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Immunohistochemical Evaluation of Vascular Endothelial Growth Factor, Endoglin, and CD31 as Angiogenic Biomarkers in Colorectal Cancer

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ABSTRACT

Background & Objective: Tumour angiogenesis, a process of new blood vessel formation, is essential for tumour progression. Angiogenic factors play a vital role in this process. In view of the rising trend of colorectal cancer (CRC), we propose this study to understand the relationship between angiogenesis and CRC, which will aid in initiating targeted therapy and halting tumour progression. We aim to assess the prognostic significance of angiogenesis using vascular endothelial growth factor (VEGF), endoglin (CD105), and CD31 in CRC.

Methods: Tumour proper sections from 39 CRC cases were stained immunohistochemically for CD31, VEGF, and CD105. CD31 and CD105 were assessed for microvessel density (MVD), and the findings were correlated with morphology, grade, stage, and prognostic parameters. The expression levels of VEGF and CD105 were compared with that of CD31.

Results: Twenty-seven cases (69.2%) had strong VEGF expression, and significant correlations of VEGF expression with tumour budding (P = 0.011) and tumour grade (P = 0.007) were observed. The median MVD values using CD31 and CD105 were 25 and 19, respectively, and were statistically correlated with tumour grade (P = 0.005 and 0.002, respectively).

Conclusion: VEGF expression showed significant correlations with tumour budding and tumour grade, supporting the potential utility of anti-VEGF targeted therapy in future therapeutic trials. In conclusion, among all three vascular endothelial markers, VEGF expression, when assessed in biopsy specimens from early colorectal cancer, can prove to be a prognostic marker. Future clinical trials on the application of anti-VEGF therapy in early CRC cases will provide further insights into this field.

KEYWORDS Colorectal cancer, angiogenesis, vascular endothelial growth factor, endoglin

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INTRODUCTION

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths in developed and developing countries (1). Effective preventive strategies aimed at inhibiting the development and progression of CRC are critical for reducing mortality. Cancer development and metastasis are dependent on various factors, one of the most significant being angiogenesis, a process involving the formation of new blood vessels from pre-existing vessels. Angiogenesis is regulated by factors such as vascular endothelial growth factor (VEGF) and endoglin (also known as CD105).

VEGF, a potent angiogenic factor, is a heparin-binding glycoprotein with angiogenic, mitogenic, and vascular permeability-enhancing activities specific to endothelial cells (2). Similarly, CD105 is a glycoprotein expressed on endothelial cells of newly formed arteries, veins, and capillaries. A soluble form of CD105 is also released during pathophysiological processes such as injury and tumour angiogenesis (3). While commonly used pan-endothelial markers such as CD31, CD34, and von Willebrand factor are ideal for staining normal blood vessels, immunohistochemical markers for vascular endothelial growth factor (VEGF) and endoglin (CD105) can be used to identify tumour neoangiogenesis, predict metastasis, or initiate targeted (antiangiogenic) therapy (4, 5).

In this study, we aim to evaluate the clinical utility of the immunohistochemical expression of vascular endothelial growth factor (VEGF), endoglin, and CD31 in colorectal cancers and their effects as prognostic markers.

METHODS

Study Population; Inclusion and Exclusion Criteria

This was a retrospective, single-centre observational study of archived colorectal carcinoma resection specimens. A waiver of consent was obtained for the archived tissue and clinical data. Patient identifiers, such as names and hospital identification numbers, were masked. The study complied with the Declaration of Helsinki and was approved by the Institutional Human Ethics Committee (IHEC) (Proposal Number: 22/314). The study population comprised resected specimens from patients with colorectal cancer received during the 1-year study period (January 2022–December 2022). All cases diagnosed as colorectal cancer in resection specimens were included in the study, and those diagnosed using small biopsies without resected specimens were excluded.

Cases were taken from the archives of the Department of Pathology. The clinical details of these cases were obtained from the Medical Records Department and hospital information system (HIS).

Clinicopathological Parameters

Age, sex, clinical presentation, and radiological findings were obtained from the HIS. Tumour location, size, and other gross findings were retrieved from the histopathology reports. Haematoxylin and eosin (H&E) blocks from all cases were retrieved. H&E slides were analysed again by two pathologists for the following: tumour histological type, grade, tumour extent (stage), lymphovascular/perineural invasion, tumour budding, and lymph node status. The presence of single cells or small clusters of fewer than five cells at the advancing front of the tumour was considered peritumoral tumour budding. Tumour budding was reported by selecting a "hotspot" chosen after reviewing all available slides with invasive tumour. The total number of buds was reported in an area measuring 0.785 mm^2, which corresponds to a 20× field. Both the total number of buds and a three-tier score (based on a 0.785 mm^2 field area) were reported: low (0–4 buds), intermediate (5–9 buds), and high (≥10 buds) (6).

Tissue Sections and Immunohistochemistry

Paraffin-embedded tumour proper blocks from all cases were sectioned at a thickness of 3 µm. Sections were placed on slides for immunohistochemical staining of CD31, CD105, and VEGF-A. Appropriate positive controls (internal vessels for CD31 and placental chorionic villous endothelial cells for CD105 and VEGF) were included. All standard operating procedures for immunohistochemistry using CD31 (PECAM-1; clone GM006; PathnSitu), CD105 (clone EP274; PathnSitu), and VEGF-A (also known as VEGF; ready-to-use, prediluted rabbit polyclonal antibody; BioGenex Life Sciences Pvt Ltd) were performed using an automated immunostaining instrument (Leica; #750-701).

Evaluation of MVD Using CD31 and CD105

Microvessel density (MVD) was evaluated using CD31 and CD105 immunohistochemical staining according to the method described by Weidner et al. Individual microvessels were identified as single brown-stained endothelial cells or small clusters of endothelial cells, with or without a visible lumen. The entire tumour section was initially examined using a Nikon E200 microscope under low-power magnification to identify areas with the highest vascular density ("hotspots"). Three hotspots with the greatest concentration of microvessels were selected, and each was evaluated under high-power magnification (×400; field diameter, 0.65 mm). The number of microvessels was counted in each field, and the highest count among the three hotspots was recorded as the MVD for that case, in accordance with the original Weidner method, as it reflects the area of maximal tumour angiogenesis. MVD was expressed as the number of microvessels per high-power field (HPF) and was not converted to vessels/mm^2. The median MVD for the study population was used as the cutoff value to categorise cases as having low MVD (≤median) or high MVD (>median).

Evaluation of VEGF Expression

VEGF expression in colorectal cancer cases showed cytoplasmic granular reactivity. Expression was assessed semiquantitatively based on the criteria outlined by Martins et al, 2013, as follows: 0 indicated 0% immunoreactive cells; 1 represented <5% immunoreactive cells; 2 corresponded to 5%–50% immunoreactive cells; and 3 denoted >50% immunoreactive cells. Staining intensity was evaluated semiqualitatively and scored as 0 for negative, 1 for weak, 2 for intermediate, and 3 for strong. The overall immunoreaction score was determined by summing the two parameters (percentage of immunoreactive cells and staining intensity) and categorised as negative (0), weak (1–2), moderate (3), or strong (4–6). For statistical analysis, scores classified as negative or weak were grouped as low expression, while strong scores were categorised as high expression and moderate groups were taken as moderate expression (7).

Statistics

Statistical analysis was performed using IBM SPSS Statistics version 22.0. Descriptive statistics were used to summarise the data. Categorical variables were expressed as frequencies and percentages. Associations between histomorphological parameters and angiogenic markers were assessed using Pearson's chi-square test. Fisher's exact test was used when the assumptions of the chi-square test were not met because of small, expected cell counts. Expected cell counts were checked before applying the chi-square test. All statistical tests were two-sided, and a P value < 0.05 was considered statistically significant. No adjustment for multiple comparisons was performed because the analyses were exploratory.

RESULTS

During the 1-year study period, gastrointestinal malignancies constituted 12.9% of all biopsies. Among the biopsies, 200 gastrointestinal (GI) malignancies were reported. Colorectal cancer accounted for 64 cases, representing 32% of all GI malignancies and 7.1% of all malignancies reported.

Sixty-four cases involving gastrointestinal biopsies and resected specimens were diagnosed as colorectal cancer. Thirty-nine resected specimens of colorectal carcinoma were selected from these 64 cases according to the inclusion and exclusion criteria. The clinicopathological details of these cases are summarised in Table 1.

Table 1. Clinicopathological Characteristics of the Patients (N = 39)

Clinicopathological parameter	Category	No. of patients, n (%)
Age, years	<55	12 (30.8)
	≥55	27 (69.2)
Sex	Male	21 (53.8)
	Female	18 (46.2)
Tumour site	Proximal colon	17 (43.6)
	Distal colon	1 (2.6)
	Rectum/rectosigmoid	20 (51.3)
	Entire colon	1 (2.6)
Histological type	Adenocarcinoma, NOS	35 (89.7)
	Mucinous adenocarcinoma	3 (7.7)
	Mixed neuroendocrine–non-neuroendocrine neoplasm	1 (2.6)

VEGF, CD31 and CD 105 expression were compared with the tumour bud score (Fig 1).

VEGF

The expression of VEGF-A in colon cancer showed cytoplasmic granular positivity (Fig. 2). Among the 39 cases, 27 (69.2%) showed strong expression, with scores ranging from 4

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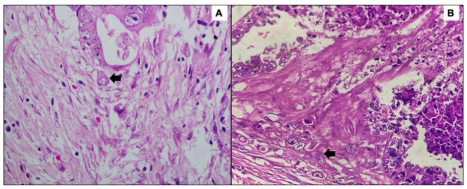

Fig 1. Tumour buds in H&E at the invasive font of the tumour (400X,800µm)- (A) single tumour cell or (B) cluster of less than 5 tumour cell .The tumour budding scoring was done as described in the methods section (6).

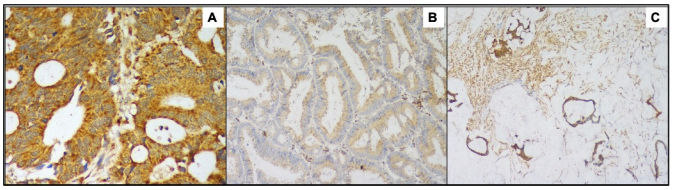

Fig 2. Immunohistochemical expression of VEGF. (A) Strong cytoplasmic expression in tumour cells. (B) Moderate cytoplasmic expression in tumour cells. (C) VEGF expression within the cytoplasm of the mucinous component. Original magnification ×100; scale bar = 200 µm.

Table 2. Association of VEGF Expression With Histomorphological and Clinical Parameters

Parameter	Category	Strong, n (%)	Moderate, n (%)	Weak, n (%)	Total, n (%)	Generalized Fisher exact test	P value
Tumour budding	High	8 (88.9)	1 (11.1)	0	9 (100.0)	4.671	0.011
	Intermediate	7 (87.5)	1 (12.5)	0	8 (100.0)		
	Low	12 (54.5)	5 (22.7)	5 (22.7)	22 (100.0)		
Grade	1	4 (36.4)	4 (36.4)	3 (27.3)	11 (100.0)	8.547	0.007
	2	20 (80.0)	3 (12.0)	2 (8.0)	25 (100.0)		
	3	2 (100.0)	0	0	2 (100.0)		
	4	1 (100.0)	0	0	1 (100.0)		
Lymphovascular invasion	Present	22 (75.9)	3 (10.3)	4 (13.8)	29 (100.0)	4.064	0.134
	Absent	5 (50.0)	4 (40.0)	1 (10.0)	10 (100.0)		
Stage (pT category)	pTis	1 (50.0)	0	1 (50.0)	2 (100.0)	6.876	0.145
	pT1	5 (71.4)	1 (14.3)	1 (14.3)	7 (100.0)		
	pT2	4 (57.1)	1 (14.3)	2 (28.6)	7 (100.0)		
	pT3	9 (64.3)	4 (28.6)	1 (7.1)	14 (100.0)		
	pT4	8 (88.9)	1 (11.1)	0	9 (100.0)		
Lymph node metastasis	Present	12 (80.0)	2 (13.3)	1 (6.7)	15 (100.0)	1.264	0.178
	Absent	15 (62.5)	5 (20.8)	4 (16.7)	24 (100.0)		

Abbreviation: VEGF, vascular endothelial growth factor. Bold P values indicate statistical significance (P value < 0.05). Percentages may not total 100 because of rounding.

to 6; 7 (17.9%) showed moderate expression (score = 3); and 5 (12.8%) showed weak expression, with scores ≤2. None of the cases were negative for VEGF-A (Table 2).

CD31

MVD was calculated using CD31 in tumour proper sections by counting microvessels at ×400 magnification in three hotspot fields; the highest value was considered. The values ranged from 8 to 89 microvessels per hotspot field, with a mean ± standard deviation of 26.69 ± 14.41. The median (interquartile range [IQR]) was 25 (IQR 18–33) microvessels/HPF. Because the MVD values were not normally distributed and the sample size was relatively small, the median value was used as the cutoff to categorise tumours into low- and high-MVD groups. Cases with an MVD greater than the median were classified as high MVD, whereas those with an MVD equal to or less than the median were classified as low MVD. In addition, MVD was analysed as a continuous variable to evaluate its association with clinicopathological parameters. Eighteen cases had high MVD, and 21 cases had low MVD (Table 3; Figs. 3 and 4).

CD105

MVD was calculated using CD105 in tumour proper sections by counting microvessels at ×400 magnification in three hotspot fields. The highest value among the three fields was considered the MVD. The values ranged from 1 to 51 microvessels per hotspot field, with a mean value of 19.8± 12.3.The median (interquartile range [IQR]) was 19 (IQR 10- 29) microvessels/HPF. Because the MVD values were not normally distributed and the sample size was relatively small, the median value was used as the cutoff to categorise tumours into low- and high-MVD groups. Cases with an MVD greater than the median

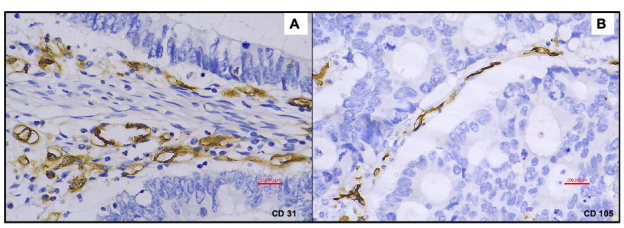

Fig 3. Microvessel density within adenocarcinoma in a hotspot. Individual endothelial cells or clusters, with or without a visible lumen, were counted as microvessels. (A) CD31. (B) CD105. Original magnification ×400; scale bar = 800 µm.

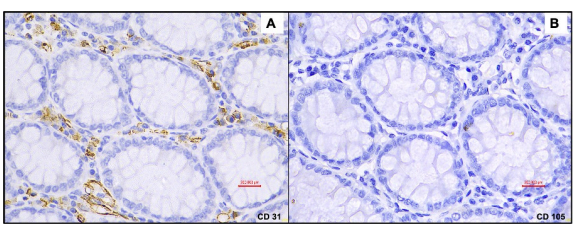

Fig 4. Normal colonic mucosa stained with immunohistochemical markers. (A) CD31 highlights endothelial cells in the normal mucosa. (B) No CD105 staining is evident in this representative field. Original magnification ×400; scale bar = 800 µm.

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Table 3. Association of CD31-Based Microvessel Density with Clinicopathological Parameters

Prognostic factor	Category	High MVD, n (%) (n = 18)	Low MVD, n (%) (n = 21)	Total, n (%) (N = 39)	Test statistic	P value
Tumour budding	High	6 (66.7)	3 (33.3)	9 (100.0)	2.832	0.113
	Intermediate	2 (25.0)	6 (75.0)	8 (100.0)		
	Low	10 (45.5)	12 (54.5)	22 (100.0)		
Grade	1	2 (18.2)	9 (81.8)	11 (100.0)	7.094	0.005
	2	14 (56.0)	11 (44.0)	25 (100.0)		
	3	2 (100.0)	0	2 (100.0)		
	4	0	1 (100.0)	1 (100.0)		
Lymphovascular invasion	Present	14 (48.3)	15 (51.7)	29 (100.0)	0.447	0.468
	Absent	4 (40.0)	6 (60.0)	10 (100.0)		
Stage (pT category)	pTis	0	2 (100.0)	2 (100.0)	1.996	0.415
	pT1	4 (57.1)	3 (42.9)	7 (100.0)		
	pT2	3 (42.9)	4 (57.1)	7 (100.0)		
	pT3	7 (50.0)	7 (50.0)	14 (100.0)		
	pT4	4 (44.4)	5 (55.6)	9 (100.0)		
Lymph node metastasis	Present	6 (40.0)	9 (60.0)	15 (100.0)	0.317	0.319
	Absent	12 (50.0)	12 (50.0)	24 (100.0)		

Abbreviations: CD31, cluster of differentiation 31; MVD, microvessel density. Bold indicates statistical significance (P value < 0.05). Percentages are calculated by row and may not total 100 because of rounding.

were classified as high MVD, whereas those with an MVD equal to or less than the median were classified as low MVD. In addition, MVD was analysed as a continuous variable to evaluate its association with clinicopathological parameters. High MVD using CD105 was observed in 21 cases, and low MVD was observed in 18 cases (Table 4; Figs. 3 and 4).

DISCUSSION

Gastrointestinal malignancies constituted 12.9% of all biopsies. This incidence was slightly higher than that reported by the Population-Based Cancer Registry of India (8). Among the biopsies, 200 malignancies were reported in the gastrointestinal (GI) system. Colorectal cancer accounted for 64 cases, representing 32% of all GI malignancies and 7.1% of all malignancies reported.

A review of the literature shows variations in the incidence of colorectal cancer among different locations and population groups, mainly related to environmental factors and lifestyle habits (9). Tamil Nadu, a state located in the southern part of a developing country (India), has factors such as obesity, family history, and low socioeconomic status associated with CRC, explaining this increased incidence (10).

In our study, the age at diagnosis of colorectal cancer ranged from 34 to 85 years, with a predominance at 61 years. Early-onset colorectal cancers in patients younger than 50 years accounted for approximately 20.5% of cases and were more common in females than in males. However, a literature survey states that early-onset disease is observed more frequently in males (11). The male-to-female prevalence ratio was 1.16:1, with a slight male predominance. A similar result was observed in a

Table 4. Association of CD105-Based Microvessel Density with Clinicopathological Parameters

Prognostic factor	Category	High MVD, n (%) (n = 21)	Low MVD, n (%) (n = 18)	Total, n (%) (N = 39)	Test statistic	P value
Tumour budding	High	5 (55.6)	4 (44.4)	9 (100.0)	2.021	0.284
	Intermediate	6 (75.0)	2 (25.0)	8 (100.0)		
	Low	10 (45.5)	12 (54.5)	22 (100.0)		
Grade	1	2 (18.2)	9 (81.8)	11 (100.0)	8.655	0.002
	2	16 (64.0)	9 (36.0)	25 (100.0)		
	3	2 (100.0)	0	2 (100.0)		
	4	1 (100.0)	0	1 (100.0)		
Lymphovascular invasion	Present	17 (58.6)	12 (41.4)	29 (100.0)	1.037	0.275
	Absent	4 (40.0)	6 (60.0)	10 (100.0)		
Stage (pT category)	pTis	0	2 (100.0)	2 (100.0)	4.461	0.278
	pT1	4 (57.1)	3 (42.9)	7 (100.0)		
	pT2	3 (42.9)	4 (57.1)	7 (100.0)		
	pT3	10 (71.4)	4 (28.6)	14 (100.0)		
	pT4	4 (44.4)	5 (55.6)	9 (100.0)		
Lymph node metastasis	Present	9 (60.0)	6 (40.0)	15 (100.0)	0.371	0.391
	Absent	12 (50.0)	12 (50.0)	24 (100.0)		

Abbreviations: CD105, cluster of differentiation 105; MVD, microvessel density. Bold indicates statistical significance (P value < 0.05). Percentages were calculated by row and may not total 100 because of rounding.

study conducted by Abotchie et al in the United States (12). This slight increase in the incidence of colorectal cancer among males is similar to that reported in various studies in the literature (13, 14). Similar to a study conducted by Gryfe et al, adenocarcinoma (89.7%) was the most common histological subtype, followed by mucinous adenocarcinoma (7.7%) (15).

As reported by Sudarshan et al, the rectosigmoid region was the most common site of colorectal cancer (7, 16). A similar incidence pattern was observed here. The majority of cases were associated with a low tumour budding score. Most tumours were moderately differentiated, similar to the findings of Mehta et al (17), and were classified as pT2 or pT3. Lymphovascular invasion was observed in 74.3% of cases, and 38.4% had lymph node metastasis. The liver is the most frequent site of metastasis (18). Five cases had metastatic disease, with the liver being the most common site.

Angiogenesis plays a key role in tumour development and metastasis (19). It has been observed that antiangiogenic therapy can work by directly affecting endothelial cells through the inhibition of proangiogenic peptide production or activity. Alternatively, it can act indirectly by stimulating the release of antiangiogenic factors within the tumour, thereby improving survival and prognosis. Therefore, markers that can be used to assess angiogenesis will provide insight into targeted therapy for decreasing tumour progression and improving patient survival. The immunohistochemical expression of vascular endothelial growth factor A (VEGF-A), CD31, and CD105 in angiogenesis and their prognostic significance in terms of their impact on tumour progression at all stages of colorectal cancer were analysed.

VEGF showed granular cytoplasmic positivity in neoplastic cells. Strong expression was observed in most cases (69.2%), similar to the findings of Salem Y. Mohamed et al, Saad et al, and Hutajulu et al (7, 20, 21). None of the cases showed negative expression, which differs from previous reports (22). VEGF expression showed a significant positive association with tumour budding, with a P value of 0.011. This finding is similar to that of a study conducted in South Korea (23). A significant positive association was also observed between tumour grade and VEGF expression (P = 0.007). Higher expression was noted in all pT4 cases. Strong VEGF expression was associated with lymphovascular invasion in 75.9% of cases and lymph node metastasis in 80% of cases. However, VEGF expression did not have a statistically significant relationship with age, tumour location, stage, lymphovascular invasion, or lymph node metastasis. Strong VEGF expression in pT1 tumours was observed in 4 of 7 cases, in agreement with a study by Balasubramanian et al (24). Other histological subtypes, such as mucinous adenocarcinomas and tumours with neuroendocrine differentiation, showed strong expression. Five cases with liver metastasis did not attain complete remission, and all showed strong expression. Studies by Chung et al and Khorana et al have also demonstrated a direct association between VEGF and poor disease-free survival (25, 26).

CD31, a pan-endothelial marker, is expressed by endothelial cells both intratumourally and outside the tumour (Fig. 4). Of the cases, 46.2% (n = 18) had high MVD, whereas 53.8% (n = 21) had low MVD based on CD31 expression. When CD31-based MVD was compared with prognostic parameters, most cases with high MVD were grade 2 tumours, with a significant direct correlation between grade and MVD (P = 0.005). However, no association was found between CD31-based MVD and stage, tumour budding, lymphovascular invasion, or lymph node metastasis in our study. Saad et al, Takebayashi et al, and Tanigawa et al found significant correlations between CD31-based MVD and tumour size and angiolymphatic invasion (20, 27, 28).

CD105 is an endothelial marker expressed only in highly active vessels. It is not expressed in endothelial cells of the normal colonic mucosa. It is also expressed only by smaller microvessels and not by larger vessels. CD105 does not show false-positive expression in non-neoangiogenic vessels, unlike CD31. Similar observations have been reported in other studies using pan-endothelial markers such as CD34 to assess MVD (28–30). Paauwe et al demonstrated that CD105 promotes tumour invasion by stimulating the proliferation of cancer-associated fibroblasts (4). In the present study, CD105 showed high MVD in 21 cases (53.8%), which was comparable to the literature, in which 68% of cases showed high MVD (7). When CD105-based MVD was correlated with prognostic parameters, a significant positive association was found with tumour grade (P = 0.002). CD105-based MVD did not show any significant association with tumour budding, tumour stage, lymphovascular invasion, or nodal metastasis. However, a study by Li Gardi described a correlation between CD105 expression and tumour stage (31), and Nogues et al demonstrated an association between tumour metastasis and CD105 expression (32). Several studies of various tumours have shown that elevated serum endoglin (CD105) levels are associated with metastatic disease and relapse (33, 34).

When CD31 was compared with CD105, the number of microvessels inside the tumour was greater with CD31 in all cases. This finding is supported by other studies stating that CD31 stains both pre-existing vessels and new tumour microvessels (35). Endoglin (CD105) plays a key role in distinguishing newly formed blood vessels from pre-existing vessels within tumours. This ability is crucial for assessing tumour angiogenesis. An extensive search for studies directly linking tumour budding with angiogenesis indicated a paucity of literature. Very few studies have used endothelial markers to understand this relationship (20, 22).

Our study directly related tumour budding to neoangiogenesis and found that VEGF was the only angiogenic marker among the three associated with tumour budding in colorectal cancer. A direct relationship between angiogenesis and tumour grade was observed, with increased angiogenesis associated with poor differentiation. Strong VEGF expression was associated with either lymphovascular invasion or lymph node metastasis, highlighting the aggressive tumour behaviour related to its expression.

In terms of microvessel density, both CD31 and CD105 were associated with tumour grade. CD31, a pan-endothelial marker, was expressed in both normal and tumour-associated blood vessels, whereas CD105 expression was observed only within the tumour and at all tumour stages (pT1–pT4). Consequently, targeting CD31 for antiangiogenic therapy may inadvertently affect adjacent normal vasculature, leading to potential adverse effects. In contrast, CD105 is more specific for tumour-associated neovasculature, making it a significant therapeutic target for future clinical trials.

The roles of CD31 and VEGF in various solid malignancies, such as prostatic adenocarcinoma, small cell lung carcinoma, and endometrial adenocarcinoma, have been studied. Shahshenas et

al described similar findings for VEGF but also described CD31 as an important prognostic indicator (36). Anke et al demonstrated that CD31 and VEGF are important prognostic factors in early-stage laryngeal cancer (37). Studies have described similar results for CD31 and VEGF in endometrial and small cell lung carcinomas (38).

Although the results of this study are encouraging, they should be interpreted in light of the relatively small sample size (39 cases), single-centre design, arbitrary MVD dichotomisation, absence of multivariable analysis, lack of validated survival analysis, and high loss to follow-up. Future studies involving larger cohorts and well-designed clinical trials evaluating targeted anti-VEGF therapy are warranted to validate these findings and establish their clinical utility.

CONCLUSION

This study attempted to elucidate the role of angiogenesis in the development and progression of colorectal cancer, which may aid in developing targeted therapies that can be used to control tumour progression, thereby increasing survival. Vascular endothelial growth factor expression showed significant correlations with tumour budding and tumour grade. CD31- and CD105-based MVD were directly associated with tumour grade. Future studies on the clinical application of anti-VEGF therapy in early CRC cases will provide further insights into this field.

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Conflict of interest

The authors declare no conflict of interest.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval

Medical research on human participants must be carried out according to the World Medical Association Declaration of Helsinki. The study was approved by the Institutional Human Ethics Committee (IHEC) (Proposal Number: 22/314).

Authors' Contributions

Conceptualization: [Abinaya Sundari[2], Shanthakumari Sivanandam[2], Preethi Muthusamy Sundar]; Methodology: [Ragavi Velusamy[1], Abinaya Sundari]; Investigation: [Ragavi Velusamy[1], Abinaya Sundari[2]]; Writing – Original Draft Preparation: [Ragavi Velusamy[1], Abinaya Sundari[2]]; Writing – Review & Editing: [Abinaya Sundari[2]]. All authors have read and agreed to the published version of the manuscript

Acknowledgments

NA

Declaration of AI Use

Generative AI and AI- assisted technologies were not used in this manuscript

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