Study of Vascular Endothelial Growth Factor (VEGF) Expression in Serous and Mucinous Surface Epithelial Ovarian Tumours in Thrissur

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ABSTRACT

BACKGROUND

Ovarian carcinoma is one of the leading causes of mortality and morbidity in females of the perimenopausal age of which surface epithelial ovarian tumours are the majority. These are often detected at late stages owing to their asymptomatic nature and have a dismal prognosis despite treatment, thus necessitating the need for novel therapeutic agents. Vascular endothelial growth factor (VEGF) is a key mediator of angiogenesis that may be a potential target. The purpose of this study was to assess the expression of VEGF in serous and mucinous surface epithelial ovarian tumours and determine whether there is any relationship between VEGF expression and tumour grade.

METHODS

H and E slides of serous and mucinous surface epithelial ovarian tumours were taken and immunohistochemical staining for VEGF was done. The intensity and percentage of staining was studied in different tumours and a score was obtained. VEGF score was also compared with the grade of the tumour.

RESULTS

Out of 50 cases, 92 % showed VEGF expression, with 60 % showing strong staining and 40 % weak to moderate staining. VEGF expression significantly increased in malignant tumours as compared to benign and borderline ones. There was no significant difference in the VEGF expression with grade of the tumour.

CONCLUSIONS

As VEGF expression is higher in malignant cases, anti-VEGF drugs like bevacizumab may have a therapeutic role in ovarian cancer. Also, further studying of the mechanisms by which VEGF is expressed in malignant ovarian tumours may open up newer potential targets. As no significant difference between VEGF expression and grade/stage of disease was obtained, further studies are needed to confirm the prognostic role of VEGF

KEYWORDS

Vascular Endothelial Growth Factor, Surface Epithelial Ovarian Tumours, Serous, Mucinous, Ovary

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DOI: 10.18410/jebmh/2021/660

How to Cite This Article:
Eappan RA, Valooran JL, Jayasree K. Study of vascular endothelial growth factor (VEGF) expression in serous and mucinous surface epithelial ovarian tumours in Thrissur. J Evid Based Med Healthc 2021;8(42):3654-3658. DOI: 10.18410/jebmh/2021/660

Submission 15-06-2021,
Peer Review 23-06-2021,
Acceptance 18-11-2021,
Published 30-11-2021.

BACKGROUND

Ovarian cancer is the seventh most common cancer worldwide. In India, it is third most common among women. Epithelial ovarian cancer comprises 90 % of ovarian cancer.[1] The incidence of ovarian cancer increases with age. The symptoms of epithelial ovarian tumours are vague and most patients present at a late stage. There is no effective screening method to detect early cases and cases detected late have a very poor prognosis. Seventy-five percent of patients who initially respond to conventional chemotherapy have a fatal relapse, [2] thus necessitating the need for newer therapeutic agents. Surface epithelial tumours are essentially tumours of perimenopausal and postmenopausal age group. Majority of them are diagnosed at an age of around 40 years with invasive carcinomas being around 60 years. Most of them are sporadic with only 5 – 10 % being familial.[3] Angiogenesis is essential for tumour growth and survival as any tumour cannot grow beyond 2 mm without vessels. Angiogenic switch i.e., transition of a tumour from an avascular state to a vascular state takes place with the help of many pro-angiogenic factors; VEGF is one such factor. Hypoxia is a major stimulus for the expression of VEGF. It develops when the proliferation of cancer cells exceeds the rate of vessel formation. The hypoxia-inducible factor is the transcriptional factor that stimulates the production of VEGF.[4] Vascular Endothelial Growth Factor is a dimeric 46-kDa, endothelial cell-specific glycoprotein[5] and one of the key mediators of angiogenesis. It helps in proliferation, survival, and migration of endothelial cells and is needed for new vessel proliferation. VEGF is reported to be synthesized and secreted by solid tumours of the lung, brain, gastrointestinal tract, kidney, and also in the ovary.[6] With the advancement of medicine, novel VEGF targeting agents have been developed for the treatment of ovarian tumours and are under clinical trial.[4]

The purpose of this study was to determine the expression of VEGF in serous and mucinous surface epithelial tumours of the ovary and to see its correlation with histological grading and clinical staging of malignant cases.

METHODS

This is a cross-sectional study conducted at the Department of Pathology, Government Medical College, Thrissur, Kerala, India from January 2018 to June 2019.

Inclusion Criteria

Ovariotomy specimens diagnosed as serous and mucinous surface epithelial tumour received in the Central laboratory of Government Medical College, Thrissur.

Exclusion Criteria

Patients already treated for ovary cancer
Specimen that has undergone torsion

Sample Size

In this study, a final sample size of 50 comprising of 20 benign, 10 borderline, and 20 malignant serous and mucinous ovarian surface epithelial tumours were taken.

The samples were collected from the ovariotomy specimens received in Central laboratory of Government Medical College, Thrissur. The specimens were fixed in formalin, dehydrated in alcohols, and cleared in xylene, and embedded in paraffin. Haematoxylin and eosin-stained sections were prepared from the formalin-fixed paraffin wax embedded blocks and examined to determine the histological tumour type. For immunohistochemical analysis, 4 µm thick sections were made on slides, kept at 37º C overnight and 60ºC for 1 hour and then dewaxed in xylene. Hydration was done followed by antigen retrieval and staining. On microscopic examination, positive staining for VEGF was seen as granular brown cytoplasmic staining in tumour cells.

The scoring was done by evaluating the intensity of cytoplasmic staining and the percentage of positive tumour cells i.e.

0 - absent staining (no colour)
1- mild staining (pale yellow)
2- moderate staining (yellow-brown)
3- strong staining (brown)

0- less than 1% positive cells
1- 1-10 % positive cells
2 – 11 – 50 % positive cells
3 – 51-100 % positive cells

The two scores were added to get the final scores. Based on the scores, the surface epithelial tumours were categorized as high VEGF expressors (Scores 5 and 6) and low VEGF expressors (Scores 4 and below). VEGF expression was also compared with the grade of the tumour.

Statistical Analysis

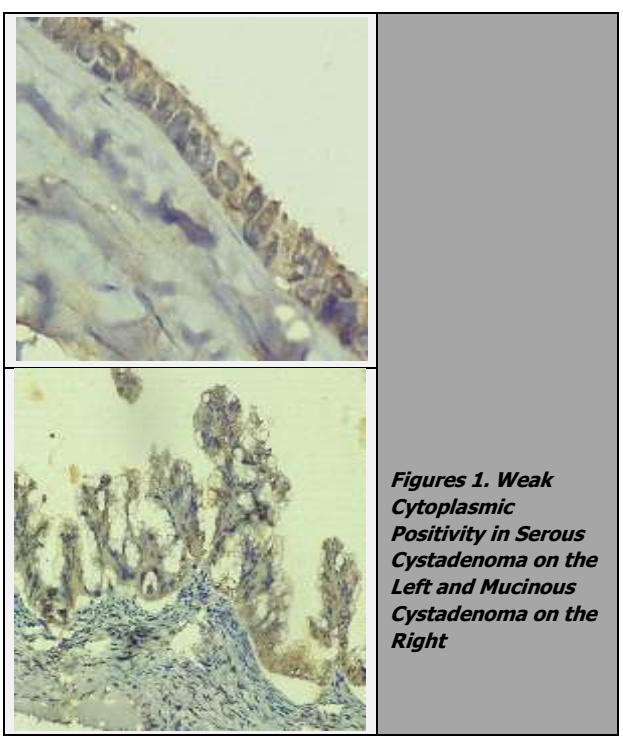
Data was coded and entered into Excel sheets and analysed using EpiInfo /Statistical Package for Social Sciences (SPSS) software. Statistical significance was tested using Fisher's exact test. The p-values of less than 0.05 were considered significant.

RESULTS

In this study, 50 cases of histopathologically diagnosed serous and mucinous epithelial ovarian tumours, comprising of 20 benign, 10 borderline, and 20 malignant were taken at random. The most frequent age group of epithelial ovarian tumours was 41 – 60 years. 60 % of the tumours were type 2 or high-grade ovarian tumours. Type two ovarian tumours included high-grade serous, high-grade endometrial and clear cell carcinomas. As in this study, only serous and mucinous tumours were included, all of the high-grade tumours were high-grade serous carcinomas. Out of the 20 malignant cases, 12 were high grade and 8 were low grade which included two low-grade

serous carcinomas and eight mucinous carcinomas. The increasing prevalence of high-grade tumours may be attributed to the increasing incidence of polycystic ovarian disease and hormonal therapies which increase ovulation. Out of 50 cases, all except 4 cases showed some expression of VEGF. The cases that showed absent VEGF expression were benign tumours. This was similar to the study conducted by C. S. Premlatha et al. where all except one case had shown VEGF expression.[1] But though almost all cases had taken VEGF stain, there was a difference in the intensity of staining. 60 % of cases were high expressors and 40 % of were low expressors. Of the 60 % that showed high expression, 63.3 % were malignant, 20 % were borderline and 16.6 % were benign.

Among the benign neoplasms, 75 % were low expressors for VEGF. In borderline neoplasm, 60 % (6 out of 10 cases) had shown high expression, and 40 % (4 out of 10 cases) had shown low expression. Among the malignant cases, ninety percent showed high expression. Thus, VEGF expression increased in malignant tumours compared to the benign and borderline cases, and this was statistically significant with $p < 0.001$. These results were comparable to other studies conducted by Premlatha et al.[1]



Figures 1. Weak Cytoplasmic Positivity in Serous Cystadenoma on the Left and Mucinous Cystadenoma on the Right

Out of 20 malignant cases, all except two were high expressors. The two of the low expressors were a low-grade serous carcinoma and a mucinous carcinoma. Therefore, this was statistically not significant. In the study conducted by Jun Wang et al. also no statistically significant difference was found between VEGF expression and grade of tumors.[7] This was in contrast to the studies done by C. S. Premlatha et al. Sudeshna Mukherjee et al. where a statistically significant difference was found between the grade of tumours and VEGF expression.[1,4]

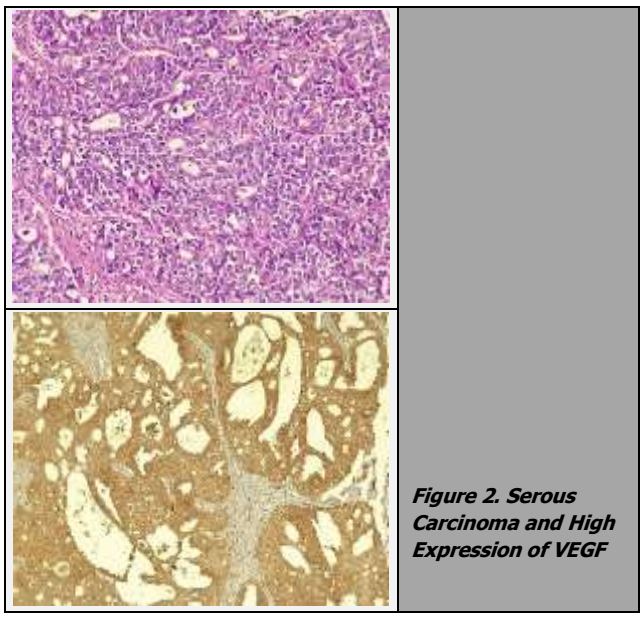


Figure 2. Serous Carcinoma and High Expression of VEGF

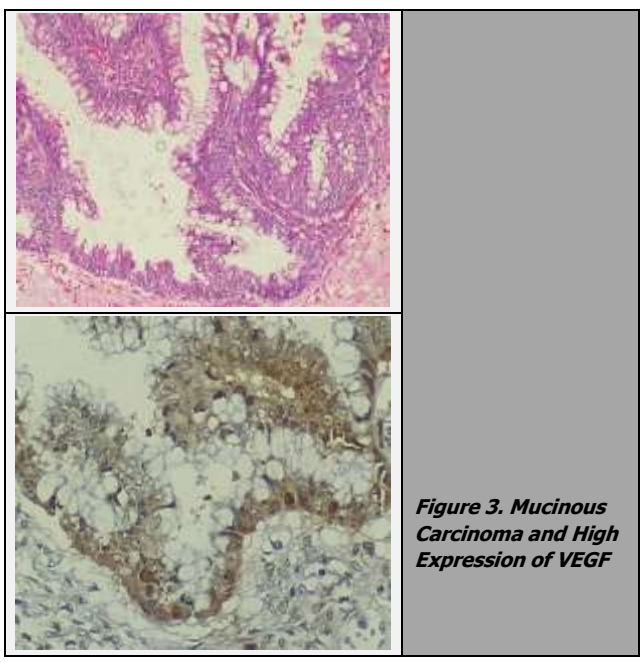


Figure 3. Mucinous Carcinoma and High Expression of VEGF

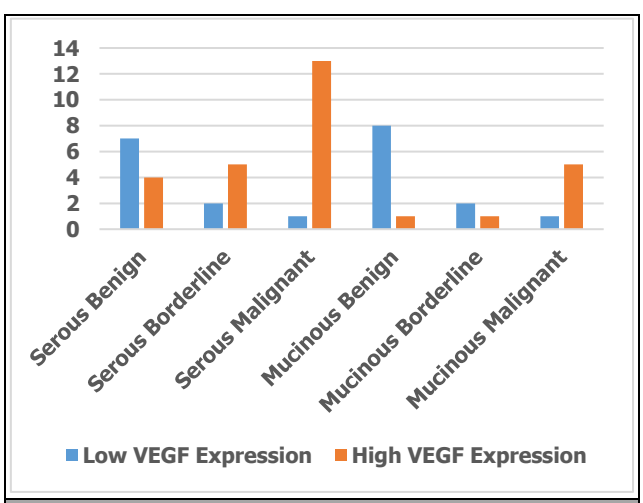


Figure 4. In Serous Neoplasms, VEGF Expression Significantly Increased in Malignant Cases as Compared to Benign and Borderline ($p < 0.001$). Mucinous Neoplasms Also Showed a Similar Expression

Therefore, in this study, it is clear that VEGF expression is significantly higher in malignant epithelial ovarian tumours as compared to benign and borderline tumours. Further studies are needed to see the different mechanisms by which VEGF is strongly expressed in malignant ovarian neoplasms and the utility of targeted anti-VEGF therapy in these patients.

DISCUSSION

The term angiogenesis was first used to describe the growth of endothelial sprouts from pre-existing post capillary venules. Now it is used to describe the growth and remodelling process of a primitive vascular network into a complex network. This involves the enlargement of venules which becomes divided by pillars of periendothelial cells which then separates to form new capillaries.[8] In 1971, Folkman emphasized the importance of angiogenesis for tumour survival and metastasis. In the absence of angiogenesis, tumour remains dormant and microscopic. Angiogenesis occurs as a final result of the net balance of many positive and negative factors.[9] Senger et al. later described a vascular permeability factor, of molecular weight 34,000 - 42,000 which was abundantly present in tumour secretions and found to have caused increased vascular permeability.[10] This was later found to be vascular endothelial growth factor. Vascular endothelial growth factors consist of VEGF -A, -B, -C, and -D and PIGF (placental growth factor). VEGF-A is referred to as VEGF and is the major angiogenic factor after injury and in tumours. VEGF-B and PIGF help in embryonic vessel development, and VEGF-C and VEGF-D are involved both in angiogenesis and lymphatic development.[11] Receptors for VEGF are two types: VEGFR-1 (flt-1) and VEGFR-2 (KDTflt-1)[7] In the ovaries, VEGF is expressed in the developing follicles and also in the cortex, which contains the surface epithelium and surface inclusions.[12] Luteinised cells and corpora lutea strongly express VEGF.[13,14] Presence of VEGF is reported in ovarian follicles and ascitic fluids in patients with hyper stimulation syndrome. This is induced by gonadotrophin treatment and characterised by massive ascites and/or hydrothorax.[15] This is because of the effect of VEGF on permeability of vascular endothelium in the peritoneum or the ovary.[16] Schiffenbauer et al. showed that loss of ovarian function promoted the growth of ovarian epithelial neoplasms in vivo by accelerating angiogenesis.[17] VEGF expression has been shown to parallel in vivo steps of tumour metastasis and development.[18] Wang et al. has studied VEGF production by cell lines of ovarian epithelial tumours under the regulation of gonadotropins in vitro. He suggested that both luteinizing hormone (LH) and follicular stimulating hormone (FSH) have promoting effects on ovarian tumour via VEGF while LH also has inhibitory effect especially in benign and borderline variety.[7] This was also supported by Schiffenbauer et al. Yanamoto et al. showed that serum level of VEGF was significantly elevated in patients with epithelial ovarian cancers compared to benign cystadenomas. VEGF accumulation in the peritoneal cavity paralleled tumour growth, increased inflow of macromolecules from the plasma into the peritoneal cavity leading to the formation of ascites. He also suggested that VEGF immunoreactivity strongly correlated with FIGO staging.[6,19] flk-1 VEGFR receptor inhibition is associated with reduction of tumour induced ascites and tumour growth in mice.[20,21]

VEGF has also been correlated with prognosis of ovarian cancer. In their study, W. Shen, H-L Li, L. Liu and J-X. Cheng positively correlated VEGF expression with clinical stage, lymph node metastasis and the degree of differentiation of tumour cells. He suggested VEGF to be an independent prognostic marker whose high levels indicated poor prognosis and lower survival rates.[22] Brustmann and Naude studied the expression of VEGF in serous ovarian carcinoma and its relationship with high mitotic activity and FIGO staging. They observed that VEGF expression was seen in weak intensity in 6 out of 10 serous cystadenomas and only 4 among 45 serous cystadenocarcinomas did not stain for VEGF. There was significant increase in VEGF staining with high mitotic index.[5] However in Indian literature, studies on VEGF in epithelial ovarian tumours are limited. C. S. Premlata et al. studied the expression of VEGF-A in epithelial ovarian cancer and its correlation with morphologic types, grade and clinical stage. They found that expression of VEGF on epithelial ovarian tumours were variable and only 33.3 % showed high positivity.[1] This is comparable to study by Sudeshna Mukherjee et al. where 19 out of 50 cases showed high reactivity.[4] Thus, angiogenesis plays a vital role in the clinical behaviour of epithelial ovarian carcinomas and is an important mediator in promoting and increasing vascularity. It also plays a role in neoangiogenesis and is thought to be responsible for the development of ascites in both animal models and patients. Vascular endothelial growth factor or VEGF is a key protein involved in angiogenesis. The elevated level of VEGF is also associated with a poor prognosis. These observations indicate that VEGF can be a potential therapeutic target for the treatment of epithelial ovarian tumours. Therefore, studying the expression of VEGF in different grades of epithelial ovarian tumours is of considerable significance. In this study, all except 4 cases were positive for VEGF, with higher intensity staining observed consistently with malignant neoplasms as compared to benign and borderline neoplasms, which was significant with a $p < 0.001$. There was no significant difference between VEGF expression and the histopathological grade of the disease. This was probably because majority of the tumours were high-grade serous and almost all malignant cases showed high expression irrespective of the grade. Literature also has conflicting observations with some studies having significant difference and some without between the expression of VEGF and grade and stage of the disease.

CONCLUSIONS

Therefore, it can be concluded that the expression of VEGF consistently increases in malignancy, making it a potential target for the treatment of epithelial ovarian carcinomas. This also warrants for further studies to explain the

mechanisms by which VEGF increases significantly in ovarian carcinomas.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

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