

## IMMUNOHISTOCHEMICAL EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND ITS CORRELATION WITH TUMOR GRADE IN BREAST CARCINOMAS

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### ABSTRACT

**Background and Objectives:** To analyse the expression of VEGF in human breast cancer; to correlate the expression of VEGF and tumor grade in breast carcinoma; to assess the clinical significance of VEGF as a predictor of aggressiveness of breast cancers.

**Methods:** This was a prospective study. 59 cases of radical mastectomy received for routine histopathological evaluation (after the histological sections were stained with H and E stain) in the Department of Pathology, MGM Hospital, Warangal, India, from June 2019 to November 2021 were studied.

**Results:** All breast carcinoma cases were subjected to VEGF immunostaining. In present study the commonest age group was from 41-50 years with majority of the lesions diagnosed as IDCC NOS. Females contributed to large number of cases (57) in the present study. Majority of cases (59.6%) in this study are poorly differentiated breast carcinomas. VEGF expression was seen in 52(88%) cases. Of the 52 cases, 16 (80%) of the breast cancers were highly differentiated, 14 (87%) were moderately differentiated, and 22 (91%) were poorly differentiated. There is a significant correlation [P< 0.01] between VEGF expression and grade of breast carcinoma but there is no significant correlation is observed with variables like age, gender, tumor size.

**Conclusion:** Recent results suggest that VEGF expression could both be considered as independent prognostic factor associated with unfavourable clinical outcome in breast carcinomas and that their expression is higher in high grade variants in comparison with low grade.

**Keywords:** Breast carcinoma, VEGF expression.

### INTRODUCTION

The most dangerous kind of cancer in women, breast cancer causes mortality in around one-fourth of cases [1]. Breast cancer is depicted in deeds and relics of human resistance to illness. The earliest known description of breast cancer was made in Egypt around 1600 BC [2]. In India, breast cancer is the most common type of cancer. Breast cancer is still the most common type of cancer among women in many parts of India, despite having an incidence that is between one-third and one-fourth that of the United States. Numerous genetic changes and oncogene protein products that disrupt the proliferation and differentiation of tumour development mechanisms have been identified and studied. Candidate prognostic biomarkers in breast cancer include increased expression of the proliferating cell nuclear antigen, amplification of the oestrogen and progesterone receptors, overexpression of HER2, cyclin D1, accumulation of P53 nuclear protein, expression of bcl2, and changes in angiogenesis proteins like VEGF [2].

Vascular endothelial growth factor [VEGF] is emerging as a predictive marker in numerous types of cancer, including breast cancer [3]. Over the past few years, many researchers have focused on tumour angiogenesis, which is a crucial phase in the formation and spread of cancer.

Numerous normal cells produce and secrete VEGF, which is a polyfunctional molecule implicated in vasculogenesis, endothelial cell migration and proliferation, vascular permeability, and stromal degradation [4]. This is because VEGF activates some proteolytic enzymes that are involved in tumour invasiveness and angiogenesis. Breast cancer carcinogenesis requires VEGF in its early phases, and this early effect is connected to the growth of the neovascular stroma [5]. The prognostic value of immuno histochemical [IHC]analysis of direct VEGF expression by tumor cells is not yet clarified. Previous studies have reported large varied expression in breast cancer [6,7]. The relations of VEGF with other clinicopathologic features of breast cancer remains uncertain. there is some molecular evidence of high VEGF mRNA levels in invasive breast ductal carcinomas [IBDC]. The correlation of tumor VEGF expression with tumor grade is uncertain and contraversal [8,9]. The present study includes the pattern of VEGF expression in malignant breast lesions.

### AIMS AND OBJECTIVES

- To analyse the expression of VEGF in human breast cancer.
- To correlate the expression of VEGF and tumor grade in breast carcinoma.
- To assess the clinical significance of VEGF as a predictor of aggressiveness of breast cancers.

**MATERIAL AND METHODS**

This was a prospective study. 59 cases of radical mastectomy received for routine histopathological evaluation (after the histological sections were stained with H and E stain) in the Department of Pathology, MGM Hospital, Warangal, India, from June 2019 to November 2021 were studied.

**INCLUSION CRITERIA**

- All MRM specimens histologically proven as breast carcinoma in the department of pathology, MGM hospital, Warangal. All age groups. Both male and female patients.

**EXCLUSION CRITERIA**

- Benign breast lesions inflammatory lesions of breast.

From the patients' case files, the comprehensive clinical history and the findings of pertinent examinations were gathered. The pathology department received the specimens from the modified radical mastectomy in 10% neutral buffered formalin. Every time, the normal procedure for surgical grossing of specimens from modified radical mastectomy was used. 24 hours were spent fixing the specimen. Multiple sections were collected from the tumour, surgical margins, nipple and areola, non-neoplastic breast, and all the lymph nodes following a thorough specimen description. Following standard processing and paraffin wax embedding, 4 mm thick slices were cut using a rotator microtome and stained with haematoxylin and eosin (H&E) for histological analysis.

**IHC INTERPRETATION**

VEGF reactivity was recognized as dark brown intracellular [cytoplasmic] precipitate. The proportion of staining is assessed by counting the percentage of positive cells for 100 malignant cells at 40X objective in 5 random fields.

**VEGF SCORING SYSTEM**

VEGF Score	Staining pattern
Score 0	Negative, none of cells revealed positivity for marker.
Score 1+	Weak or mild staining, [5-10%] positive of tumor cells.
Score 2+	Moderate staining, less than 25% of tumor cells are stained positive.
Score 3+	Strong staining [25-50%] of tumor cells are stained positive
Score 4+	Highly strong staining, over 50% of tumor cells stained positive.

**RESULTS**

During the period of study from June 2019 to November 2021, 59 modified radical mastectomies were received in the Department of Pathology, Kakatiya Medical College, and Warangal.

**TABLE 1: HISTOLOGIC TYPES OF CARCINOMA**

	Frequent	Percentage
Invasive ductal carcinoma (NOS)	31	52.9
Invasive ductal carcinoma + ductal carcinoma insitu	1	1.9
Invasive ductal carcinoma + pagets disease	1	1.9
Invasive ductal carcinoma with mucinous component	8	13.5
Lobular carcinoma	5	8.2
Medullary carcinoma	10	16.4
Metaplastic carcinoma	1	1.9
Papillary carcinoma	2	3.3
Total	59	100

The most common histologic type of breast carcinoma was Invasive Ductal carcinoma (NOS) type. 31 patients out of total 59 (52.5%) had IDC (NOS) type.

**TABLE 2: Histologic Grade (MSBR) Of Breast Carcinoma**

	Frequent	Percent
Grade I	20	33.9
Grade II	16	27.2
Grade III	23	38.9
Total	59	100

Majority of the patients presented with Grade 3 tumors (38.9%)

**TABLE 3: Lymph Node Status**

	Frequency	Percent
<b>Metastasis present</b>	<b>15</b>	<b>25.4</b>
<b>Metastasis absent</b>	<b>44</b>	<b>74.6</b>
<b>Total</b>	<b>59</b>	<b>100</b>

25.4% (15 patients out of 59) presented with lymph node metastasis. Among 15 patients 8 showed deposits in 1-3 lymph nodes, 7 showed deposits in 4-9 lymph nodes.

**TABLE 4: Tumour Size**

	Frequent	Percent
<b>&lt; 2</b>	<b>13</b>	<b>22</b>
<b>2-5</b>	<b>33</b>	<b>56</b>
<b>&gt;5</b>	<b>13</b>	<b>22</b>
<b>Total</b>	<b>59</b>	<b>100</b>

13 out of 59 patients (22%) had tumour size less than or equal to 2cms, 33 out of 59 patients [56%] had tumor size 2-5 cm, 13 out of 59 patients [22%] had tumor size >5 cm.

**TABLE 5: Age distribution**

Age	Frequent	Percent
1-10 yr	0	0
11-20 yr	0	0
21-30 yr	0	0
31-40 yr	8	13.5
41-50 yr	28	47.4
51-60 yr	11	18.6
61-70 yr	10	16.9
71-80 yr	2	3.6
81-90 yr	0	0
91-100 yr	0	0
<b>Total</b>	<b>59</b>	<b>100</b>

Highest incidence is in 5<sup>th</sup> decade 28 cases out of 59 [47%].

#### VEGF SCORE

VEGF scoring was done by counting at least 100 tumour cells. VEGF positive was seen in 52 cases.

VEGF negative was seen in 7 cases.

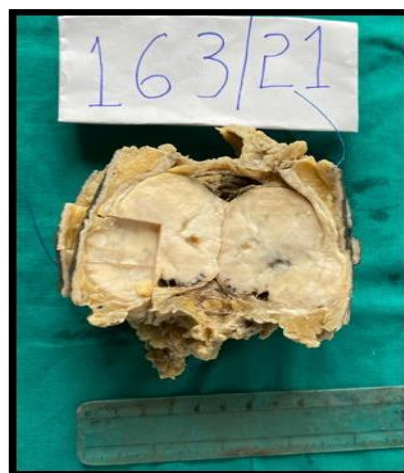
**TABLE 6: VEGF SCORE in positive cases in relation to histologic grade**

Histologic grade	VEGF score 1	score 2	score 3	Score 4	Total	Percentage
Well differentiated	9	4	2	1	16	80% [20]
Moderately differentiated	1	3	8	2	14	87% [16]
Poorly differentiated	4	5	6	7	22	91.3% [23]
	14	12	16	10	52	

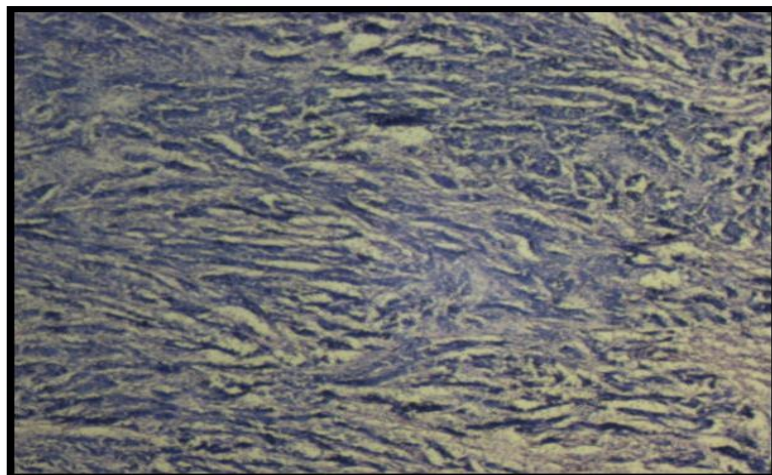
Out of 20 cases of grade I 16 cases shows positive VEGF expression [80%], out of 16 cases of grade II 14 shows positive expression of VEGF [87%], out of 23 cases of grade III 22 shows positive expression [91%].



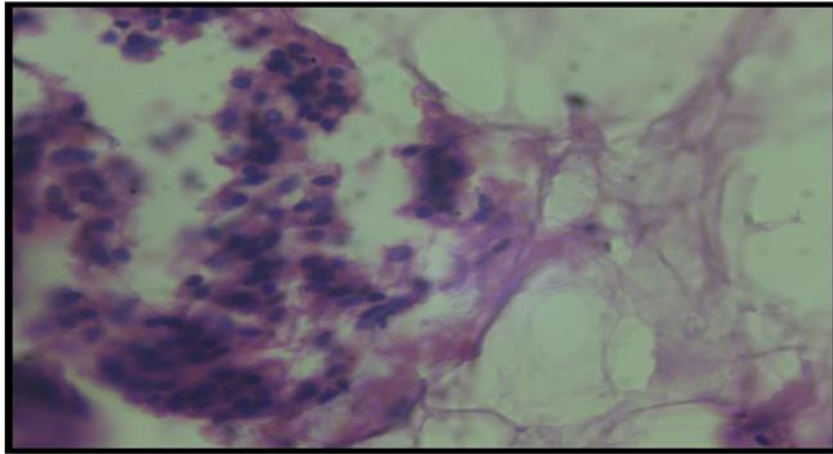
**FIGURE 1: GROSS PICTURE OF BREAST CARCINOMA**



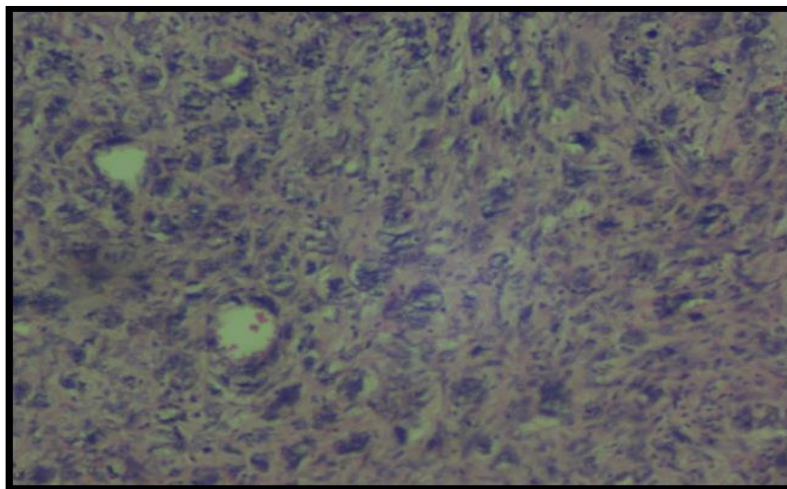
**FIGURE 2: CUT SECTION OF BREAST CARCINOMA**



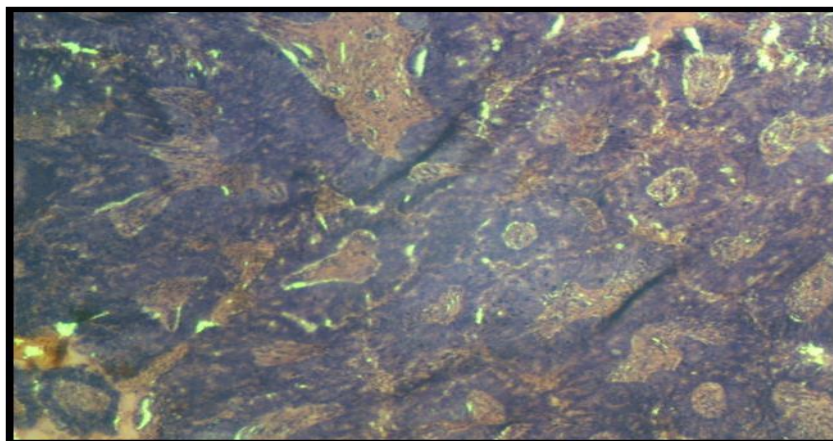
**FIGURE 3: IDC BREAST H&E stained [10X]**



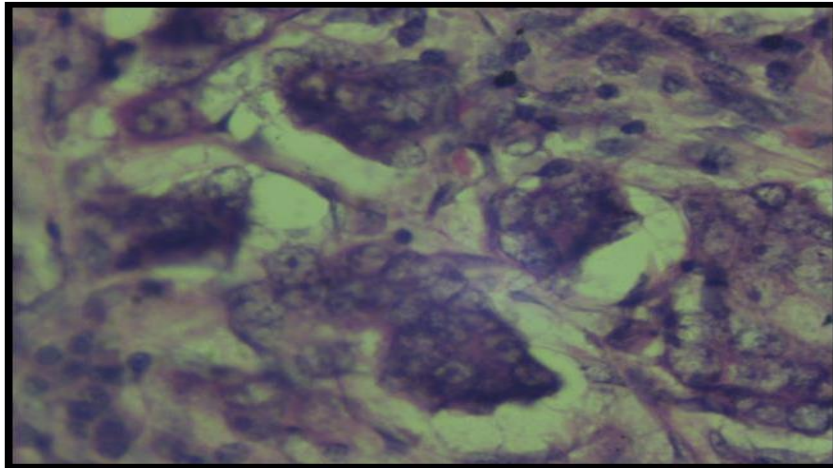
**FIGURE 4: MUCINOUS CARCINOMA H&E stained [40X]**



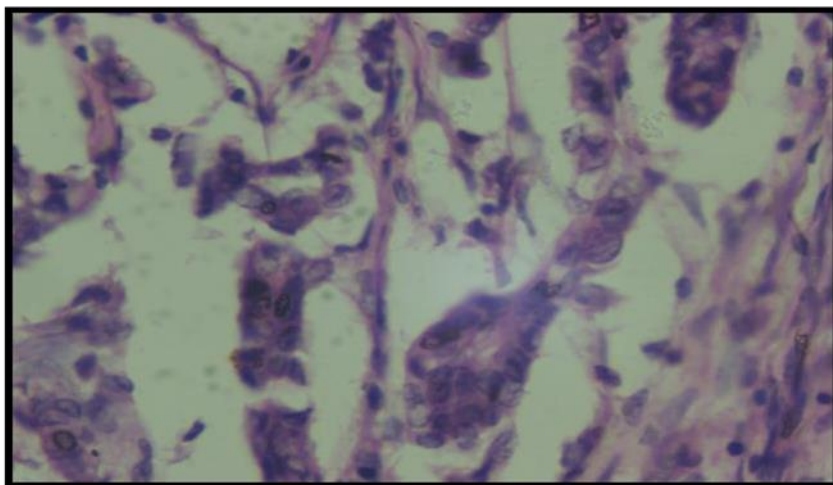
**FIGURE 5: METAPLASTIC CARCINOMA H&E stained[20X]**



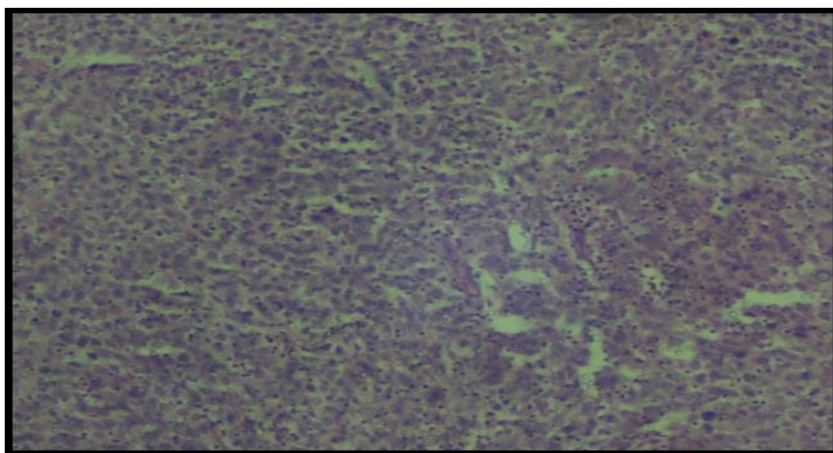
**FIGURE 6: PAPILLARY CARCINOMA H&E stained [20X]**



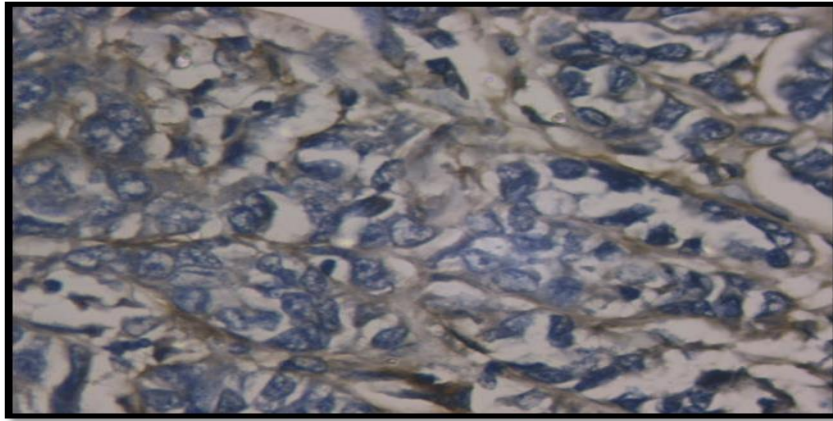
**FIGURE 7: Well differentiated breast carcinoma H& E STAIN [40X]**



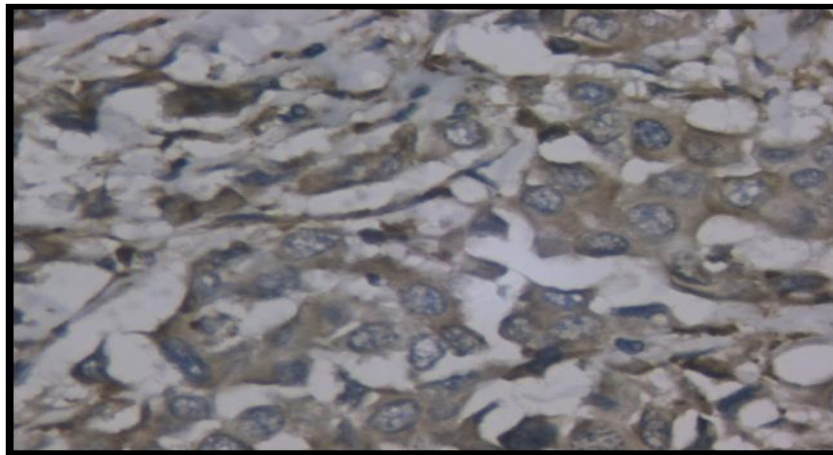
**FIGURE 8: Moderately differentiated breast carcinoma H&E stained [40X]**



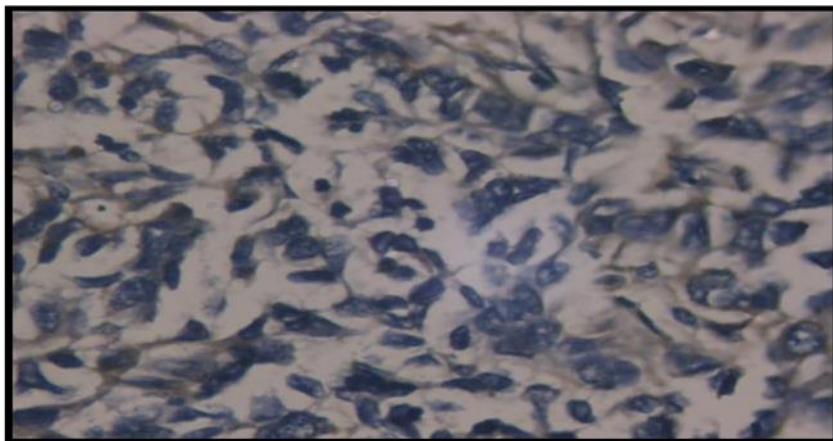
**FIGURE 9: Poorly differentiated carcinoma H&E stained [10X]**



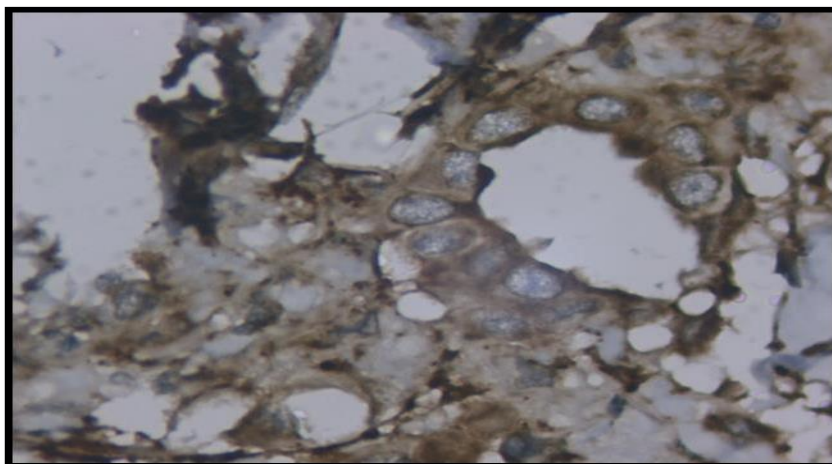
**FIGURE 10: VEGF SCORE 0 IHC [40X]**



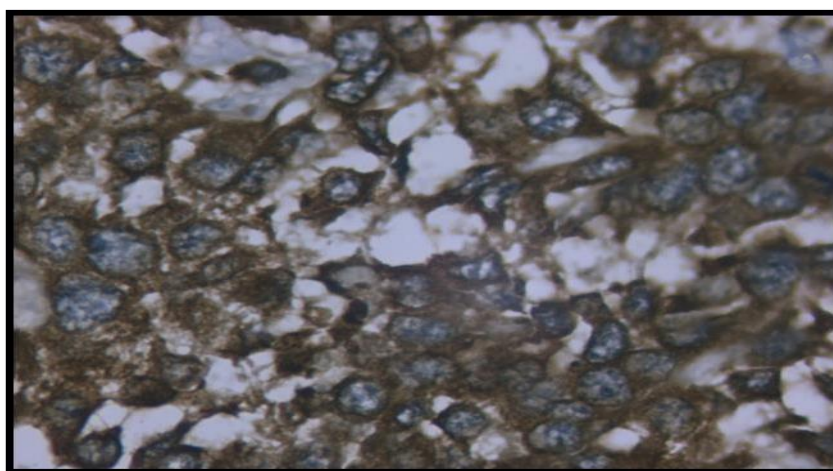
**FIGURE 11: VEGF SCORE 1 IHC [40X]**



**FIGURE 12: VEGF SCORE 2 IHC [40X]**



**FIGURE 13: VEGF SCORE 3 IHC [40X]**



**FIGURE 14: VEGF SCORE 4 IHC [40X]**

## DISCUSSION

The most prevalent malignant tumour in females and a heterogeneous collection of tumours is breast cancer [10,11]. For a woman's breast to develop normally, ovarian steroid hormone is required, and an imbalance might hasten the development of cancer [12]. The prognostic variables offer significant information that aids the oncologist in selecting the best course of action for each patient. Numerous factors, including age, tumour size, tumour grade, histologic type, lymph node status, and receptor status, have been linked to the prognosis of breast cancer.

Initially dependent on oestrogen for growth and progression, human breast cancer is an angiogenesis-dependent tumour [13]. Despite our limited understanding of specific prognostic variables, breast cancer biology continues to be poorly understood [14]. In addition to the conventional /nodal/ metastasis staging variables, IHC evaluation of ER/PR and VEGF are critical in determining the course of treatment for breast cancer patients [15].

The current study represents study of VEGF expression for appropriate or better treatment.

## VEGF EXPRESSION AND AGE

In this study VEGF immune-expression was not always increased with increasing age. There was no significant difference among these age groups. This corresponds to natural frequency of breast cancer. This finding is consistent with that of Gasparini G et al [2], Obermair A et al [16] and Li J et al [17], who suggested that there is no correlation between age of patient and VEGF expression.

**TABLE 7: VEGF Expression and age in comparison with other studies**

Study	VEGF Relation with age
Gasparini G et al [2]	No association
obermair A et al [16]	No association
Li J et al [17]	No association
Present study	No association
Greb R et al [18]	Higher expression in younger age



Most common type of breast carcinoma In present study invasive ductal carcinoma accounts for 52.9 % and most common form, this corresponds to H.L. Krishan Prasad et al [19] 58% Michael Strierer [20] et al 68%.

**TABLE 8: most common type of breast carcinoma in comparison with other studies**

Author	Common type
H.L Krishan Prasad et al [19]	IDC [NOS] 58%
Michael Strierer et al [20]	IDC [NOS] 68%
Mehrdad Nadji et al [21]	IDC [NOS] 73%
Present study	IDC [NOS] 52%

Most common grade at presentation In present study most common grade is grade III [38%], this corresponds to Lakmini K.B.Mudduwa [22] [49%].

**TABLE 9: most common grade at presentation in comparison with other studies**

Author	Grade
Lakmini K.B. Mudduwa [22]	Grade 3 (49%) Grade 2 (36.4%) Grade 1 (14.6%)
H.L Krishan Prasad et al [19]	Grade 1 (44.4%) Grade 2 (39.7%) Grade 3 (15.9%)
Adedayo . et al [23]	Grade 2 (38.4%) Grade 3(35.9%) Grade 1 (21.2%)
Present study	Grade 3[38], Grade 1[33], Grade 2[27]

In this study grade 3 cases are 38/59, 33/59 are grade 1, 27/59 are grade 2.

**TABLE 10: Comparison of VEGF expression in breast carcinomas with other studies**

Author	Percentage
Melanie et al [21]	60%
Anca maria et al [22]	87%
Yasushi Nakamura et al [23]	83%
Shuaeb Bhat [3]	75%
Present study	88%

In this study VEGF was expressed in 52 out of 59 cases [88%], corresponding with Anca Maria Cimpean et al [22] [87%].

**TABLE 11: Correlation of VEGF with tumor grade in positive cases**

Grade	1	2	3	4	Total
Well	9	4	2	1	16
Moderate	1	3	8	2	14
Poorly	4	5	6	7	22
Total	14	12	16	10	52

P value 0.01; VEGF immune expression increases with increasing grade and there is significant association with p value < 0.01.

**TABLE 12: VEGF expression in relation to grade in comparison with other studies**

Author	GRADE I	GRADE II	GRADE III	P Value	Association
Shuaebhat [3]	0%	75%	90%	<0.05	Significant association
Mais almumen [23]	0%	50%	65.7%	<0.05	Significant association
This study	80%	87%	91%	0.01	Significant association
Shamimshera [2]	0%	75%	90%	<0.05	Significant association
AL Harris Eet al [24]	100%	55%	61%	<0.05%	Significant association

Studies by shuaeb bhat [3], Mais almumen [24], Shamim shera [2] demonstrated a statistically significant association between tumor grade and VEGF correlating with present study.

**TABLE 13: correlation of VEGF expression and number of lymph node metastasis**

Lymph node	VEGF +	VEGF -	No of cases
Nil	38 [86%]	6 [13.6%]	44
1-3	7 [87.5%]	1[12.5]	8
4-9	7[100%]	0[0]	7

>10	0	0	0
Total	52	7	59

In this study 44 cases showed no lymph node metastasis in which 38[86%] cases were positive for VEGF.7[87.5%] out of 8 cases with 1-3 LN were positive for VEGF.All the 7 [100%] cases with 4-9 LN were positive for VEGF.

**TABLE 14: VEGF expression and node positive breast cases incomparison with other studies.**

Author	Node status	VEGF POSITIVE	Association
Shuaeb Bhat [3]	14 positive	13/14	Significant association
Nahidasarbovic [24]	29 positive	27/29	Significant association
This study	15 positive	14/15	Significant association

In this study 15 out of 59 cases showed lymph node positivity, 14 out of 15 node positive cases showed increased VEGF expression, corresponding to Shuaeb Bhat [3].

## CONCLUSION

One of the most prevalent female cancers and a leading cause of death for women globally is breast cancer. The death rate of breast cancer has stayed mostly stable over the past few decades, despite improvements in early detection techniques. Invasive breast cancers have been found to have several genetic changes, many of which have potential prognostic or predictive value. Others forecast the possibility that a patient would benefit from a certain therapy. Some provide treatment independent statistics on patient survival. In present study we have 59 cases of breast carcinoma studied by light microscopy for histologic type, grade, lymph node status and immuno histochemical for VEGF were correlated with clinical parameters like age and tumour size. Invasive ductal carcinoma was the most common histologic type of breast cancer in both pre and post-menopausal females in our study. Majority of the patients at the time of presentation had grade 3 tumour and were less than 5 cm in diameter at the time of diagnosis. Out of 59 cases, 52 cases are VEGF positive and 7 cases are VEGF negative. There is significant expression of VEGF in poorly differentiated and moderately differentiated carcinomas, when compared to well differentiate breast carcinoma. Hence VEGF have a definite role in breast cancer pathogenesis. Therefore, there is significant correlation between VEGF expression and grade of tumour since grade is a proven prognostic marker, VEGF expression and scoring will also be another good prognostic marker in breast carcinomas. But there is no association between VEGF expression and other variables like Age, gender and size of tumour. Recent results suggest that VEGF expression could both be considered as independent prognostic factor associated with unfavorable clinical outcome in breast carcinomas and that their expression is higher in high grade variants in comparison with low grade.

## FUNDING SUPPORT

Nil

## CONFLICT OF INTEREST

None

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