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ANALYSIS OF TUMOUR VASCULARIZATION BY MICROVESSEL DENSITY AND ITS PROGNOSTIC SIGNIFICANCE IN SURFACE EPITHELIAL OVARIAN NEOPLASMS

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ABSTRACT

Ovarian malignancy is one of the most common cancers contributing to significant morbidity and mortality. Tumour vascularization is critical to growth and metastasis of the tumour determining the course of disease. Microvessel density is a reliable indicator of degree of vascularization in a tumour and can be used as a parameter to compare aggressiveness. The purpose of this study was to evaluate the degree of tumour vascularization across a spectrum of surface epithelial ovarian tumours by calculating microvessel density. Immunohistochemistry was used to identify CD34 and quantify the microvessels observed. Our study revealed that malignant ovarian tumours have a higher microvessel density as compared to benign and borderline tumours. This finding is significant in context of treatment of advanced ovarian cancers by anti-angiogenic drugs to improve survival rates. Hence, microvessel density is an important prognostic factor in ovarian malignancy and can be used to monitor treatment using novel targeted therapy.

KEYWORDS

Ovarian carcinoma, Surface epithelial ovarian tumours, Tumour vascularization, CD34, Microvessel density.

INTRODUCTION

Ovarian carcinomas account for 3% of all cancers in females.[1] Ovarian carcinomas represent sixth most common female cancers and fourth leading cause of death due to cancers in women. Surface epithelial tumours are commonest variety of ovarian tumours followed by germ cell tumours.[2]

Epithelial ovarian cancers originate from normal ovarian surface epithelium or from inclusion cysts arising from surface epithelium.[3] Recent evidence shows that high grade serous carcinoma arises from fallopian tube.[4] Due to its insidious onset and paucity of symptoms, most patients present with advanced disease and 5 year survival rates are approximately 20%.[5]

Angiogenesis is a critical factor in tumour growth and metastasis because tumour proliferation is severely limited by the nutrient supply to proliferating cells.[6] Hence tumorigenesis of malignant neoplasms is associated with extensive neovascularization.

Analysis of tumour vascularization by microvessel density and its prognostic significance has been evaluated in many tumours in earlier studies by Toi M et al, Weidner et al, Macchiarini P et al.[7-12] But there is a paucity of literature regarding characteristics of tumour vascularization in ovarian surface epithelial tumours. Hence the present study aims to evaluate microvessel density in ovarian surface epithelial tumours by using Immunohistochemistry.

CD34 is a cell surface protein expressed on endothelial cells. Upregulation of CD34 is associated with increased neovascularization during tumour development.[13] In this study we have evaluated CD34 by antibodies to it using Immunohistochemistry. The purpose of this study was to evaluate the microvessel density in ovarian surface epithelial tumours and to study the difference in angiogenesis between benign and malignant tumours.

Studies have shown that antiangiogenic agents have an important role in treatment of ovarian cancer[14] and the most promising group of drugs are anti-VEGF drugs especially Bevacizumab.[15] Recent advances in treatment have resulted in improvement in 5 year survival in patients with epithelial ovarian cancer.[16] The greatest success in epithelial ovarian cancer has come from targeting angiogenesis.[17,18,19]

MATERIALS & METHODS

This cross-sectional, retrospective study was performed at Gandhi Medical College, Secunderabad in the state of Telangana, India. The study was performed in the Department of Pathology from June 2015 to July 2017. All specimens of ovarian surface epithelial tumours with adequate clinical history were included and other ovarian tumours were excluded from the study.

The specimens were fixed in 10% formalin for 24 hours and then meticulously grossed. Representative bits were taken from the specimens and submitted for processing. The tissue bits were routinely processed and sections of 3-4 micron thickness were cut and stained with Haematoxylin and Eosin stains. The sections were then studied under microscopy and the results were recorded. Imntimunohistochemistry was done using Dako antibody to Cd34.

Microvessel Density was calculated from CD34 stained sections by using the method proposed by Weidner et al.[20] In each section, three most vascular areas were chosen using low power field (10X)-HOTSPOTS. Number of microvessels in each hotspot was counted using high power field (40X) and average of three hotspots was recorded. Brown staining of endothelial cells, separated from adjacent microvessels, connective tissue and tumour cells was considered as single countable microvessel.[13] Counts were expressed as total number of microvessels per hpf40X (0.196mm^2). This data was referred to as Microvessel Density count (MVD). MVD was calculated for all the tumours and compared between benign and malignant tumours. P value was calculated using student t test.

This study was approved by the institutional Human Ethics Committee.

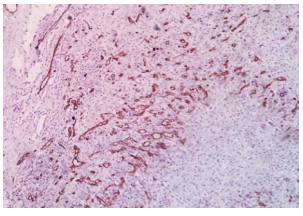

Figure 1: IHC Stain- CD34 - 10X- serous carcinoma showing Hotspot

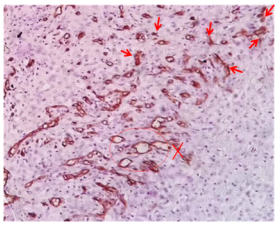

Figure 2: IHC Stain - CD34 - 40X - Serous Carcinoma.

Large lumen vessels are excluded. Few microvessels which can be counted are shown by arrows

RESULTS

Out of the 100 epithelial ovarian tumours studied, the youngest age at diagnosis was 29 years and the oldest was 65 years. Our study revealed that of the 100 cases, 62 were benign, 8 were borderline and 30 were malignant. 56 of the tumours were classified as serous, 40 were classified as mucinous and 4 were determined to be Brenner tumours.

Table No 1: Age Distribution

Age	Number of Patients
20-29	01
30-39	11
40-49	56
50-59	28
60-69	04
Total	100 Cases

Table No 2: Histomorphological Distribution Of Surface Epithelial Tumours Of Ovary

Type of Tumour	Benign n= 62 Cases(62%)	Borderline n= 8 Cases (8%)	Malignant n = 30 Cases (30%)
SEROUS TUMOURS n=56(56%)	36	04	16
MUCINOUS TUMOURS n=40(40%)	24	04	12
BRENNER TUMOURS N=4(4%)	02	--	02

Table No 3: Frequency Distribution of Surface Epithelial Tumours of Ovary

Histomorphological Type	Number of Cases (n=100)
Serous Cystadenoma	28
Serous Adenofibroma	08
Serous Borderline Tumour	04
Serous Carcinoma	16
Mucinous Cystadenoma	24
Mucinous Borderline Tumour	04
Mucinous Carcinoma	12
Brenner Tumour	02
Malignant Brenner Tumour	02

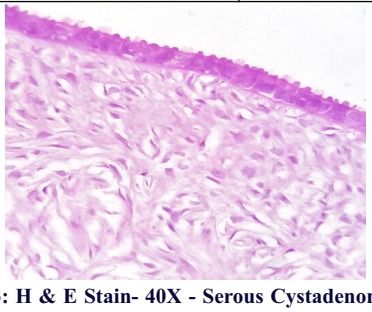

Figure No 3: H & E Stain- 40X - Serous Cystadenoma Showing Cyst Wall Lined By Cuboidal Epithelium

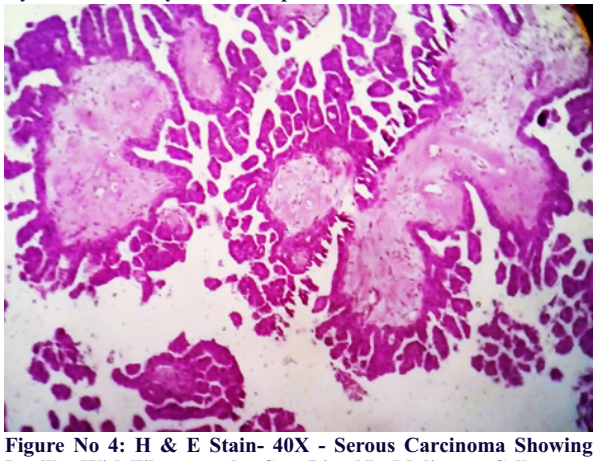

Figure No 4: H & E Stain- 40X - Serous Carcinoma Showing Papillae With Fibrovascular Core Lined By Malignant Cells

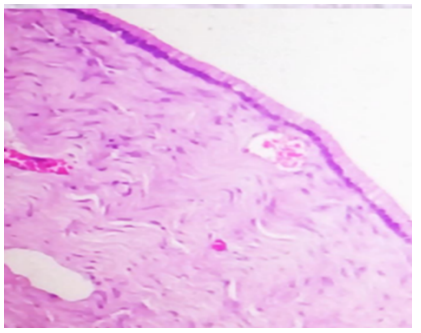

Figure No 5: H & E Stain- 40X - Mucinous Cystadenoma Showing Cyst Wall Lined By Simple Mucinous Epithelium

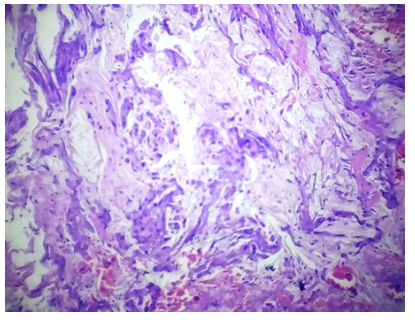

Figure No 6: H & E Stain- 40X - Mucinous Carcinoma Showing Pools Of Mucin

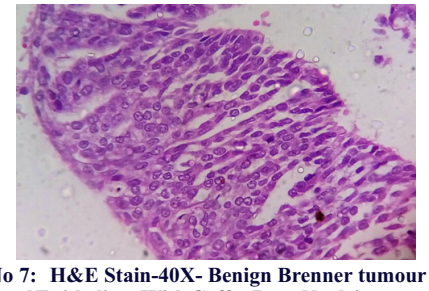

Figure No 7: H&E Stain-40X- Benign Brenner tumour Showing Transitional Epithelium With Coffee Bean Nuclei

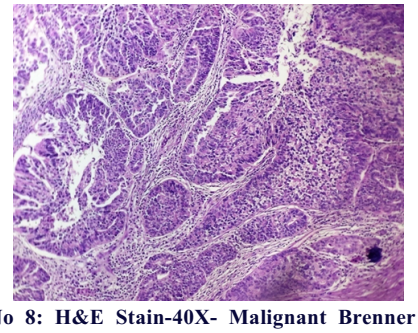

Figure No 8: H&E Stain-40X- Malignant Brenner Tumour Showing Malignant Transitional Epithelium With Focus Of Benign Island

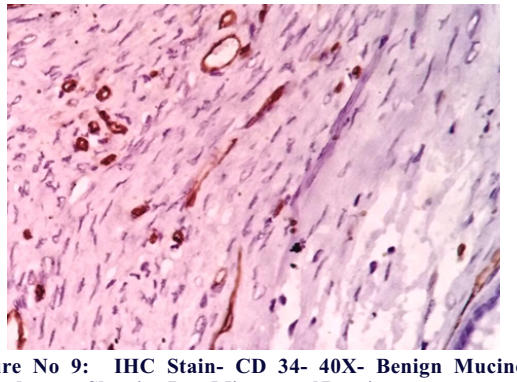

Figure No 9: IHC Stain- CD 34- 40X- Benign Mucinous Cystadenoma Showing Low Microvessel Density

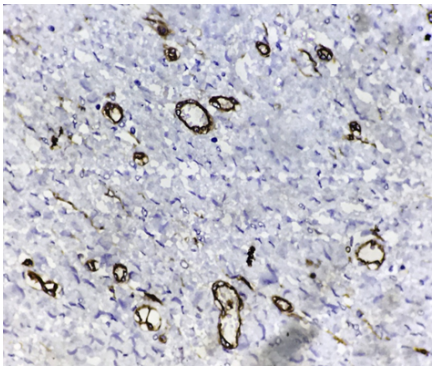

Figure No 10: IHC stain - CD 34- 40x- Benign Serous Cystadenoma Showing Low Microvessel Density

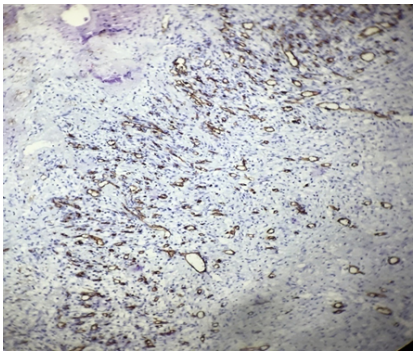

Figure No 11: IHC Stain- CD 34 –10X- serous Carcinoma Showing Hot Spot

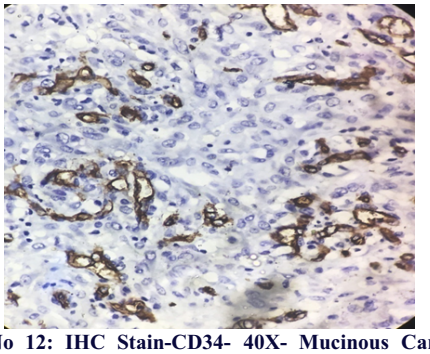

Figure No 12: IHC Stain-CD34- 40X- Mucinous Carcinoma Showing High Microvessel Density

Table No 4: Micro Vessel Density of Benign ovarian surface epithelial Tumours

Benign Tumours	Number of Cases	Mean MVD / HPF (SD)
Serous Cystadenomas	36	4.50
Mucinous Cystadenoma	24	4.33
Brenners	02	06
Total	62	4.48(0.99)

NOTE: Mean microvessel density per high power field of benign ovarian surface epithelial tumours is 4.48 ± 0.99

Table No 5: Micro Vessel Density Of Borderline Ovarian Surface Epithelial Tumours

Borderline Tumours	Number of Cases	Mean MVD/HPF
Serous Borderline Tumour	04	7.5
Mucinous Borderline Tumour	04	7.5
Total	08	7.5

NOTE: Mean micro vessel density per high power field of borderline ovarian surface epithelial tumours is 7.5

Table No 6: Micro Vessel Density Of Malignant Ovarian Surface Epithelial Tumours

Malignant Tumours	Number of cases	Mean MVD /HPF (SD)
Serous Carcinoma	16	17.37
Mucinous Carcinoma	12	16.33
Malignant Brenner Tumour	02	11
Total	30	16.53(2.36)

NOTE: Mean Micro vessel density per high power field of malignant ovarian surface epithelial tumours is 16.53 ± 2.36

Table No 7: Comparision Of Micro Vessel Density Of Ovarian Surface Epithelial Tumours

Type of Tumour	Mean MVD/HPF
Benign	4.48
Borderline	7.5
Malignant	16.5

NOTE: Mean Micro Vessel Density of malignant Ovarian Surface Epithelial Tumours (16.5) is high compared to benign (4.48) and borderline tumours (7.5)

Table No 8: Comparison Of Micro Vessel Density Between Benign And Malignant Ovarian Surface Epithelial Tumours

Parameter	Benign Tumours	Malignant Tumours	P Value Student T Test
Mean of MVD/HPF	4.45	7.5	<0.001

NOTE: P Value By Student T Test Is Less Than 0.001 Indicating Statistical Significance Of The Study

DISCUSSION

Ovarian cancer is a malignancy with high mortality and aggressive course associated with poor prognosis in majority of the patients. The aim of the present study was to evaluate the microvessel density and compare it across a spectrum of ovarian surface epithelial tumours ranging from benign to malignant. The result obtained were reviewed in the light of other publications.

Our study revealed serous tumours to be the commonest (56%) similar to studies of Hollingsworth et al[21], Arjunan et al[13], Sehgal et al[22]. Abulafia et al[23] studied angiogenesis on ovarian tumours in 83 patients by calculating microvessel density using antibodies against vWF. Study showed that angiogenic switch occurs between benign and borderline and malignant tumours and microvessel density might help to differentiate between benign and invasive tumours. Wang et al[24] did a study on characteristics of contrast enhanced ultrasound and its utility in assessing microvessel density in ovarian tumours which showed that microvessel density is higher in malignant tumours as compared to benign. Sehgal et al[22] conducted a study on 42 cases of ovarian surface epithelial tumours. Microvessel density was calculated using antibodies against CD 34. Results showed that malignant tumours showed higher microvessel density as compared to benign ovarian tumours and similar findings were obtained in our study. In our study 8 cases of borderline surface epithelial tumours were diagnosed; 4 each of serous and mucinous type. In these borderline tumours, the microvessel density was found to be higher than benign tumours and lower than malignant tumours.

The idea of neo-angiogenesis in solid tumour provided an impetus for development of therapeutic agents that block angiogenesis. Role of angiogenesis in ovarian carcinoma was uncertain until Hollingsworth et al[21] study suggested that the degree of neovascularization is a useful prognostic factor which determines disease free survival. Our study concluded that average microvessel density was significantly higher in malignant ovarian surface epithelial tumours and high microvessel density was associated with transformation and acquisition of invasive phenotype of advanced epithelial ovarian cancers. Studies done by Hollingsworth et al[21] and Arjunan et al[13] corroborate our findings.

Ogawa et al[25] study using antibodies to CD34 in surface epithelial tumours of ovary showed that microvessel density is an independent prognostic factor. Rossochacka-Rostalska[26] study using antibodies to CD31 and CD34 in ovarian carcinomas and Van Diest et al[27] study in advanced epithelial ovarian cancer showed that increased microvessel density is associated with bad prognosis. Gasparini et al[28] reported that microvessel density is the most significant predictor of metastatis of breast carcinoma.

Anti-angiogenic therapy is a very exciting and promising new treatment modality for advanced ovarian cancers and is being used as frontline therapy for aggressive malignant surface epithelial ovarian

cancers currently[17,18,19]. Bevacizumab, a humanized anti-VEGF monoclonal antibody is the most widely studied drug. Novel approach of treatment of advanced ovarian cancers by using combination of Bevacizumab and other targeted drugs by modifying tumour microenvironment have led to improvement in 5 year survival rates[16].

Table No 9: Supporting Studies of Micro Vessel Density

Study	Sample size	Mean MVD / hpf - Beingn	Mean MVD / hpf – Malignant	P value
Present study	50	4.48 / 0.196 mm^2	16.53 / 0.196 mm^2	< 0.001
Sehgal et al[49]	42	16.53/ 0.375 mm^2	34.5/ 0.375 mm^2	< 0.001
Arjunan et al[14]	50	14.88/ 0.375 mm^2	26.09/ 0.375 mm^2	0.002

CONCLUSION

Microvessel density is useful in assessing the degree of angiogenesis in a tumour with high microvessel density being directly linked to local growth and distant metastasis. Our study concluded that microvessel density is an independent prognostic factor and a predictive tool for determining outcome in ovarian malignancy. In view of these results, new therapy should aim to alter the tumor microenvironment leading to decreased angiogenic capacity of tumour cells.

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