm and /or membrane staining. The percentage of tumor cells taking up the stain and the intensity with which they stain were also analyzed.

Statistical Analysis

Performed with package for social science software version 11.5. The expression of EGFR, and VEGF were correlated and studied using student t-test and chi-square test.

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RESULTS

Table 1: Frequency Of Ovarian Specimen Among Total Histopathological Specimen

	Count	Percentage
Ovarian Specimen	2435	26.15%
Others	6878	73.85%

Among ovarian lesions, 846 were non-neoplastic and 171 were neoplastic

Table 2: Frequency Of Nonneoplastic And Neoplastic Lesions Ovary

	Count	Percentage
Neoplastic	171	7.02%
Non neoplastic	846	92.98%

A total ovarian specimen of 2435, normal ovaries were 1418 constituting 58.23%, non-neoplastic ovaries were 846 constituting 34.74% and neoplastic ovaries were 171 constituting 7.02%.

Table 3:Frequency Of Normal, Neoplastic And Non-Neoplastic Ovaries

	Count	Percentage
Normal	1418	58.23%
Neoplastic	171	7.02%
Non neoplastic	846	34.74%

Among 171 ovarian neoplasms, 92 were surface epithelial ovarian neoplasms that constituted 53.801% of total ovarian neoplasms, and hence topped the list of total ovarian neoplasms and were statistically significant.

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Table 4: Frequency Of Epithelial Ovarian Neoplasms

	Count	Percentage
Epithelial-Ovarian Neoplasms	92	53.8%
Others	79	46.2%

Among 92 surface epithelial ovarian neoplasms, 62 were benign, 4 were borderline tumors and 26 were malignant.

Table 5: Frequency Of Benign, Borderline And Malignant Epithelial Ovarian Neoplasms.

	Count	Percentage
Benign	62	68%
Borderline	4	4%
Malignant	26	28%

Among the 62 benign ovarian surface epithelial tumors, the frequency of distribution of different histopathological types.

Table 6: Histomorphological Distribution Of Benign Surface Epithelial Ovarian Neoplasms

	Count	Percentage
Papillary serous cystadenoma	17	27.41%
Benign serous cystadenoma	21	33.87%
Benign mucinous cystadenoma	19	30.64%
Benign Brenner	5	8.06%

Among the 4 borderline tumors, 2 were atypical proliferating serous tumors (50%), 2 were atypical proliferating mucinous tumors (50%)

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Table 7:Histomorphological Distribution Of Surface Epithelial Ovarian Malignancies

	Count	Percentage
Papillary serous cystadenocarcinoma	9	34.61%
Mucinous adenocarcinoma	4	15.38%
Endometroid adenocarcinoma	8	30.76%
Clear cell carcinoma	4	15.38%
Adenosquamous carcinoma	1	3.81%

Table 8: Grade-Wise Distribution Of Malignant Epithelial Ovarian Neoplasms

Grade	Number of cases	Percentage
I	5	19.23%
II	9	34.61%
III	12	46.16%
Total cases	26	100%

Table 9: Percentage Of Positive Expression Of EGFR, VEGF Among Borderline Ovarian Neoplasms

IHC marker	Positive Cases	Negative Cases
EGFR	2 (50%)	2 (50%)
VEGF	3 (75%)	1 (25%)

Out of the four borderline ovarian neoplasms, 50% showed positivity for EGFR and another 50% showed negativity for EGFR.Out of four borderline epithelial ovarian neoplasms, 75% showed positivity for VEGF.

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Table 10: Distribution Of Positivity Of EGFR and VEGF Among Types Of Borderline Epithelial Ovarian Neoplasms.

IHC marker	APST Positive (%)	APMT Positive (%)	Total
EGFR	2 (100%)	Nil positive	4
VEGF	2 (100%)	1 (50%)	4

Table 11: Distribution Of Positivity Among Malignant Epithelial Ovarian Neoplasms

IHC marker	Positive cases (%)	Negative cases (%)	Total
EGFR	21 (80.76%)	5 (19.23%)	26 (100%)
VEGF	22 (84.62%)	4 (15.38%)	26 (100%)

Out of the total 26 malignant epithelial ovarian neoplasms, 21 (80.76%) of them showed positivity for EGFR and 19.23% of them were negative for EGFR.

Table 12:Distribution Of Positivity Of EGFR and VEGF Among Types Of Malignant Epithelial Ovarian Neoplasms.

Histopathological type of malignant ovarian neoplasm	EGFR Positive	EGFR Negative	VEGF Positive	VEGF Negative	Total
Papillary serous cystadenoma carcinoma	8 (88.89%)	1 (11.11%)	8 (88.89%)	1 (11.11%)	9 (100%)
Endometroid adenocarcinoma	7 (87.5%)	1 (12.5%)	7 (87.5%)	1 (12.5%)	8 (100%)
Mucinous adenocarcinoma	2 (50%)	2 (50%)	3 (75%)	1 (25%)	4 (100%)
Clear cell	4 (100%)	Nil	4 (100%)	Nil	4

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carcinoma					(100%)
Adenosquamous carcinoma	Nil positive		Nil positive		1 (100%)

88.89% of papillary serous cystadenocarcinoma ovary showed positivity for both EGFR and VEGF.87.5% of endometroid adenocarcinoma ovary showed positivity for both EGFR and VEGF. Only 50% of mucinous adenocarcinoma showed positivity for EGFR while 75% of them showed positivity for VEGF. All the clear cell carcinomas – (100% of them) showed positivity for both EGFR and VEGF. The adenosquamous carcinoma that was evaluated did not show positivity for either EGFR or VEGF.

Table 13: Table For Comparison Of Intensity Of Expression Of EGFR and VEGF Among Borderline Tumors And Malignant Epithelial Ovarian Tumors.

		EGFR			Total
		NEGATIVE	2+	3+	
MALIGNANT TUMOURS	Count	5	5	16	26
	% within EGFR	71.4%	71.4%	100.0%	86.7%
BORDERLINE TUMOURS	Count	2	2	0	4
	% within EGFR	28.6%	28.6%	0.0%	13.3%
Total	Count	7	7	16	30
	% within EGFR	100.0%	100.0%	100.0%	100.0%

Table 14: Percentage Of Expression Of EGFR In Malignant Epithelial Ovarian Neoplasms.

HPE		EGFR			Total
		NEGATIVE	2+	3+	
Papillary Serous Cystadenocarcinoma	Count	1	3	5	9
	%	11.11%	33.33%	55.56%	100.00%
Endometrioid adenocarcinoma of ovary	Count	1	2	5	8
	%	12.50%	25.00%	62.50%	100.00%

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Mucinous adenocarcinoma ovary	Count	2	0	2	4
	%	50.00%	0.00%	50.00%	100.00%
Clear cell carcinoma ovary	Count	0	0	4	4
	%	0.00%	0.00%	100.00%	100.00%
Adenosquamous carcinoma ovary	Count	1	0	0	1
	%	100.00%	0.00%	0.00%	0.00%
Borderline tumors	Count	2	2	0	4
	%	50.00%	50.00%	0.00%	13.30%
Total	Count	7	7	16	30
	%	23.33%	23.33%	53.33%	100.00%

From this table we infer that nearly 100% of clear cell carcinomas studied, 62.5% of endometroid carcinomas studied, 55.56% of papillary serous carcinomas studied and 50% of mucinous carcinomas studied showed EGFR positivity.

Table 15: Correlation Of Tumor Grade With EGFR Expression

			EGFR			Total
			NEGATIVE	2+	3+	
Tumor grade	I	Count	4	2	0	6
		% within EGFR	57.1%	28.6%	0.0%	20.0%
	II	Count	2	5	3	10
		% within EGFR	28.6%	71.4%	18.8%	33.3%
	III	Count	1	0	13	14
		% within EGFR	14.3%	0.0%	81.2%	46.7%
Total		Count	7	7	16	30
		% within EGFR	100.0%	100.0%	100.0%	100.0%

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Table 16: Correlation Of Tumor Stage With EGFR Expression

			EGFR			Total
			NEGATIVE	2+	3+	
Stage		Count	2	2	0	4
		% within EGFR	28.6%	28.6%	0.0%	13.3%
	II A	Count	3	3	4	10
		% within EGFR	42.9%	42.9%	25.0%	33.3%
	II B	Count	1	1	0	2
		% within EGFR	14.3%	14.3%	0.0%	6.7%
	III B	Count	0	1	5	6
		% within EGFR	0.0%	14.3%	31.2%	20.0%
	III C	Count	1	0	7	8
		% within EGFR	14.3%	0.0%	43.8%	26.7%
Total		Count	7	7	16	30
		% within EGFR	100.0%	100.0%	100.0%	100.0%

In this study, 75% of stage III tumors showed 3+ positivity. The higher the stage, the higher the expression of EGFR, and this correlation was statistically significant since the P value was 0.039.

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Table 17: Table For Comparison Of Intensity Of VEGF Expression Among Borderline And Malignant Epithelial Ovarian Tumors

		VEGF				Total
		Negative	1+	2+	3+	
MALIGNANT TUMOURS	Count	4	0	4	18	26
	% within VEGF	80.0%	0.0%	66.7%	100.0%	86.7%
BORDERLINE TUMOURS	Count	1	1	2	0	4
	% within VEGF	20.0%	100.0%	33.3%	0.0%	13.3%
Total	Count	5	1	6	18	30
	% within VEGF	100.0%	100.0%	100.0%	100.0%	100.0%

From this, we infer that 86.7% of malignant epithelial ovarian tumors showed varying degrees of positivity for VEGF while only 13.3% of borderline tumors showed VEGF positivity. The P value 0.009 shows that this correlation was statistically significant

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Table 18: Correlation Of VEGF With Histopathological Types Of Malignant Epithelial Ovarian Neoplasms

			VEGF				Total
			NEGATIVE E	1+	2+	3+	
HPE	Papillary Serous Cystadeno carcinoma	Count	1	0	1	7	9
		% within VEGF	11.11%	0.00%	11.11%	77.78%	100.00%
	Endometrioid adenocarcinoma of ovary	Count	1	0	2	5	8
		% within VEGF	12.50%	0.00%	25.00%	62.50%	100.00%
	Mucinous adenocarcinoma ovary	Count	1	0	1	2	4
		% within VEGF	25.00%	0.00%	25.00%	50.00%	100.00%
	Clear cell carcinoma ovary	Count	0	0	0	4	4
		% within VEGF	0.00%	0.00%	0.00%	100.00%	100.00%
	Adenosquamous carcinoma ovary	Count	1	0	0	0	1
		% within VEGF	100.00%	0.00%	0.00%	0.00%	100.00%
	Borderline tumors	Count	1	1	2	0	4
		% within VEGF	25.00%	25.00%	50.00%	0.00%	100.00%
Total		Count	5	1	6	18	30
		% within VEGF	16.67%	3.33%	20.00%	60.00%	100.00%

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This table shows that nearly 100% of clear cell carcinomas studied, 77.78% of papillary serous carcinomas studied, 62.5% of endometroid carcinomas studied, and 50% of mucinous carcinomas studied showed VEGF positivity.

Table 19: Correlation Of Tumor Grade With VEGF

EXPRESSION

Crosstab							
			VEGF				Total
			NEGATIVE	1+	2+	3+	
Grade	I	Count	2	1	3	0	6
		% within VEGF	40.0%	100.0%	50.0%	0.0%	20.0%
	II	Count	2	0	3	5	10
		% within VEGF	40.0%	0.0%	50.0%	27.8%	33.3%
	III	Count	1	0	0	13	14
		% within VEGF	20.0%	0.0%	0.0%	72.2%	46.7%
Total		Count	5	1	6	18	30
		% within VEGF	100.0%	100.0%	100.0%	100.0%	100.0%

In this study, 72.2% of grade 3 tumors showed 3+ VEGF positivity. The higher the grade, the higher the expression of VEGF, and this correlation was found to be statistically significant as the P value was 0.006

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Table 20: Correlation Of Tumor Stage With Vegf Expression

			VEGF				Total
			NEGATIVE	1+	2+	3+	
Stage		Count	1	1	2	0	4
		% within VEGF	20.0%	100.0%	33.3%	0.0%	13.3%
	IIA	Count	2	0	3	5	10
		% within VEGF	40.0%	0.0%	50.0%	27.8%	33.3%
	IIB	Count	1	0	1	0	2
		% within VEGF	20.0%	0.0%	16.7%	0.0%	6.7%
	IIIB	Count	0	0	0	6	6
		% within VEGF	0.0%	0.0%	0.0%	33.3%	20.0%
	IIIC	Count	1	0	0	7	8
		% within VEGF	20.0%	0.0%	0.0%	38.9%	26.7%
Total		Count	5	1	6	18	30
		% within VEGF	100.0%	100.0%	100.0%	100.0%	100.0%

In this study, 72.2% of stage III tumors showed 3+ VEGF positivity. The higher the stage, the higher the expression of VEGF, and this correlation was found to be statistically significant since the P value was 0.043.

DISCUSSION

VEGF as an angiogenic factor plays a critical role in tumor angiogenesis and neovascularization The clinicopathologic and prognostic value of VEGF in ovarian cancer has been investigated in different studies. Many of these studies confirmed that intratumoral VEGF is overexpressed in ovarian cancer and it could be considered to be a prognostic factor.[8] However, the association between VEGF expression and other prognostic factors including tumor stage and grade has been shown some controversies. Findings showed more frequent expression of VEGF in ovarian adenocarcinoma than in borderline and benign tumors. They found a significant, strongly-positive VEGF expression in late-stage and high-grade tumors. Some other studies also showed that VEGF overexpression is related to advanced tumor stages in patients with ovarian cancer [9]. However, some reports failed to identify a significant association between VEGF and clinicopathologic factors. Consistent with these results, our study revealed positive expression of VEGF at a significantly higher frequency in ovarian tumor specimens relative to the control group. No association between VEGF expression and age, tumor stage, and grade was found. Although it was not statistically significant, we observed positive expression of VEGF in a higher percentage of early-stage tumors (33.3%) than in late-stage tumors (20%). High expression of VEGF in early-stage disease has been reported in some tumors. In the other study researchers showed that protein and gene expression levels of VEGF are higher in early-stage patients of prostate cancer. They concluded that VEGF, as an angiogenic isoform, is overexpressed in early stages while in advanced stages of the disease, the lymphangiogenic isoform VEGF-D is up-regulated. Findings of a meta-analysis showed that intratumoral overexpression of VEGF is a significant prognostic factor in early stages, but not in late stages of ovarian cancer.[10]The correlation of EGFR expression level with aggressive phenotypes, metastasis, and poor prognosis of solid tumors including breast, gastric, and colorectal carcinoma has been reported in several studies Overexpression of EGFR in ovarian tumors has also been reported. The results of a study performed on patients with advanced ovarian tumors could not confirm the prognostic significance of EGFR and its association with clinical parameters [11]. Several studies have investigated protein expression, gene amplification, and mutations of EGFR in ovarian cancer. EGFR amplification and overexpression are related to patients' age, high tumor grade, and poor prognosis. No mutations in the EGFR gene were observed. They suggested that EGFR amplification has a greater prognostic value than EGFR protein overexpression. It also has been shown that EGFR protein expression is related to its gene amplification in primary ovarian tumors.[12]

CONCLUSION

To conclude, we can say that like all other studies even in this study – the surface epithelial ovarian neoplasms were found to be statistically the most significant one contributing 53.81% of the total ovarian neoplasms. Even among surface epithelial tumors, benign neoplasms significantly out numbered the borderline and the malignant ones and they mostly occurred in the 30 to 40 years age group. Malignant surface epithelial ovarian tumors showed peak incidence in the post-menopausal age group of 50 to 60 years. In this study, maximum cases presented at an advanced stage III. With Immunohistochemical analysis, the percentage of EGFR and VEGF expression showed a significant increase in malignant tumors compared to borderline tumors. Even among malignancies, EGFR and VEGF showed a significant correlation with tumor grade and FIGO stage. High-grade and advanced-stage tumors showed EGFR and VEGF overexpression compared to low-grade and early-stage carcinomas. EGFR and VEGF both have diagnostic and therapeutic implications. Both markers were found to be independent prognostic factors in ovarian neoplasms. Identifying these markers may also be useful for chemopreventive and chemotherapeutic strategies for patients with malignant ovarian tumors. However larger scale investigation with more samples at different stages and grades can support the results of present study.

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