Original Article

Received on 29.07.2016, **Accepted on** 10.08.2016

Indian Journal of Pathology: Research and Practice
Volume 5 Number 3, September - December 2016
DOI: http://dx.doi.org/10.21088/ijprp.2278.148X.5316.8

Implication of VEGF (Vascular Endothelial Growth Factor) in Epithelial Ovarian Neoplasms

K. Rama*, D. Kanmani**

*Professor, Dept of Pathology, Govt. Kasturba Gandhi Hospital for Women, Chennai, Tamil Nadu. **MD Pathology, Madras Medical College, Chennai, Tamil Nadu.

Abstract

Introduction: The silent killer disease-Ovarian carcinoma ranks 6th among carcinomas in women. Surface epithelial tumours constitute 90-95% of them. Vascular Endothelial Growth Factor is a Tumour angiogenesis factor. Bevacizumab-anti VEGF antibody shows promise in the treatment of ovarian cancer. Compared to benign ovarian lesions,early stage ovarian cancer patients showed raised levels of VEGF. When used in combination with CA 125, sensitivity increased upto 96%, specificity increased upto 77%. *Aims:* To study the expression of VEGF (Vascular Endothelial Growth Factor) in epithelial ovarian neoplasms which could thence be, used as therapeutic targets in future. Methods and Material: In the 3 year study from June 2012 to june 2015, out of 92 surface epithelial ovarian neoplasms received, 26 ovarian malignancies and 4 borderline tumors were randomly selected for VEGF immunohistochemistry and statistical analysis was done. *Results:* Maximum number of patients presented only at an advanced stage of ovarian carcinoma. Among malignancies, 84.02% showed VEGF positivity. Carcinomas showed higher degree of VEGF positivity compared to borderline tumors. Higher the stage and grade, greater was the expression of VEGF and these were found to be statistically significant. *Conclusions:* VEGF has both diagnostic and therapeutic implications. There is a wide arena of community based studies and research activities being carried out with this marker - opening up newer dimensions and horizons in the early diagnosis and chemotherapeutic approaches with anti VEGF antibodies in the battle against this silent killer called Cancer Ovary.

Keywords: Vascular Endothelial Growth Factor; Tyrosine Kinase; Tumor Angiogenesis.

Introduction

Neoplasms of the ovary occupy the 6th position among carcinomas in women [1]. And it is the 5th most common cause of cancer death in women [2]. Surface epithelial neoplasms form the major bulk of 90 to 95% among ovarian malignancies [3].

Surface epithelial tumours, 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 [4]. It has been suggested that majority of the surface tumours arise from the portion of the epithelium that has invaginated to produce surface epithelial glands and cysts [5]. Another proposed origin of some ovarian epithelial tumours (especially serous type) is the epithelium of the tubal fimbriae and fimbriae are the most common sitIntroduction:es of early serous carcinoma in women with BRAC mutations [6]. A new strategy divides surface epithelial tumours into 2 broad categories: Type 1 and Type 2, based on their clinicopathological features and characteristic molecular genetic changes [7].

Type 1 tumors are slow growing, generally confined to the ovary at the time of diagnosis and developing from well-established precursor lesions [8]. Type 2 tumours are rapidly growing, highly aggressive neoplasms for which well-defined precursor lesions have not been identified. More than 75% of them have TP53 mutations [9].

Corresponding Author: D. Kanmani, 5, Maria Street, Sathyanagar Extension, Padi, Chennai-600050 Tamil Nadu.
E-mail: kdurairaj1953@gmail.com

VEGF (Vascular Endothelial Growth Factor)

VEGF is a dimeric glycoprotein with the structural homology to platelet derived growth factor and may function as a tumour angiogenesis factor. VEGF has been known to play a crucial role in new vessel formation in tumors, providing nourishment for the highly metabolic tumor cells and providing access to the host vasculature [10,11].

The Prognostic and Therapeutic Impact of VEGF(Vascular Endothelial Growth Factor)

A multivariate cox analysis regression model showed that high serum VEGF expression in stage I patients is correlated with 8 fold increase in cancer mortality [10]. 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 upto 96% and specificity up to 77% [11]. Higher levels of VEGF is associated with metastases, development of ascites and poorer prognosis. Bevacizumab – Anti VEGF antibody, shows promise in the treatment of ovarian cancer.

Aims and Objectives

1. To study the expression of VEGF (Vascular Endothelial Growth Factor) in epithelial ovarian neoplasms, which could thence be, used as therapeutic targets in future.

Materials and Methods

This study is a retrospective one conducted at Institute of Social Obstetrics and Govt Kasturba Gandhi Hospital for Women and Children, Madras Medical College, Chennai for a 3 year study period from 2013 to 2015. Out of the total 9313 cases of histopathological specimens received, 192 were ovarian neoplasms of which 162 were benign, 4 were borderline and 26 were malignant.

Data Collection

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 ovarian neoplasms, 26 ovarian malignancies and 4 borderline tumors selected and their corresponding paraffin tissue blocks obtained for immunohisto-chemical analysis of EGFR and VEGF.

Procedure of Immunohistochemistry

1. 4 micron thick sections were cut from formalin fixed paraffin embedded tissue blocks and transferred onto gelatin –chrome-alum coated glass slides

Antigen	Vendor	species (clone)	Positive Control
VEGF	PathnSitu	Mouse Monoclonal	Kidney

2. The glass slides were kept in an incubator at 58 degree Celsius overnight.
3. Depanaffinisation in xylene for 15 minutes x 2 changes
4. Dehydration with absolute alcohol for 5 minutes x 2 changes
5. Washing of sections done in tap water for 10 minutes
6. Then in distilled water for 5 minutes
7. Retrieval of antigen done with microwave oven with sections immersed in Tris EDTA buffer for 20 minutes
   a. 800 watts – 5 minutes
   b. 600 watts – 10 minutes
   c. 400 watts – 5 minutes
8. Cool the slides to room temperature and then washed with distilled water for 10 minutes.
9. Then washed in phosphate buffer for 5 minutes x 2 changes
10. Application of peroxidase block over the sections for 10 minutes
11. Slides washed with phosphate buffer for 5 minutes.
12. Appropriate primary antibody was applied over the sections and incubated for half an hour.
13. After washing with wash buffer, polyexcel target binder reagent applied for 15 minutes.
14. Slides were washed with 2 changes of buffer for 2 minutes.
15. Sections were covered with HRP micropolymer for 15 minutes
16. Washed with phosphate buffer for 2 minutes
17. 1 drop of DAB chromogen (prepared by diluting 1 drop of DAB chromogen to 1 mL of DAB buffer) was applied for 2-5 minutes

18. Counterstaining was done with hematoxylon, washed in running tap water, air dried, cleared with xylene and mounted.

Interpretation and Scoring

The IHC slides were analysed for the presence of the reaction, cellular localization of the staining – VEGF shows cytoplasm and / or membrane staining. Percentage of tumor cells taking up the stain and the intensity with which they stain were also analysed [21].

Statistical Analysis

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

Observation and Results

In the 36-month study performed from June 2012 to June 2015, 9313 specimen were received at Department of Pathology, Institute of social obstetrics and Govt. Kasturba Gandhi Hospital for women and children for histopathological examination. Out of the total 9313 cases, Ovarian specimen were 2435, of which, 171 were neoplastic, 1418 were normal and 846 were non-neoplastic.

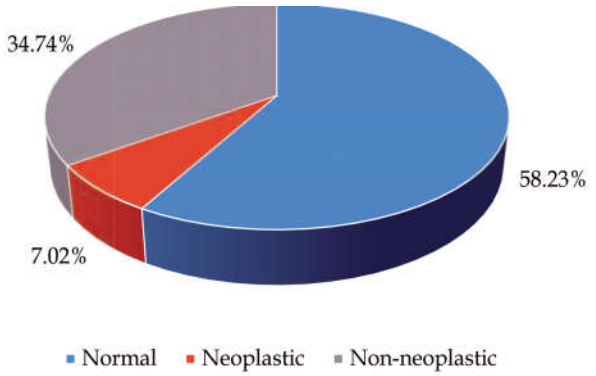

Chart 1: Percentage of normal,neoplastic and non neoplastic lesions among total ovarian specimen

Amidst 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 .

Amidst 92 surface epithelial ovarian neoplasms, 62 were benign, 4 were borderline tumours and 26 were malignant. Amidst the 62 benign ovarian surface epithelial tumours, the frequency of distribution of different histopathological types were:

Table 1: Frequency of epithelial ovarian neoplasms

	Count	Percentage
Epithelial Ovarian Neoplasms	92	53.8%
Other	79	46.2%

Papillary serous cystadenoma	70%
Benign serous cystadenoma	4%
Benign mucinous cystadenoma	30.64%
Benign Brenner	8.06%.

Amidst the 26 surface epithelial ovarian malignancies, the different histopathological types were:

Papillary serous cystadenocarcinoma	34.61%
Mucinous adenocarcinoma	15.38%
Endometrioid adenocarcinoma	30.76%
Clear cell carcinoma	15.38%
Adenosquamous carcinoma	3.81%.

Benign epithelial ovarian neoplasms had a peak incidence at age group of 31 – 40 years that constitutes about 40.24% followed by the age group of 41 – 50 years that formed about 24.38%. Mean age was about 33.33 years .

Maximum incidence of borderline epithelial ovarian neoplasms was found in the age group of 41-50 years. Mean age affected was found to be 47.21 years .

Maximum incidence of malignant epithelial ovarian tumours was found in the age group of 51 to 60 years followed by 41 to 50 years. Mean age affected was found to be 54.5 years. Maximum number of malignant tumours presented in grade III .

Results of Immunohis to Chemical Analysis

All the 26 malignant epithelial ovarian neoplasms and 4 borderline epithelial tumors were subjected to immunohistochemical marker VEGF(Vascular Endothelial Growth Factor)

Out of four borderline epithelial ovarian neoplasms, 75% showed positivity for VEGF.

Out of the total 26 malignant epithelial ovarian neoplasms, 22 (84.62%) of them showed positivity for VEGF while only 4 (15.38%) of them were negative for VEGF.

Positivity of VEGF among types of malignant epithelial ovarian neoplasms.

- 88.89% of papillary serous cystadenocarcinoma ovary showed positivity for VEGF.
- 87.5% of endometrioid adenocarcinoma ovary showed positivity for VEGF.
- 75% of mucinous adenocarcinomas showed positivity for VEGF.
- All the clear cell carcinomas – (100% of them) showed positivity for VEGF.

This table shows that nearly 100% of clear cell carcinomas studied, 77.78% of papillary serous

Table 2: Distribution of malignant epithelial ovarian neoplasms according to the FIGO (International Federation of Gynaecology and Obstetrics) stage

Stage	Number of cases	Percentage
I	4	15.38%
IIA	8	30.76%
IIB	2	7.69%
IIIB	5	19.23%
IIIC	7	26.92%
Total cases	26	100%

Table 3: Correlation of VEGF with histopathological types of malignant epithelial ovarian neoplasms

			VEGF				Total
			NEGATIVE	1+	2+	3+	
HPE	Papillary Serous Cystadenocarcinoma	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%

Table 4: Correlation of tumour stage with VEGF expression

Crosstab							
			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%
	II A	Count	2	0	3	5	10
		% within VEGF	40.0%	0.0%	50.0%	27.8%	33.3%
	II B	Count	1	0	1	0	2
		% within VEGF	20.0%	0.0%	16.7%	0.0%	6.7%
	III B	Count	0	0	0	6	6
		% within VEGF	0.0%	0.0%	0.0%	33.3%	20.0%
	III C	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%

P=0.043

carcinomas , 62.5% of endometrioid carcinomas , and 50% of mucinous carcinomas showed VEGF positivity .

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

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

COLOR PLATES

Fig. 1: HPE NO:1842/15,Papillary serous cystadeno carcinoma ovary

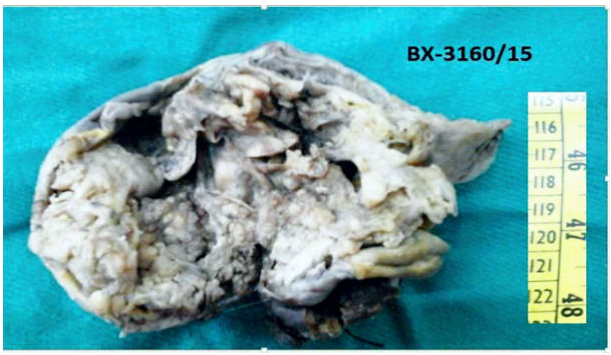

Fig. 2: HPE NO:3160/15,Endometrioid adenocarcinoma ovary

Fig. 3: HPE NO:877/15 Mucinous adenocarcinoma ovary

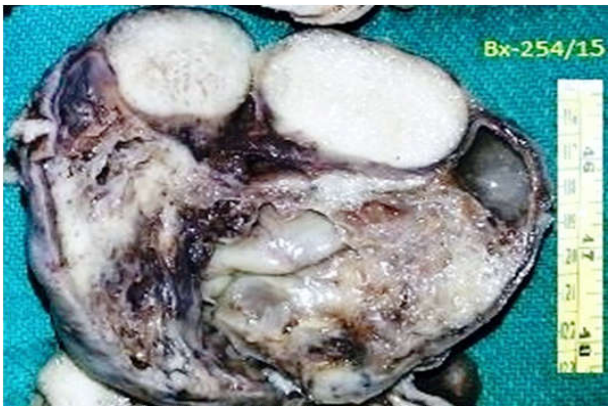

Fig. 4: HPE NO:254/15 Clear cell adenocarcinoma ovary

Fig. 5: HPE NO:404/14 Adenosquamous carcinoma ovary

Fig. 6: HPE NO:305/13-Atypical Proliferating Serous

IHC Profile of papillary serous cystadenocarcinoma

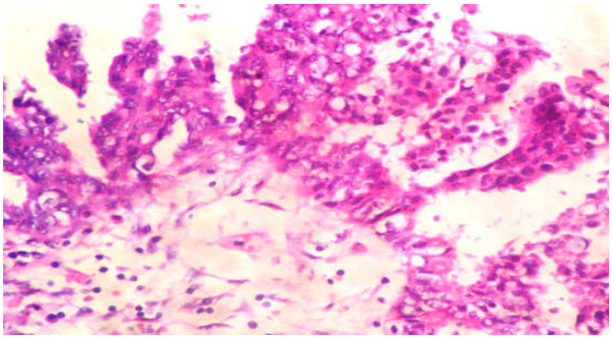

Fig. 7: H&E-High power view

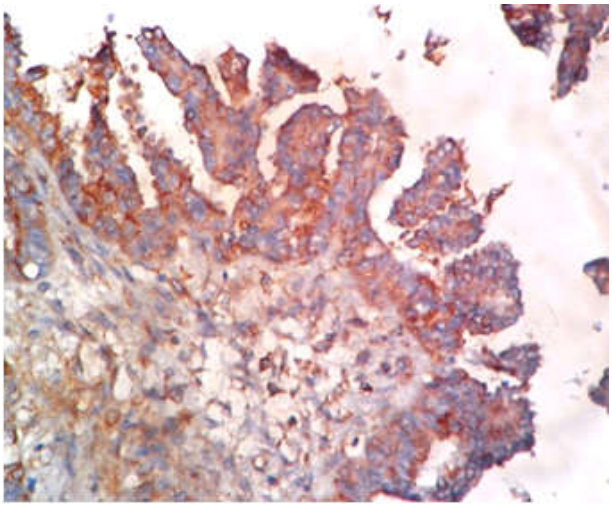

Fig. 8: VEGF Score 3+

IHC Profile of endometrioid adenocarcinoma ovary

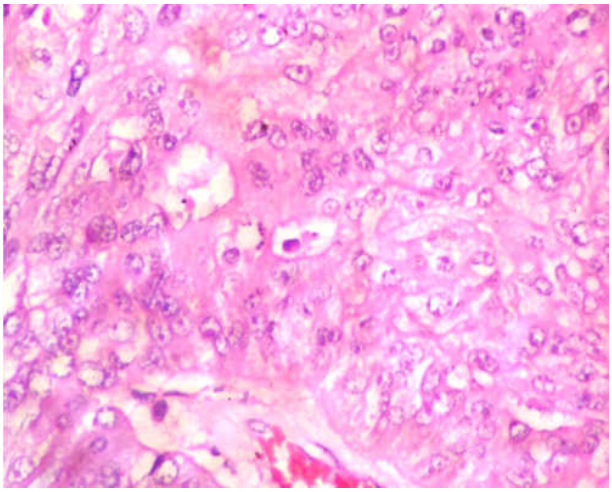

Fig. 9: H&E High power view

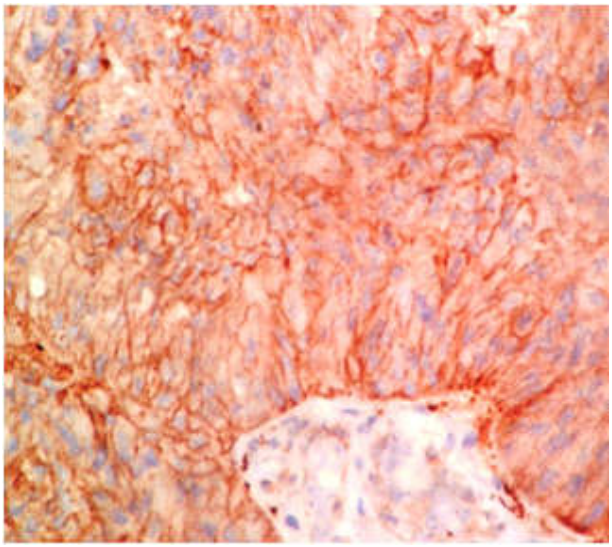

Fig. 10: VEGF Score 3+

IHC Profile of mucinous adenocarcinoma ovary

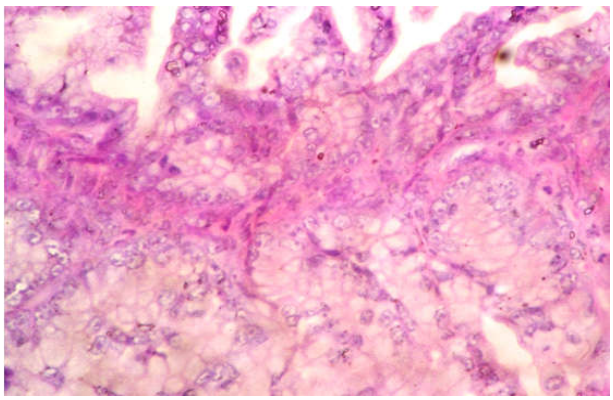

Fig. 11: H&E High power view

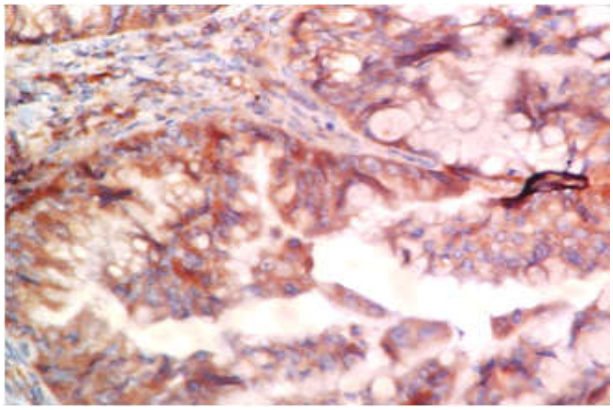

Fig. 12: VEGF Score 3+

IHC Profile of clear cell adenocarcinoma

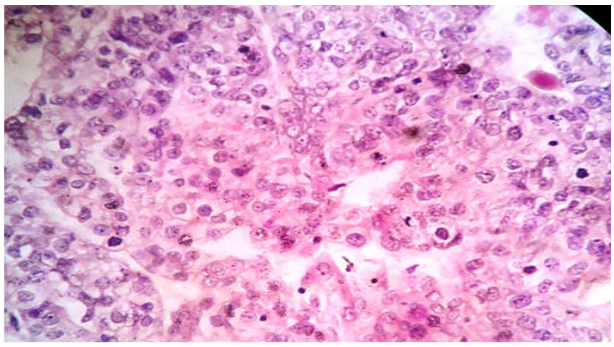

Fig. 13: H&E High power view

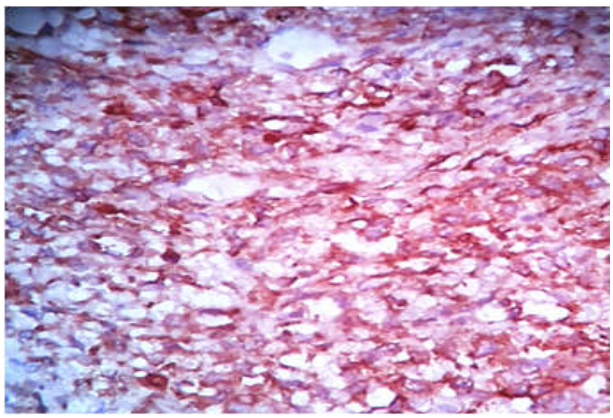

Fig. 14: VEGF Score 3+

IHC Profile of adenosquamous carcinoma

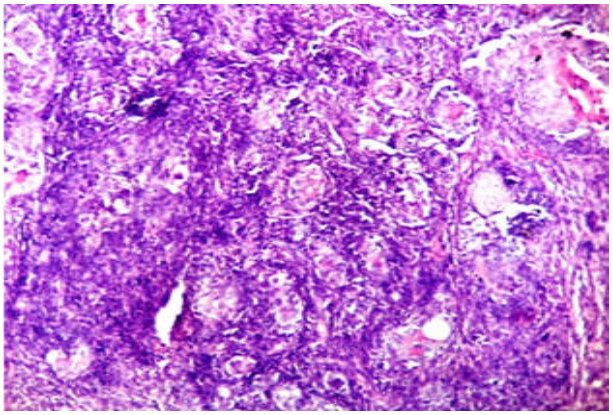

Fig. 15: H&E High power view

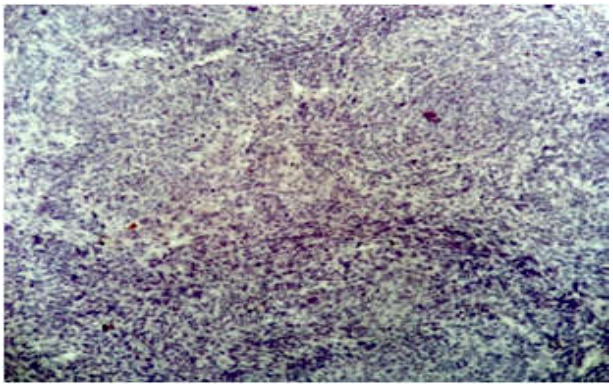

Fig. 16: VEGF Negative

IHC Profile of borderline tumors ovary

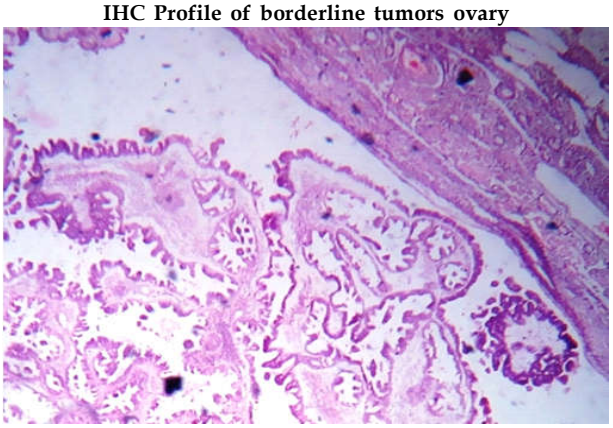

Fig. 17: H&E Low power view

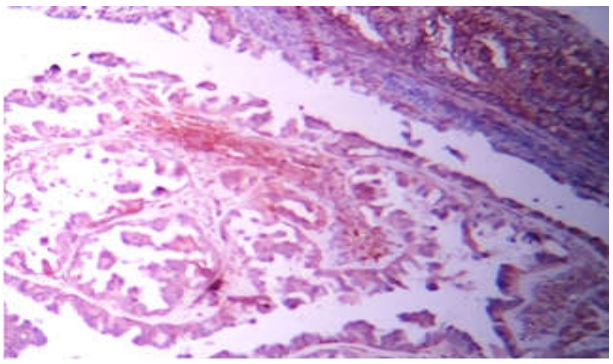

Fig. 18: VEGF Positive, low power view

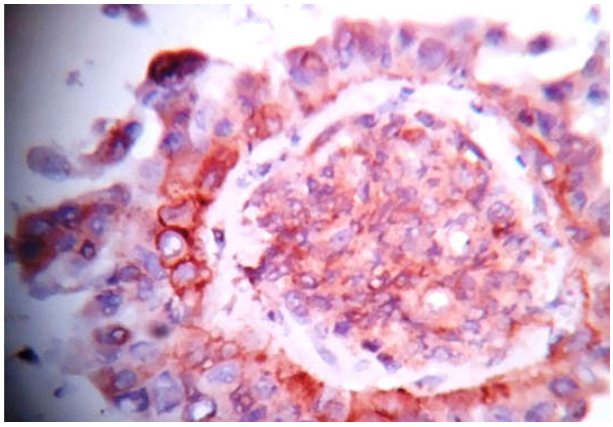

Fig. 19: VEGF Score 3+ High power view

Discussion

Amidst neoplasms of female genital tract, ovarian carcinoma carries maximum morbidity and mortality as there are no easy or effective screening techniques and most of the ovarian neoplasms present at an advanced stage, since early stages are predominantly asymptomatic.

Tumor Grade

In the study by Bashir Ahmad et al, poorly differentiated (grade III) tumours topped the list among different malignant epithelial ovarian neoplasms which are similar to the current study where the frequency of grade 3 tumors was found to be 46.7%.

Tumor Stage

In the study by Mohamed Farouk et al, percentage of epithelial ovarian carcinomas presenting at Stage III was given as 50.6% [10,11] which are similar to the current study where maximum cases presented in the late stage III constituting about 46.15%.

Immunohistochemical Analysis

VEGF

Vascular Endothelial Growth Factor a dimeric polypeptide has got potent mitogenic effect on endothelial cells. It plays an important role in regulation of angiogenesis process during embryogenesis. It also plays a vital role in cancer – neoangiogenesis [8,9]. VEGF is a multifunctional cytokine that causes increase in microvascular permeability and density, nourishing the highly metabolic tumour cells and also provides access to the host vasculature [12].

In the study by Jun Wang et al, only 30% of borderline epithelial ovarian tumours and 80% of malignant epithelial ovarian tumours were positive for VEGF expression [13].

In the study by S.Yamamoto et al, 97% of ovarian carcinomas showed positive immunostaining for VEGF while 52% of borderline epithelial ovarian tumours showed positivity [13].

In the study by Hel, Zhao X et al, 80% of ovarian carcinomas, 21% of borderline epithelial ovarian tumours showed positive VEGF immunostaining [14].

In our present study, positive VEGF expression was found in 75% of borderline and 84.62% of malignant epithelial ovarian tumours.

VEGF expression showed statistically significant correlation between FIGO stage and lymph node metastasis.

Even in our present study, there was a positive correlation of VEGF with tumour grade and stage with P value of 0.006 and 0.043 respectively.

Conclusion

To conclude, we can say that, the epithelial ovarian neoplasms were found to be statistically the most significant one contributing 53.81% of the total ovarian neoplasms [15]. As in the society even in this study, maximum cases presented at an advanced stage III.

With Immunohistochemical analysis, the percentage of VEGF expression showed a significant increase in malignant compared to borderline tumours. Even among malignancies, VEGF showed a significant correlation with tumour grade and FIGO stage. High grade and advanced stage tumours showed VEGF overexpression compared to low grade and early stage carcinomas.

Thus VEGF has got both diagnostic and therapeutic implications in the treatment of surface epithelial ovarian neoplasms.

References

1. Journal of Oncology Volume 2012, ID 540791, 14 pages, 2012, DOI 10.1155/2012/540791.
2. "Ovarian Cancer Statistics" cancer research UK retrieved 28 Oct 2014.
3. Bradshaw, KarenD, Scharge, JohnO, Schaffer, Joseph, Lisa M, Halvorson, Haffman, Barbara G. Williams' Oncology. McGraw-Hill Professional; 2008. ISBN-0-07-147257-6.
4. 4 Jarboe E, Folkins A, Serous carcinogenesis in the fallopian tube: a descriptive classification. Int. J. Gynecol. Pathol 2008; 27:1-9.
5. Kurman RJ, Shih I-M. The origin and pathogenesis of epithelial ovarian cancer: a proposal unifying theory. AmJSurgPathol 2010; 34:433-443.
6. Colgan TJ, Norris HJ, Ovarian epithelial tumors of low malignant potential. A review. IntJ.Gynecol Pathol 1983; 1:367-382.
7. Crum CP, Dropkin R, Kindelberger D, Medeiras F, Miron A, Lery. Lessons from BRCA: the tubal fimbria emerges as an origin from pelvic serous cancer. Clin Med Red 2007; 5:35-44.
8. Neufold G, Tessler R, Gitay-Goren H, Cohen T, LeviB2. Vascular endothelial growth factor and its receptors. Prog Growth Factor Red 1994; 5:89-97.
9. Senger DR, Vandacuoter L, Brown LF et al. Vascular permeability factor in tumor biology cancer metastasis Rev 1993; 12:303-24.
10. Mohammed Farouk Mostafa et al 'Retrospective analysis evaluating ovarian cancer cases presented at the clinical oncology department, Alexandria university' – Alexandria Journal of Medicine 2012 Dec; 48:353-360.
11. Michelle A Roest. Ovarian cancer: An overview American Family Physician. 2009 Sep 15; 80(6): 609-616
12. JunWang et al – Int J. Cancer 2002 97, 163-167. Neufeld Getal – VEGF and its receptors. Prog growth factor Res 1994; (5):89-97.
13. Yamamoto et al – Expression of VEGF in epithelial ovarian neoplasms Br J Cancer 1997; 76(9):1221-1227.
14. Hel et al – Expression of VEGF in epithelial ovarian cancer and its relationship to lymphatic metastasis.
15. Hatak , expression of VEGF gene in epithelial ovarian cancer – Anin Cancer Res 2011 Feb; 31(2): 731-737
16. Raica M, Cimpean AM, Anghel A. Immunohisto-chemical expression of vascular endothelial growth factor (VEGF) does not correlate with microvessel density in renal cell carcinoma. Neoplasma 2007; 54:278-84.