Section: Pathology

Original Research Article

STUDY OF MICROVESSEL DENSITY(MVD) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AS PROGNOSTIC INDICATOR IN COLORECTOL CANCER AND CORRELATE WITH PTNM STAINING

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ABSTRACT

Background: The aim & objective is to study the Microvessel density (MVD) and Vascular endothelial growth factor (VEGF) as prognostic indicator in colorectal carcinoma and correlate with PTNM staging.

Materials and Methods: The present study is an observational study and cross sectional done on 50 Colorectal resection specimens received by the Department of Pathology, Gandhi Hospital, Secunderabad for a period of 18 months from October 2022- March 2024. Relevant clinical details of all the 50 patients diagnosed with Colorectal carcinoma are documented. The specimens received are fixed, processed and embedded in paraffin wax. Serial sections of 4-5 µ thickness are obtained and stained with H&E.

Results: Routine processing and H&E staining were done followed by immunohistochemistry with MVD and VEGF. In the present study, the age of the patients with Colorectal carcinoma was ranging from 36-70 years and majority of the subjects were males with M:F ratio of 1.77:1. Majority of the tumours were moderately differentiated and Poorly differentiated adenocarcinoma belonged to stage III and IV. MVD and VEGF immunoexpression was correlated with clinicopathological parameters like grade and stage of the tumour to analyse the usefulness of these immunomarkers in prognosis. In Present study, MVD and VEGF expression was more in Moderately differentiated tumours and Poorly differentiated tumours than in Well differentiated tumors. Present study shows that there is a statistically significant correlation of MVD and VEGF positivity with stage of the tumor. There is higher expression of MVD and VEGF in higher stage tumours (Stage III and Stage IV).

Conclusion: The present study concluded that, MVD and VEGF represent important prognostic indicators in colorectal carcinoma. As the predominant angiogensis factors in the growth and maturation of new vessels- MVD, VEGFs are associated with incidence of metastases and decresed survival. Combined targeting of MVD and VEGF pathways may offer a novel and potentially promising chemotherapeutic strategy for treatment and/or prevention of Colorectal neoplasia.

Keywords: MVD, VEGF, Colorectal Carcinoma, Adenocarcinoma, Immunohistochemistry.

INTRODUCTION

Colorectal carcinoma is one of the most frequent malignancies in the world.[1] Compared to the western world, the incidence rates of colorectal cancer are low in India; but apart from geographical variations,[2] the incidence is rising rapidly in India. Worldwide, it is the second most common cancer

among men and third most common cancer among women according to the recent GLOBOCAN cancer Statistics.[3] Colorectal cancer is a multifactorial disease process. Genetic factors, environmental exposures (including diet) and inflammatory conditions of digestive tract are all involved in the development of Colorectal cancer.[4] Populations differ in the risk of development of colorectal cancer depending upon the race and ethnicity, e.g.,Ashkenazi Jews are at a slightly increased risk,[5] of colorectal cancer. In USA, the incidence of CRC is higher in African-Americans compared to Caucasians which in turn are at higher risk than Asian American, Native Americans,[6] and Hispanic Americans. Increased caloric intake and decreased intake of fibre containing foods are among the possible dietary influence,[7] for causing Colorectal cancer. Majority of the cases are diagnosed in patients greater than 50 years of age but now the incidence in younger population is increasing and they present at an advanced stage.[8] The prognosis is related to various clinical and pathological parameters.

Microvessel density (MVD) and vascular endothelial growth factor (VEGF) in tumor tissue may be reliable markers of tumor angiogenesis.[9-11] One potential indicator in Colorectal cancer is tumor induced angiogenesis.It is a prerequisite for tumor growth and metastasis. Spread of tumor cells is quantitatively related to microvessel density (MVD) and VEGF. Microvessel density can facilitate assessment of the degree of angiogenic activity of the tumor and the prognosis.[12]

MVD is a measure of the number of vessels per high power field. More than 15 microvessels/ high power field view is considered as High MVD. Less than 15 microvessels/ high power field view is considered as Low MVD.MVD is calculated by evaluating immunohistochemically stained vessels, or vascular hot spots by using CD34 antibodies.[13]

VEGF is secreted to the greatest extent by endothelial cells but it can also secreted by thrombocytes, macrophages, astrocytes, osteoblasts and tumor cells.Vascular endothelial growth factor (VEGF) has significant impact on angiogenesis.[14] In tumor cells it stains the cytoplasm and stromal cells.

The present study is conducted to analyse clinical features, tumor type and tumor differentiation and to study the Microvessel density (MVD) and Vascular endothelial growth factor (VEGF) as prognostic indicator in colorectal carcinoma and correlate with PTNM staging.

Aim and Objectives of the study

1. To analyse clinical features, tumor type and tumor differentiation.
2. To study the Microvessel density (MVD) and Vascular endothelial growth factor (VEGF) as prognostic indicator in colorectal carcinoma and correlate with PTNM staging.

MATERIALS AND METHODS

The present study is an observational study and cross sectional done on 50 Colorectal resection specimens received by the Department of Pathology, Gandhi Hospital, Secunderabad for a period of 18 months from October 2022- March 2024.

Inclusion Criteria

- Colorectal resection specimens in patients diagnosed with colorectal carcinoma are included.
- All ages and both sexes are included

Exclusion Criteria

- Poorly preserved specimens and specimens with artifacts.
- Patients with unobtainable clinical data.
- Patients with concurrent other malignant tumor or on any immunomodulation therapy.

Methodology

Relevant clinical details of all the 50 patients diagnosed with Colorectal carcinoma are documented.

The specimens received are fixed, processed and embedded in paraffin wax. Serial sections of 4-5 µ thickness are obtained and stained with H&E.

RESULTS

- Nuclei: Blue
- Cytoplasm: Varying shades of pink

Sections are studied under light microscope and histopathological findings are documented.

Tumors are graded as well differentiated (G1) or moderately differentiated (G2) or poorly differentiated (G3) according to the percentage of gland formation.

Pathological stage is determined according to TNM staging.

IHC Procedure

For performing IHC, sections of 4 to 5 micron thickness were prepared from the corresponding paraffin blocks on Poly L- lysine coated slides. Primary antibody (Anti-CD34, Mouse monoclonal antibody ready to use) of BioGenex company and secondary antibody (DBS mouse monoclonal antibody) of DBS company were used for CD34 expression.

Primary antibody (VEGF, mouse monoclonal antibody, 1:100 dilution) ofDiagnostic BioSystems company and secondary antibody (DBS mouse monoclonal antibody) of DBS company were used for VEGF expression.

Clinicopathological parameters like age, gender, grade of the tumor and stage of the tumor is correlated with MVD and VEGF immunoexpression to analyses the prognostic significance of MVD and VEGF.

Statistical Analysis: The SPSS 22 software was used for statistical analysis. The data is presented in frequency and percentage. The Fishers exact test will be used to test the association between categorical

groups. A p value of <0.05 is considered as a statistically significant correlation.

Observations and Results

The present study is an observational study and cross sectional done on 50 Colorectal resection specimens received by the Department of Pathology, Gandhi Hospital, Secunderabad for a period of 18 months from October 2022- March 2024.

Age Distribution: Among 50 patients of Colorectal carcinoma included in the study, majority of the patients (n= 38, 76%) belonged to the age group of >50 years.

12 patients belonged to the age group of ≤50 years.

The youngest case was 36 years old while the eldest case was 70 years old.

The mean age of the patient in our study was 55 years and majority of the patients belonged to the age group of 51-60 years.

Table 1: Distribution of cases according to age group.

	Frequency	Percentage
31 – 40	4	8%
41 – 50	8	16%
51 – 60	24	48%
61 – 70	14	28%
Total	50	100%
Mean Age	55.08 ± 9.04	

Gender Predominance: Among 50 cases, majority of the cases were males (n= 32, 64%) 18 cases were females (36%) with M:F ratio of 1.77:1.

Table 2: Distribution of cases according to gender.

	Frequency	Percentage
Male	32	64%
Female	18	36%
Total	50	100%

Clinical Symptoms: Majority of cases showing symptoms with altered bowel habits (n=33) and least number of cases showing with Perforation (n=1).

Table 3: Distribution of cases according to clinical symptoms

Sl.no	Symptoms	Number of cases	Percentage
1.	Altered bowel habits	33	66%
2.	Bleeding per rectum	27	54%
3.	Abdominal pain	21	42%
4.	Weight loss	21	42%
5.	Perforation	1	2%

Immunoexpression Of VEGF: Number of cases showing positive with VEGF n= 36(72%) and number of cases negative with VEGF n=14(28%).

Table 4: Distribution of cases according to immunoexpresssion VEGF

	Frequency	Percentage
Positive	36	72%
Negative	14	28%
Total	50	100%

Immunoexpression of MVD: Number of cases High with MVD n=32(64%) and number of cases Low with MVD n=18(36%).

Table 5: Distribution of cases according to MVD

	Frequency	Percentage
High	32	64%
Low	18	36%
Total	50	100%

Grade of the tumor: Majority of the tumors are Moderately differentiated belonging to grade 2(n=30, 60%).

Grade 1 tumors are seen in 24%(n= 12) of the cases and grade 3 tumors are seen in 16% (n= 8) of the cases.

Table 6: Distribution of cases according to grade of the tumor

	Frequency	Percentage
Well differentiated	12	24%
Moderately differentiated	30	60%
Poorly differentiated	8	16%
Total	50	100%

Relation of tumor grade with MVD: There is statically significant correlation with MVD and grade of the tumor. Number of cases showing High MVD are associated with Moderately differentiated adenocarcinoma n=23(71.9%).

Table 7: MVD in relation to tumor grade

	MVD			
	HIGH		LOW	
	N	%	N	%
Well differentiated	2	6.3%	10	55.6%
Moderately differentiated	23	71.9%	7	38.9%
Poorly differentiated	7	21.9%	1	5.6%
Total	32	100.0%	18	100.0%
Fishers exact test= 18.17, p=0.0001*, Statistically significant				

Relation of tumor grade with VEGF: There is statically significant correlation between VEGF immunoexpression and grade of the tumor.
Expression of vegf in Well differentiated cases n=3(8.3%), Moderately differentiated n=25(69.4%) and poorly differentiated n= 8(22.2%). Maximum number of cases seen in Moderately differentiated adenocarcinoma grade 2 n=25(69.4%).

Table 8: VEGF expression in relation to tumor grade

	VEGF			
	Positive		Negative	
	N	%	N	%
Well differentiated	3	8.3%	9	64.3%
Moderately differentiated	25	69.4%	5	35.7%
Poorly differentiated	8	22.2%	0	0.0%
Total	36	100.0%	14	100.0%
Fishers exact test= 18.17, p=0.0001*, Statistically significant				

Tumor stage: Number of cases seen Stage I n=8(16%), Stage II n=6(12%), Stage III n=30(60%), Stage IV n=6(12%). Maximum number of cases seen in Stage III n =30(60%).

Table 9: Distribution of cases according to tumor stage

	Frequency	Percentage
I	8	16%
II	6	12%
III	30	60%
IV	6	12%
Total	50	100%

Relation of tumor stage with MVD
There is a statistically significant correlation between MVD and stage of the tumor. Number of cases showing High MVD are associated with Stage III n=21(65.6%).

Table 10: MVD in relation to tumor stage

	MVD			
	HIGH		LOW	
	N	%	N	%
I	1	3.1%	7	38.9%
II	4	12.5%	2	11.1%
III	21	65.6%	9	50.0%
IV	6	18.8%	0	0.0%
Total	32	100.0%	18	100.0%
Fishers exact test= 13.07, p=0.0001*, Statistically significant				

Relation of tumor stage with VEGF
There is a statistically significant correlation between VEGF & stage of the tumor. Expression of vegf in Stage I n=1(2.7%), Stage II n=4(11.1%) and Stage III n= 25(69.4%), Stage IV n=6 (16.7%). Maximum number of cases seen in Stage III n= 25(69.4%).

Table 11: VEGF expression in relation to tumor stage

	VEGF			
	Positive/ over expression		Negative/ no expression	
	N	%	N	%
I	1	2.7%	8	57.1%
1II	4	11.1%	1	7.1%

III	25	69.4%	5	35.7%
IV	6	16.7%	0	0.0%
Total	36	100.0%	14	100.0%
Fishers exact test= 25.19, p=0.0001*, Statistically significant				

Figure 1: Gross Image External Surface and Cut Section in Colo rectalcancer

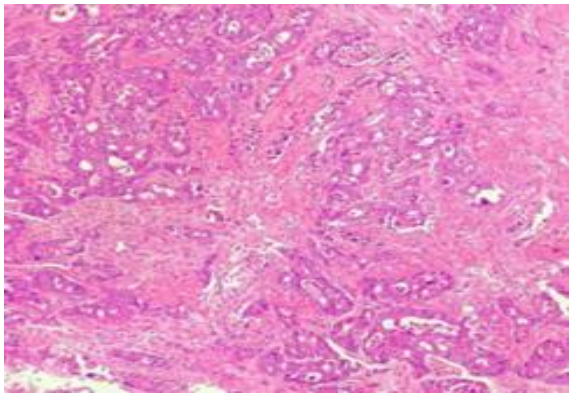

Figure 2: Adenocarcinoma, H&E10X

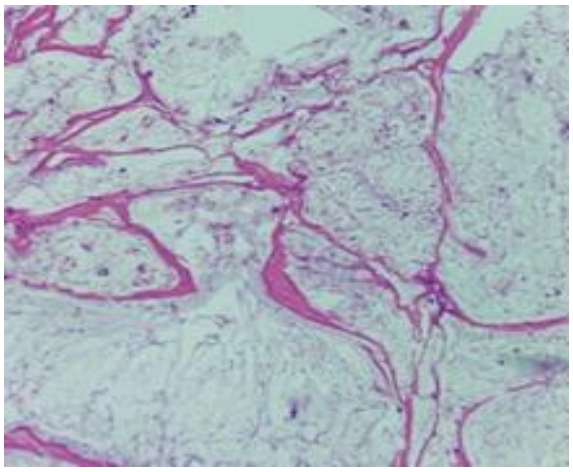

Figure 3: Mucinous Adenocarcinoma, H&E 10X

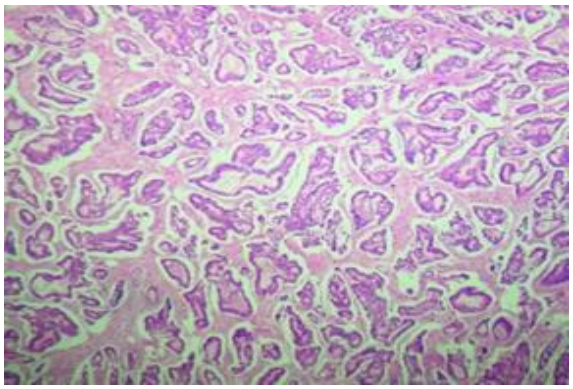

Figure 4: Well Differentiated Adenocarcinoma, H&E10X

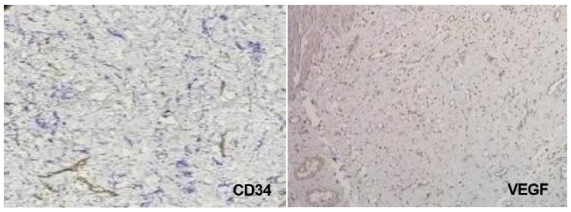

Figure 5: Expression of CD34 and VEGF in well differentiated adenocarcinoma

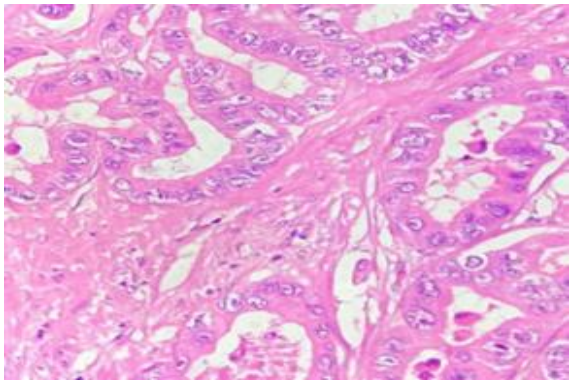

Figure 6: Moderately Differentiated Adenocarcinoma, H&E40X

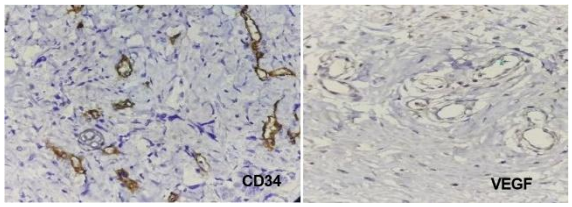

Figure 7: Expression of CD34 and VEGF in moderately differentiated adenocarcinoma

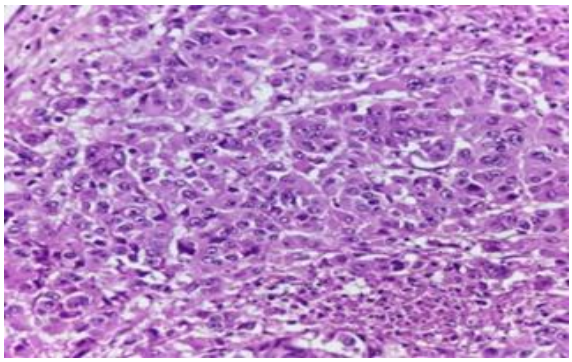

Figure 8: Poorly Differentiated Adenocarcinoma, H&E40X

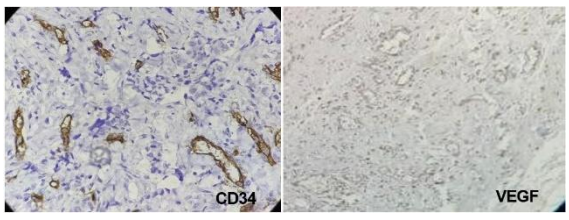

Figure 9: Expression of CD34 and VEGF in poorly differentiated adenocarcinoma

DISCUSSION

Colorectal carcinoma is one of the most frequent malignancies in the world1. Mostly CRC occurs after the fifth decade of life. However, the incidence of CRC is increasing in young age due to life style Changes.

Young-onset CRC indicates the possibility of genetic predisposition, such as hereditary nonpolyposis colorectal cancer (HNPCC), characterized by microsatellite instability (MSI). An individual with a history of adenomatous polyps or inflammatory bowel disease has an increased risk of developing colorectal cancer compared to an individual with no history of either.

Microvesseldensity (MVD)12, defined as the density of microvessels within the tumor microenvironment, serves as a surrogate marker for angiogenesis and is associated with tumor aggressiveness and patient prognosis. MVD is commonly assessed by immunohistochemical staining of endothelial cell markers, such as CD31 or CD34, and quantifying the number of microvessels per unit area within the tumor tissue.

Vascular endothelial growth factor (VEGF)14is the most widely studied and best characterized angiogenic factor, secreted by almost all solid cancers. It stimulates endothelial proliferation and migration, vascular permeability and is the most potent angiogenic protein known. The effect of VEGF depends on tumor cell expression of VEGF and its receptors in the endothelial cells. When overexpressed, VEGF is associated with advanced tumor stage or tumor invasiveness in various types of human cancer.

In our study, majority of the patients were over 50 years of age. The commonly affected age group was 51-60 years with a mean age of 55 years. The youngest patient was 36 years old whereas the oldest was 70 years.

Wen-Li Zhang, Xue-Qin Gao, Jin-Xiang Han et al,[15] (2009) observed that the age at diagnosis ranged from 38- 72 years. Youngest patient at the time of diagnosis was 38-year old.

Antonacopoulou AG et al,[16] observed that the age of presentation in their study was 25-82 years. Youngest patient at the time of diagnosis was 25 years old.

In the present study, males were more frequently affected than females with a M:F ratio of 1.77:1. 64% of the cases were males whereas 36% cases were females in our study.

Antonacopoulou AG et al,[16] in their study observed that 64% patients were males and 36% patients were females with M:F ratio of 1.75:1.

Comparison of grade of the tumour in the present study with other studies

In our study, 24% cases were diagnosed as well differentiated colorectal adenocarcinoma, 60% cases were diagnosed as moderately differentiated adenocarcinoma & 16% cases were diagnosed as poorly differentiated adenocarcinoma.

Lim SW et al,[17] observed that in their study group 32.6% cases were well differentiated adenocarcinoma, 61.1% cases were diagnosed as moderately differentiated adenocarcinoma & 6.3% cases were diagnosed as poorly differentiated adenocarcinoma.

Al-Maghrabi j et al[18]: In their study observed that 26% cases were well differentiated adenocarcinoma, 59.5% cases were diagnosed as moderately differentiated adenocarcinoma and 11.7% cases were poorly differentiated adenocarcinoma.

Wu QB et al,[19] in their study had 15% cases diagnosed as well differentiated adenocarcinoma, 57.4% cases were diagnosed as moderately differentiated adenocarcinoma and 27.4% cases were poorly differentiated adenocarcinoma.

Comparison of stage of the tumour in the present study with other studies: In our study, majority of the patients had a stage III (60%) tumor at presentation. 16% of the study subjects had stage I, 12% of the subjects had stage II tumor whereas 12% of the study subjects had stage IV tumor.

Ce´ line Hamelin, Emilie Cornut, Florence Poirier et al,[20] (2011) In their study observed that 6.6% cases were stage I, 6.6% cases were stage II, 80% cases Were stage III & 6.6% cases were stage IV tumor

Donna O'Dwyer, Lynda D. Ralton, Aisling O'Shea et al,[21] (2011) in their study observed that 82% cases were stage III and 8% cases were stage IV tumor.

Al-Maghrabi J et al,[18] in their study observed that majority of the patients had a stage III/IV tumor. In their study, 34% patients had stage I/II tumor and 66% had stage III/IV tumor at presentation.

MVD in relation to grade and stage of the tumor present study: MVD is significantly related to the grade of the tumor.MVD is High in Moderately differentiated tumors and Poorly differentiated than in well differentiated tumors.

MVD is significantly correlated with Stage of the tumour. MVD is more in Stage III and IV than stage I, stage II.

Kimura et al,[22] observed that there is association between MVD with grade of the tumor concordance to the findings of the present study and discordance with stage of tumor.

They observed that MVD was high in Moderately and poorly differentiated adenocarcinoma.

They observed that high MVD associated with stage I discordance with present study which is showing high MVD in Stage III and IV tumors.

Zheng S et al,[23] observed that there was significant correlation between MVD and grade of the tumor.

They noticed MVD was high in Poorly differentiated adenocarcinoma which is concordance with the present study.

VEGF Expression in Relation to Grade and Stage of the Tumor Present Study

VEGF expression is significantly related to the grade of the tumor. VEGF expression is more in moderately differentiated tumors andpoorly differentiated adenocarcinoma than well and poorly differentiated tumors. VEGF expression is significantly correlated

with stage of the tumour. VEGF expression is more in Stage III and IV than stage I, stage II.

Kimura et al,[22] observed that there is association between VEGF expression with grade and stage of the tumour concordance to the findings of the present study.

They observed that VEGF expression was high in Moderately and poorly differentiated adenocarcinoma and more in advanced stage tumor (Stage III and IV) concordance with the current study.

Zheng S et al,[23] observed that there was significant correlation between VEGF expression and grade of the tumor.

They noticed VEGF expression was high in Poorly differentiated adenocarcinoma and concordance with the present study.

Kamel AA et al,[24] observed that there is association between VEGF expression with grade and stage of the tumour concordance to the findings of the present study.

They observed that VEGF expression was high in Moderately and Poorly differentiated adenocarcinoma concordance with the current study. VEGF expression also related significantly with the higher stage of the tumor (Stage III and IV), which is in concordance with current study.

The present study showed increased VEGF expression in Moderately and poorly differentiated adenocarcinoma similar to the studies done by Kimura et al, Zheng S et al,Kamel AA et al.

The present study showed increased VEGF expression in Higher stage (Stage III and IV) concordance to the studies done by Kimura et al, Kamel AA et al.

Limitations of the present study

It is to be noted that the variations can be attributed to multiple factors like technical variability in the IHC performance (differences in tissue fixation, processing, epitope retrieval, primary antibody, interpretation and reporting of pathologist, ununified and widely acceptable scoring systems for evaluation of MVD and MVD expression) sample size, heterogeneity of study population, racial differences, and varied experimental designs.

As various studies use different antibodies and techniques to demonstrate MVD and VEGF expression, correlating the results with other techniques like FISH is recommended.

As environmental and genetic factors play a role in the development of colorectal carcinomas, the studies are recommended in a larger subset of population living in various geographical locations.

Also, long term follows up of the patients is needed for accurate results about the prognosis.

CONCLUSION

MVD and VEGF represent important prognostic indicators in colorectal carcinoma. As the predominant angiogensis factors in the growth and maturation of new vessels- MVD, VEGFs are associated with incidence of metastases and decresed survival. Combined targeting of MVD and VEGF pathways may offer a novel and potentially promising chemotherapeutic strategy for treatment and/or prevention of Colorectal neoplasia.

REFERENCES

1. Patra T, Mandal S, Alam N, Murmu N. Clinicopathological trends of colorectal carcinoma patients in a tertiary cancer centre in Eastern India. Clinical Epidemiology and Global Health. 2018 Mar 1;6(1):39-43.
2. Mohandas KM, Desai DC. Epidemiology of digestive tract cancers in India. V. Large and small bowel. Indian J Gastroenterol. 1999;18:118–121.
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, BrayF. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2021 May;71(3):209-49.
4. Bhat SA, Chowdri NA, Khan MA, Parray FQ, Wani RA, Mehraj A. Clinicopathological profile of colorectal cancer in Kashmir. Clin Surg. 2019; 4. 2019;2368.
5. Feldman G. Do Ashkenazi Jews have a higher than expected cancer burden?Implications for cancer control prioritization efforts. Isr Med Assoc J. 2001;3(5):341 -46.
6. Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, et al. Cancer statistics, 2004. CA Cancer J Clin. 2004;54(1):8-29.
7. Quddus MA, Alimunzzaman M, Rahman MZ, Alam MK. Clinicopathological Study of 50 Cases Colorectal Carcinoma At Tertiary Care Hospital. Journal of Shaheed Suhrawardy Medical College. 2012;4(2):53-6.
8. Saha M, Shil BC, Saha SK, Banik RK, Perveen I, Chowdhury MS, Islam AN, Saifullah AN. Study of Clinicopathological Profile of Sporadic Cases of Colorectal Cancer. Euroasian Journal of Hepato-Gastroenterology. 2016 Jul;6(2):134.
9. Des Guetz G, Uzzan B, Nicolas P, Cucherat M, Morere JF, Benamouzig R, Breau JL, Perret GY. Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature. British journal of cancer. 2006 Jun;94(12):1823-32.
10. Anannamcharoen S, Nimmanon T. Study of the vascular endothelial growth factor (VEGF) expression and microvascular density (MVD) in primary colorectal cancer specimens. Journal of the Medical Association of Thailand. 2012 Aug 1;95(8):1041.
11. Perrone G, Vincenzi B, Santini D, Verzı A, Tonini G, Vetrani A, Rabitti C. Correlation of p53 and bcl-2 expression with vascular endothelial growth factor (VEGF), microvessel density (MVD) and clinico-pathological features in colon cancer. Cancer letters. 2004 May 28;208(2):227-34.
12. Abdalla SA, Behzad F, Bsharah S, Kumar S, Amini SK, O'Dwyer ST, Haboubi NY (1999) Prognostic relevance of microvessel density in colorectal tumours. Oncol Rep 6: 839–842.
13. Banner BF, Whitehouse R, Baker SP, Swanson RS. Tumor angiogenesis in stage II colorectal carcinoma: association with survival. American journal of clinical pathology. 1998 Jun 1;109(6):733-7.
14. Zheng S, Han MY, Xiao ZX, Peng JP, Dong Q. Clinical significance of vascular endothelial growth factor expression and neovascularization in colorectal carcinoma. World journal of gastroenterology: WJG. 2003 Jun 6;9(6):1227.
15. Zhang WL, Gao XQ, Han JX, Wang GQ, Yue LT. Expressions of heat shock protein (HSP) family HSP 60, 70 and 90alpha in colorectal cancer tissues and their correlations to pathohistological characteristics. Ai zheng= Aizheng= Chinese journal of cancer. 2009 Jun;28(6):612-8
16. Antonacopoulou AG, Tsamandas AC, Petsas T, Liava A, Scopa CD, Papavassiliou AG, Kalofonos HP. EGFR, HER-2 and COX-2 levels in colorectal cancer. Histopathology. 2008 Dec;53(6):698-706.

17. Lim SW, Kim HR, Kim HY, Huh JW, Kim YJ, Shin JH, Suh SP, Ryang DW, Kim HR, Shin MG. Over-expression of Her-2 in colorectal cancer tissue, but not in serum, constitutes an independent worse prognostic factor. Cellular Oncology. 2013 Jul;36(4):311-21.
18. Al-Maghrabi J, Buhmeida A, Emam E, Syrjänen K, Sibiany A, Al-Qahtani M, Al- Ahwal M. Cyclooxygenase-2 expression as a predictor of outcome in colorectal carcinoma. World journal of gastroenterology: WJG. 2012 Apr 4;18(15):1793.
19. Wu QB, Sun GP. Expression of COX-2 and HER-2 in colorectal cancer and their correlation. World Journal of Gastroenterology: WJG. 2015 May 28;21(20):6206
20. Hamelin C, Cornut E, Poirier F, Pons S, Beaulieu C, Charrier JP, Haïdous H, Cotte E, Lambert C, Piard F, Ataman-Önal Y. Identification and verification of heat shock protein 60 as a potential serum marker for colorectal cancer. The FEBS journal. 2011 Dec;278(24):4845-59.
21. O'Dwyer D, Ralton LD, O'Shea A, Murray GI. The proteomics of colorectal cancer: identification of a protein signature associated with prognosis. PloS one. 2011 Nov 18;6(11): e 27718.
22. Kimura Y, Morohashi S, Yoshizawa T, Suzuki T, Morohashi H, Sakamoto Y, Koyama M, Murata A, Kijima H, Hakamada K. Clinicopathological significance of vascular endothelial growth factor, thymidine phosphorylase and microvesseldensity in colorectal cancer. Molecular medicine reports. 2016 Feb 1;13(2):1551- 7.
23. Zheng S, Han MY, Xiao ZX, Peng JP, Dong Q. Clinical significance of vascular endothelial growth factor expression and neovascularization in colorectal carcinoma. World journal of gastroenterology: WJG. 2003 Jun 6;9(6):1227.
24. Kamel AA, Yossef WT, Mohamed M. Correlation of vascular endothelial growth factor expression and neovascularization with colorectal carcinoma: A pilot study. J Adenocarcinoma. 2016;1(1):5.