Original Research Article

Correlation of vascular endothelial growth factor (VEGF) expression with histological grade and stage of colorectal carcinoma: An experience from a tertiary care centre of West Bengal, India

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Abstract

Background: Angiogenesis plays a crucial role in the development and progression of colorectal carcinoma (CRC). Vascular endothelial growth factor (VEGF) is the most potent angiogenic factor in colorectal cancer and has association with metastasis.

Aim: The study was conducted with an aim to determine correlation of VEGF expression with histological grade and stage of colorectal carcinoma cases and to compare it with the VEGF expression of normal colonic mucosa.

Materials and methods: An institution based cross sectional observational study was done in a tertiary care centre of West Bengal among 50 colorectal cancer cases and corresponding specimens using a pre-designed semi-structured schedule on clinicopathological variables like dietary history, macroscopic findings, histological grade, type, degree of differentiation, stage etc. Sections from formalin fixed, paraffin-embedded blocks were evaluated for VEGF expression by immunohistochemistry using a scoring system. Data was analyzed by mean ± SD, $\chi 2$ test, Pearson's correlation test etc. using SPSS-16.

Results: All the colorectal carcinoma cases (100%) and the corresponding normal colonic mucosa (100%) were positive for VEGF immunostaining. All cases of normal colonic mucosa (100%) were found to be low VEGF expressor. 54% of the carcinoma had high VEGF expression and the remaining (46%) had low VEGF expression. A positive linear correlation between VEGF expression of the malignant tissue and the corresponding normal colonic mucosa was observed and this was statistically significant ($r = 0.523$; $p < 0.001$). A significant positive linear correlation was obtained between VEGF expression and histopathological grade ($r = 0.344$; $p= 0.014$) as well as AJCC stage ($r = 0.486$; $p < 0.001$) of tumor.

Conclusion: VEGF expression increases linearly with the increase in histological grade and stage of CRC and hence may be considered as a potential biomarker for predicting tumor behavior.

Key words

Colorectal carcinoma, Correlation, Histological grade, Stage, VEGF.

Introduction

Colorectal Carcinoma (CRC) is the third most common cancer and has recorded second highest number of cancer related deaths in the west [1]. Tumor invasion and metastasis are the leading cause of death in colorectal carcinoma; therefore identification of factors that regulate metastasis, prognosis and optimal treatment are of great significance.

Tumor angiogenesis, also referred to as angiogenic switch, is regarded as one of the defining event in the neoplastic process. The molecular basis of this angiogenic switch involves increased production of angiogenic factors and/or loss of angiogenic inhibitors. VEGF is one such angiogenic cytokine involved in both vasculogenesis and angiogenesis [2-5]. VEGF gene, located on chromosome 6p12, is a heparin-binding glycoprotein, belonging to the platelet-derived growth factor (PDGF) family with a molecular weight of 45 kDa. There are six types of VEGF: VEGF-A, B, C, D, E and F [4]. The activity of VEGF protein is mainly on the vascular endothelial cells [5-7]. Antiangiogenic therapy targeting the VEGF inhibits the growth of colon carcinoma and its metastasis [3].

Thus, the present study was designed with an aim to assess the expression of VEGF in colorectal carcinoma in relation to different clinicopathological parameters, to determine the relationship between VEGF expression and histological grade as well as stage of the tumor and to compare it with the VEGF expression of corresponding normal colonic mucosa.

Materials and methods

An institutional based cross sectional observational study was conducted in a tertiary care centre of West Bengal in the Department of Pathology in collaboration with Department of General Surgery. The work was initiated after obtaining ethical clearance from Institutional Ethical Committee and informed consent from the study population. Specimens obtained by all types of colectomy, sigmoidectomy, anterior resection and abdominoperineal resection for colorectal growth were included in the study with the exclusion of colorectal biopsies, history of chemotherapy and non-epithelial colorectal malignancies. All cases meeting the inclusion criteria within the study period were included. Hence a total of 50 cases were enrolled. Census method of sampling was used. Data was collected using a pre-designed semi-structured schedule on dependent variables like VEGF expression and independent variables like clinicopathological profile including dietary history, macroscopic findings, histological grade, histological type, stage and other relevant parameters. Data was collected by interview, observations, record review and laboratory

techniques including histopathology and immunohistochemistry.

Histopathology

All tissue samples were collected in 10% buffered formalin and processed for routine histopathological examination. Four to five micrometers thick sections from formalin fixed paraffin embedded (FFPE) blocks were cut and stained with Hematoxylin and Eosin for histopathological diagnosis. After histological confirmation of the diagnosis (colorectal carcinoma), following parameters were analyzed: histological type, degree of differentiation, tumor grade, lymphovascular and perineural invasion, nodal involvement, status of resection margins, staging (American Joint Committee on Cancer 2010, modified Dukes). For normal mucosa, an apparently uninvolved mucosa at a distance of minimum 5 cm away from the tumor was taken in all the cases and subsequently confirmed on microscopy.

Immunohistochemistry (IHC)

For IHC staining, 2-3 μm thick sections from FFPE tissues were taken on poly L Lysine coated slides. IHC was done manually using rabbit monoclonal VEGF antibody and the steps mentioned in the kit supplied were followed. Capillary hemangioma was taken as control for VEGF immunohistochemistry. Expression of VEGF protein in the cells was seen both in normal mucosa and the malignant tissue. The criterion for a positive immune reaction was brown cytoplasmic staining.

Scoring of immunohistochemistry

The pattern of VEGF expression was assessed by scoring system which included two parameters namely, overall percentage of the tumor cells stained positive (0–100%) and the staining intensity (score 1–3). A three - scaled scoring for percentage positivity of cells (**Table - 1**) was done as follows: 1 = < 30%, 2 = 31% -60%, 3 = > 60%. The intensity scoring (**Table - 2**) was done as: 1 = weak, 2 = moderate, 3 = strong staining. The final scoring of VEGF was done by adding percentage positivity score of cells (1–3) to the intensity score (1–3) and then the final score (2-6) was sub classified as high expressor (> 4) and low expressor (≤ 4).

Table - 1: Percentage score (PS).

Percentage (%) of tumor cells stained	VEGF Score
< 30%	1
30-60%	2
> 60%	3

Table - 2: Intensity score (IS).

Intensity of staining	VEGF Score
Weak	1
Moderate	2
Strong	3

Statistical analysis

Data was entered in MS excel. For descriptive purposes mean ± SD, range and percentage were used while for testing the relationship between variables chi-square ($\chi 2$) test and Pearson's correlation test was used using SPSS-16. Significance level was considered at p value < 0.05.

Results

A total of 50 colorectal cancer cases were enrolled during the entire study period. Clinical and pathologic characteristics of the study population have been shown in **Table - 3**. Age of the study population ranged from 21- 80 years with a mean of 49.56 ± 15.51 years. Nearly three fourth of the study population (74%) were Hindu. Majority (92%) of the cases were non vegetarians. Most frequent surgical procedure performed for colorectal cancer was right hemicolectomy (34%) followed by abdominoperenial and anterior resection (20% each). The most common site of colorectal carcinoma in the study population was rectum (40%), followed by caecum (20%) and sigmoid colon (16%). Nearly two third (66%) of the cases had exophytic macroscopic appearance with predominant intraluminal growth (**Figure - 1**).

Table – 3: Clinicopathological profile of colorectal carcinoma cases (n = 50).

Clinicopathological parameters		**Number (%)**
Age in years	< 40	16 (32)
	≥ 40	34 (68)
Sex	Female	22 (44)
	Male	28 (56)
Religion	Hindu	37 (74)
	Muslim	13 (26)
Diet	Non veg	46 (92)
	Veg	4 (8)
Size in cm	< 5	28 (56)
	≥ 5	22 (44)
Histological type	Adenocarcinoma NOS	40 (80)
	Mucinous adenocarcinoma	6 (12)
	Signet ring cell carcinoma	4 (8)
Histological grade	Low	38 (76)
	High	12 (24)
AJCC stage	I	9 (18)
	II	17 (34)
	III	21 (42)
	IV	3 (6)
LN involvement	Present	23 (46)
	Absent	27 (54)
CRM / Radial margin	Involved	9 (18)
	Not involved	41 (22)
LVSI	Seen	41 (82)
	Not seen	9 (18)
PNI	Seen	12 (24)
	Not seen	38 (76)

Figure - 1: Macroscopic appearances of colorectal growth: A- exophytic / fungating, B- endophytic with intraluminal growth, C- circumferential annular growth, D- mucinous / gelatinous foci.

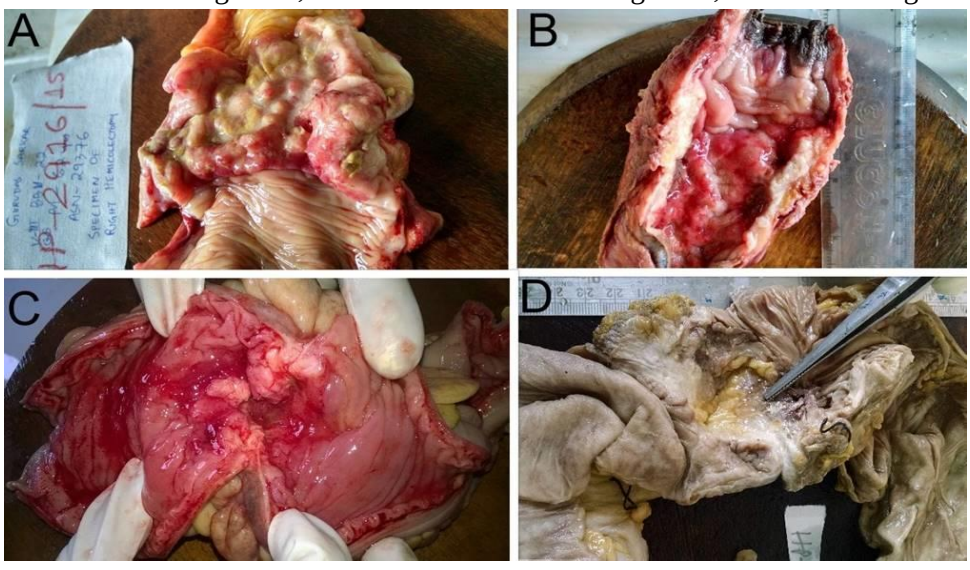


Table - 4: Distribution of VEGF expression with respect to pathological parameters (n = 50).

Pathological parameters		**VEGF Expression**		**Number (%)**
		Low Number (%)	**High Number (%)**	
Histological grade	Low	20 (52.6)	18 (42.4)	38 (76)
	High	3 (25)	9 (75)	12 (24)
AJCC Stage	I	7 (77.8)	2 (22.8)	9 (18)
	II	11 (64.7)	6 (35.3)	17(34)
	III	4 (19)	17 (81)	21 (42)
	IV	1 (33.3)	2 (66.7)	3 (6)
Histological Type	Adenocarcinoma NOS	21 (52.5)	19 (47.5)	40 (80)
	Mucinous adenocarcinoma	0 (0)	6 (100)	6 (12)
	Signet ring cell carcinoma	2 (50)	2 (50)	4 (8)
Differentiation* (* n = 40)	Well	2 (50)	2 (50)	4 (10)
	Moderate	18 (52.9)	16 (47.1)	34 (85)
	poor	1 (50)	1 (50)	2 (5)
LN involvement	Present	4 (17.3)	19 (82.7)	23 (46)
	Absent	19 (70.4)	8 (29.6)	27 (54)
LVSI	Seen	16 (39.1)	25 (60.9)	41 (82)
	Not seen	7 (77.8)	2 (22.2)	9 (18)
PNI	Seen	4 (33.3)	8 (66.7)	12 (24)
	Not seen	19 (50)	19 (50)	38 (76)
CRM / Radial margin / Mesenteric margin	Involved	4 (44.4)	5 (55.6)	9 (18)
	Not involved	19 (46.3)	22 (53.7)	41 (82)

* Differentiation was only for the Adenocarcinoma NOS category

(NOS- not otherwise specified, CRM- circumferential, LVSI- lymphovascular space invasion, PNI- perineural invasion)

Figure - 2: A (Hematoxylin and Eosin), E (Immunostain for VEGF) normal mucosa X100), B, C and D shows well, moderate and poorly differentiated colorectal carcinoma, Hematoxylin & Eosin X 400; F,G and H shows immunostain for VEGF of each respectively X 400.

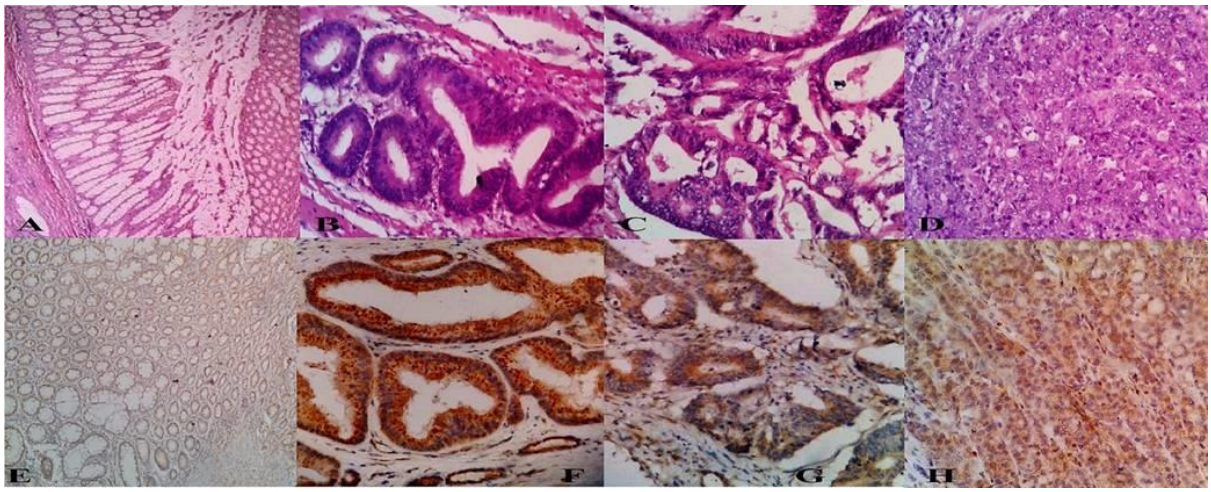


Figure - 3: A and B shows Mucinous adenocarcinoma and signet ring cell carcinoma, Hematoxylin & Eosin X 400; C and D shows immunostain for VEGF of each respectively X 400.

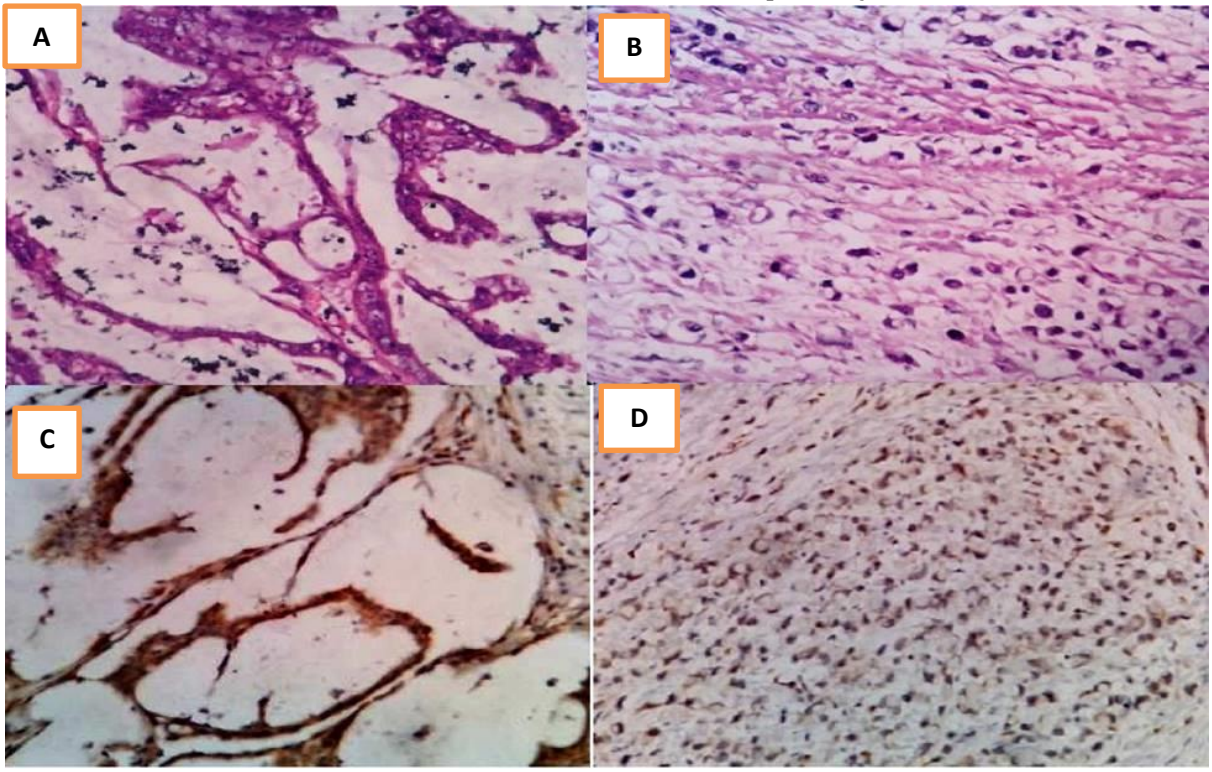
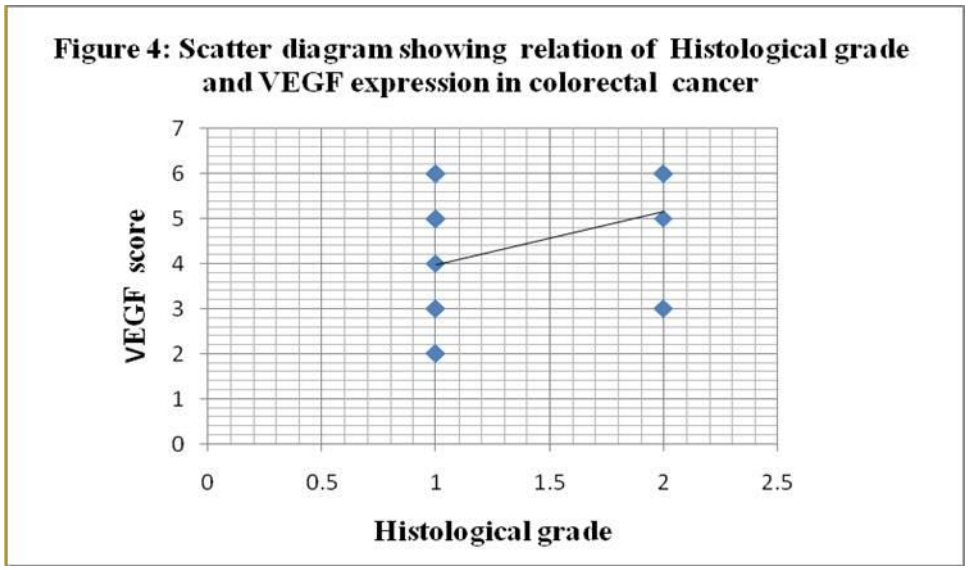


Figure - 4: Scatter plot showing correlation of VEGF expression of colorectal carcinoma with tumor grade (n=50).



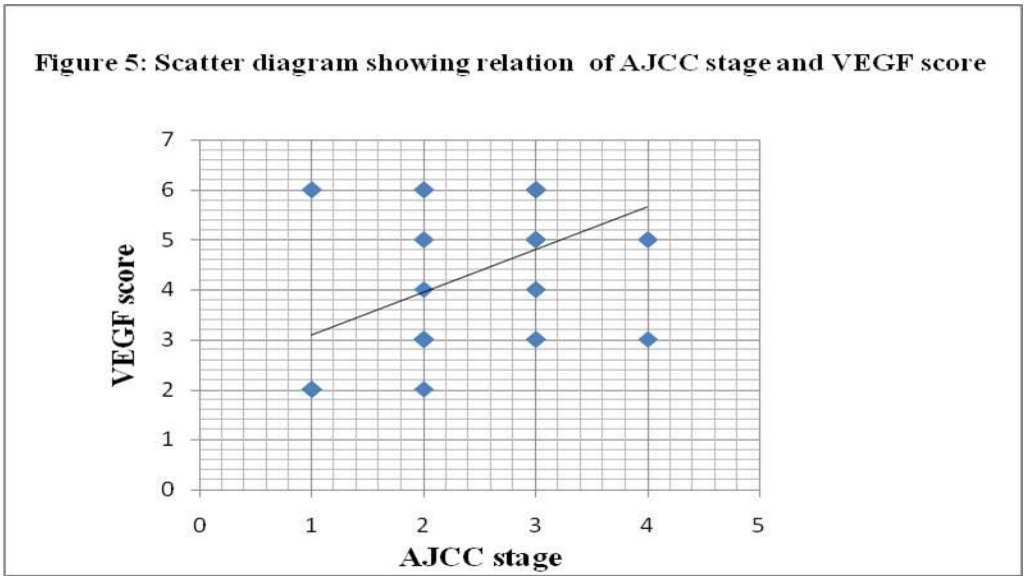
Comment: Positive linear correlation between histological grade and VEGF score (r = 0.344; p= 0.014)

The size of the tumor ranged from 2 to 10 cm with a mean of 4.50 ± 1.54 cm. Macroscopic tumor perforation was seen only in 8% of the specimens.

Adenocarcinoma not otherwise specified (NOS) was the most common histopathological type accounting for 80% of cases in the present study. Mucinous and signet ring cell carcinoma constituted 12% and 8% respectively. Nearly

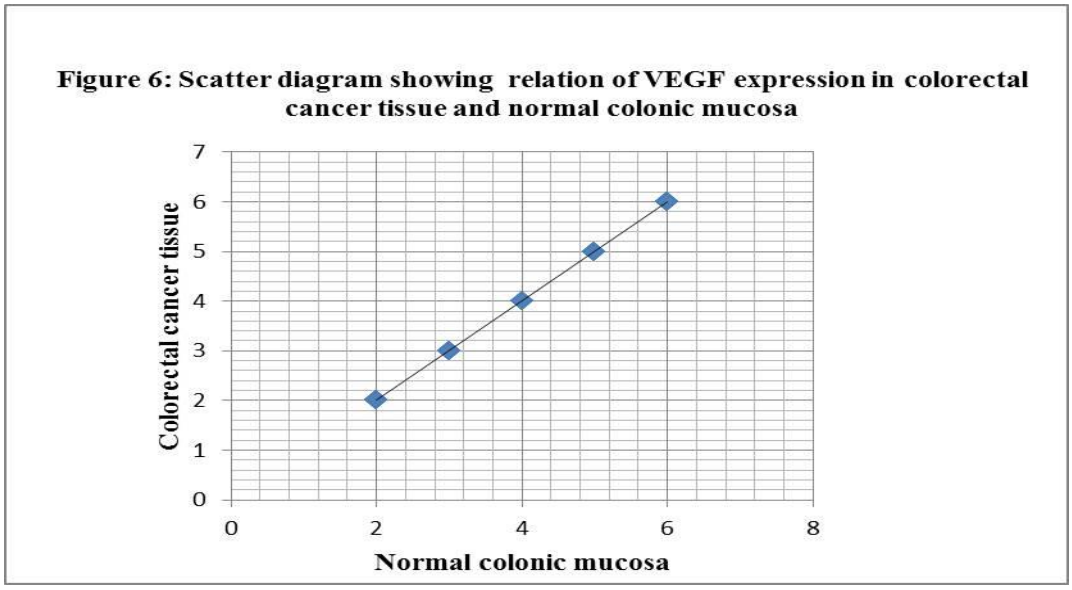
three fourth (76%) of the colorectal carcinoma had low grade histology. Modified Dukes C2 was the most common (40%) stage followed by B2 (34%). The most common (30%) TNM stage was pT3N0M0. Most (42%) of cases belonged to AJCC stage III. Three (6%) case among the study population had metastasis (M1) to liver and peritoneum corroborating with 6% each of modified Dukes D and AJCC IV. Intra-tumoral lymphocytes and Crohn- like response both were seen in 8 % cases. 23 cases (46%) had regional nodal metastasis. Only 18% of the total study population showed involvement of circumferential resection margin (CRM) / radial / mesenteric margin by the tumor. Majority (82%) of the tumor had evidence of lymphovascular space invasion (LVSI) in contrast to perineural invasion (PNI) which was seen only in 24% cases. Extranodal tumor deposits were seen only in 6% of cases.

Figure - 5: Scatter plot showing correlation of VEGF expression of colorectal carcinoma with AJCC-UICC stage (n=50).



Comment: Positive linear correlation between AJCC stage and VEGF score ($r = 0.486$; $P < 0.001$)

Figure - 6: Scatter plot showing correlation of VEGF expression of colorectal cancer tissue with that of corresponding normal colonic mucosa (n=50).



The relation between pathological parameters and VEGF expression has been shown in **Table - 4**. There was an association between lymph node involvement and VEGF expression among the study population and this was statistically significant ($\chi 2 = 14.034$, df = 1, $p < 0.001$). However there was no association between VEGF expression and histological type as well as degree of differentiation. All the Mucinous adenocarcinoma (100%) in the study population was high VEGF expressors.

All cases of normal mucosa (100%) were found to be low VEGF expressors. 54% of the carcinoma had high VEGF expression while the remaining (46%) had low VEGF expression. Variability of VEGF expression according to the degree of differentiation has been shown in **Figure - 2** and **Figure - 3**. In the present study, a significant positive linear correlation was obtained between VEGF expression and histopathological grade ($r = 0.344$; $p= 0.014$) as well as AJCC stage ($r = 0.486$; $p < 0.001$) of tumor (**Figure – 4**, **Figure – 5**). There was a positive linear correlation between VEGF expression of the malignant tissue and the corresponding normal colonic mucosa and this was statistically significant (Pearson's correlation coefficient (r) value = 0.523; $p < 0.001$) as shown in **Figure - 6**.

Discussion

Tumors recruit new blood vessels from the existing circulation (angiogenesis) and this is the basis of tumor invasion and metastasis. VEGF, an important cytokine for angiogenesis, is not only required for the tumor growth but also its metastasis. In this scenario, an institutional based cross sectional observational study was done among the colorectal carcinoma cases with a purpose to assess the correlation between histological grade and stage of the tumor versus VEGF expression by immunohistochemistry.

Mean age of the study population was found to be 49.56 ± 15.51 years. This is consistent with the figures mentioned by Hedaya, et al. [8] and Mohamed, et al. [9] However, Hashim, et al. [1], Nakasaki, et al. [10] and Taggarshe, et al. [11] found the mean as 58.1 to 62 years. In the present study there is a shift of mean to the left compared to the previous studies done by Hashim, et al.; Nakasaki, et al. and Taggarshe, et al. indicating the rising trend of involvement of younger age group in CRC, probably attributable to rising affluence which has caused a change in dietary habits rich in animal protein and fat and low in dietary fibre and other related factors. Rectum was the most commonly involved site in the present study which was supported by Malik, et al. [12] and Taggarshe, et al. [11].

There was a significant association between lymph node involvement and VEGF expression which was supported by Mohamed, et al. [9]. There was a positive linear correlation between VEGF expression of the malignant tissue and the corresponding normal colonic mucosa indicating that VEGF has a fundamental role in the angiogenesis of CRC. This was also supported by Hashim, et al. [1] As grouped in **Table - 4**, the present study shows that there was a gradual increase in the VEGF expression in accordance with the increase in the grade of tumor (42.4% high expressor in low grade and 75% high expressor in high grade). However, no significant differences among the three degrees of differentiation and histological types ($p > 0.05$) were noted. This observation is supported by Ono, et al. [13] and Gunsilius, et al. [14] who reached the same conclusion. Furthermore, there was a significant positive linear correlation between the stage of tumor and the VEGF immunostaining ($r = 0.486$; $p < 0.001$). This was in accordance to Hashim, et al. [1]; Galizia, et al. [15]; Mohamed, et al. [9] and Taggarshe, et al. [11] stating that high expression of VEGF is seen in high grade and advanced stage of tumor. This finding indicates equivocally that VEGF expression is directly proportional to the degree of spread of colorectal tumor.

The study population also showed an incidental finding of all Mucinous adenocarcinoma to be

high expressor of VEGF suggesting a definitive role of targeted therapy. The limitations of the study were lack of external validity, observation bias and staining heterogeneity of VEGF in some cases. Further, analytical studies might be done focusing on other ancillary markers of angiogenesis.

Conclusion

VEGF is the driving force behind angiogenesis in colorectal carcinoma. There is a definite positive correlation between VEGF expression of colorectal cancer tissue and the corresponding normal mucosa. VEGF expression increases linearly with the increase in histological grade and stage of colorectal carcinoma which may be a target of the newer generation monoclonal antibody against VEGF.

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