Angiogenesis in Colorectal Carcinoma: An Immunohistochemical study

Swechha Maskey, Smriti Karki, Anju Pradhan, Punam Paudyal, C S Agrawal

Nepal Cancer Hospital and Research Center, Lalitpur, Nepal

Abstract

Background: Angiogenesis plays an important role in carcinogenesis. Angiogenesis is studied by calculating the microvessel density. The purpose of this study is to determine whether angiogenesis can be documented in colorectal tumor progression and to assess whether the quantification of microvessels can be correlated to tumor aggressiveness.

Methods: This is a hospital based descriptive cross-sectional study, done from August 2015 to July 2016 after obtaining ethical approval from Institutional Review Committee. We quantified microvessel density in colorectal carcinoma. An immunohistochemical study was performed using mouse monoclonal antibody against CD34, which was used for localizing endothelial cell lined blood vessels & cluster of endothelial cells without lumen formation. Counting was done in 10 consecutive high power fields (40x). The data were analyzed after the counting was done.

Results: We compared microvessel density with age, gender, tumor size, histologic differentiation, tumoral invasion, lymph node metastasis and tumor stage. No significant correlation was found between microvessel density and the aforementioned parameters ($p>0.05$).

Conclusions: The correlation between microvessel density and tumor progression was non-significant. Hence we conclude that there is a need to undertake studies involving larger samples, and also assessment of the other factors associated with angiogenesis should be done to have a better information on prognostic values

Keywords : *Angiogenesis; Colorectal carcinoma; Microvessel density; CD 34*

Introduction

Colorectal cancer (CRC) has long been considered a western disease. There has been clear evidence that CRC incidence rates have been increasing in Asians population.[1] The cause and pathogenesis of colorectal carcinoma are related to both environmental and genetic factors.[2]

Correspondence: Dr. Swechha Maskey,Nepal Cancer Hospital and Research Center, Lalitpur, Nepal. Tel : 9849071967, email: dr.swechha@gmail.com

Angiogenesis is an important hallmark in the development of tumor. A monoclonal antibody against CD34 has been most frequently employed in immunohistochemical evaluation of endothelial cells.[3] CD34 is an antigen composed of glycoprotein, which is present in hematopoietic progenitor cells and endothelial cells and has been studied as a marker for vascular tumors.[4]

Microvessel quantification in primary colorectal carcinoma helps to predict tumoral invasion, metastasis and histologic differentiations.[5] This study aims to determine whether angiogenesis can be documented in colorectal tumor progression and to assess whether the quantitation of microvessels can be correlated to tumor aggressiveness and to study the association between angiogenesis with nodal metastasis and staging of CRC.

Methods

This is a descriptive cross sectional hospital based study carried out in the Department of Pathology, B.P. Koirala Institute of Health Sciences, Nepal from August 2015 to July 2016. Non probability purposive sampling technique was used. Patients diagnosed with colorectal carcinoma and who had undergone surgery were recruited from department of Surgery. Thirty two samples of large colorectal resection specimens were taken. Consent for the study was taken from the Institutional Ethical Board, B.P. Koirala Institute of Health Sciences before the commencement of the study.

The gross sampling technique for colorectal specimen was performed and sectioned (Figure 1). After sectioning tissues were processed in standard manner for histological examination. The tissue section of 4-6 mm thick were cut and stained by Hematoxylin and Eosin (Figure 2). After thoroughly seeing the Hematoxylin and Eosin stained slides of the specimen, the diagnosis were made and the tumor slides were separated for staining with CD34. An immunohistochemistry technique using monoclonal antibody against CD 34 for visualizing the microvessels and calculating the microvessel density was performed (Figure 3 and 4).

Determination of microvessel density: Microvessel density were assessed in tumor areas showing the highest density of staining, as determined by an initial scan with high magnification (x40) then the average counts were recorded. Any brown staining endothelial cell or endothelial cell cluster, clearly separated from adjacent microvessels, were regarded as a single, countable microvessel. A vessel lumen were required for identification of a microvessel. The areas of the highest density of staining were predominantly at the invading edge of the tumor mass. A light microscope (Nikon Eclipse E600) was used for microscopic examination.

The microvessel count were divided into two groups: Hypervascular (≥ 14 microvessel/field) Hypovascular (< 14 microvessel/field).[5] Microvessel density was correlated with age, gender, tumor size, histological differentiation, tumoral invasion, lymph node metastasis and tumor staging.

Results

Microvessel count was further subdivided into hypovascular and hypervascular with maximum number of cases falling under hypervascular category i.e. 90.6% (Table 1).

Table 1: Microvessel count

Microvessel count	No of cases	%
Hypovascular	3	9.4
Hypervascular	29	90.6
Total	32	100.0

No significant association was observed between microvessel count and age, gender, tumor size, histologic differentiation, tumor invasion, lymph node metastasis and tumor stage. When lymph node metastasis was taken into account no significant difference in the value of microvessel count and lymph node metastasis has been found [p=0.253]. Although all the cases with lymph node metastasis were under hypervascular group (100.0%) (Table 2).

Discussion

Colorectal carcinoma remains a major cause of morbidity in the form of recurrence and mortality. Angiogenesis is the process by which new vessels are formed from preexisting capillaries or post capillary venules and is known to be one of the fundamental phenomenon necessary for solid tumors to grow, expand and metastasize.[6]

Table 2: Microvessel count and histopathological variables

Histopathological Variables	Groups	Hypovascular	Hypervascular	P value
Age groups in years	≤40	1 (16.7)	5 (83.3)	0.656
	41 – 60	1 (7.7)	12 (92.3)	
	>60	1 (7.7)	12 (92.3)	
Gender	Male	1 (4.5)	21 (95.5)	0.224
	Female	2 (20.0)	8 (80.0)	
Tumor size (millimeter)	<500	1 (11.1)	21 (88.9)	0.125
	≥500	2 (8.6)	8 (91.4)	
Histologic differentiation	Well differentiated	3 (23.1)	10 (76.9)	0.089
	Moderately differentiated	0 (0)	17 (100.0)	
	Poorly differentiated	0 (0)	2 (100.0)	
Tumoral invasion	Muscularis propria	1 (25.0)	3 (75.0)	0.276
	Subserosa	0 (0)	12 (100.0)	
	Serosa	2 (12.5)	14 (87.5)	
Lymphnode metastasis	Absent	3 (15.8)	16 (84.2)	0.253
	Present	0 (0)	13 (100.0)	
Tumor stage	I	1(20.0)	4(80.0)	0.6
	IIA	2(14.3)	12 (85.7)	
	IIC	0(0)	1(100.0)	
	IIIA	0(0)	1(100.0)	
	IIIB	0(0)	11(100.0)	

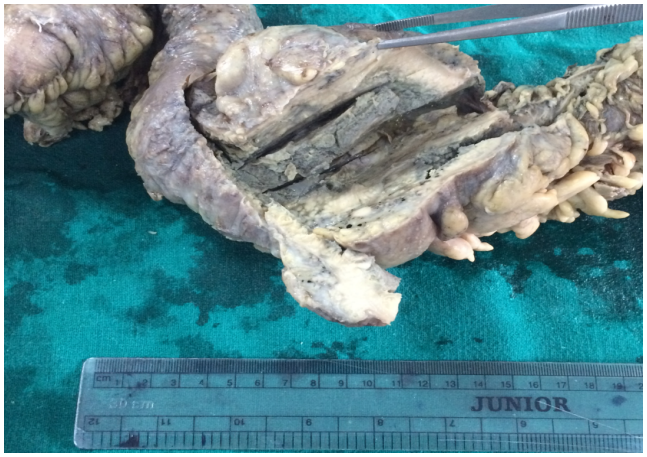

Figure 1: Colorectal carcinoma (Gross)

There is a prognostic importance of angiogenesis in colorectal carcinoma. There are a lot of reports showing that the higher microvessel density is associated with the development of metastasis and poor prognosis in colorectal cancer patients. We have undertaken this study to correlate the quantitation of microvessels to tumor aggressiveness with presence of lymph node metastasis, distant metastasis and higher tumor stage.

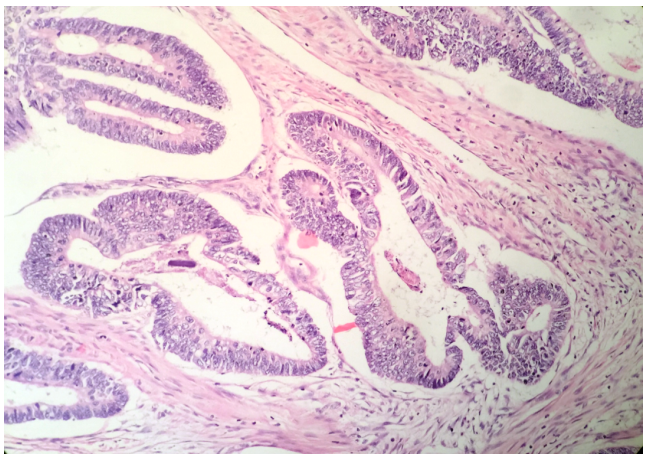

Figure 2: Colorectal carcinoma showing tumor cells in glandular pattern (H&E; 40x)

The age range in this study was from 27 to 82 years. The maximum number of patient were found to be in the age group 41 to 60 years and more than 60 years. In the study done by Choi et al., Nobuhiko et al. and Nakasaki et al., the maximum number of cases were seen in more than 50 years of age. Maximum number of patients were male as compared to female which is comparable to the study done by Choi et al., Nobuhiko et al. and Claudio et al. with male preponderance. Association of age and sex with MVD was not found in this study. This was in accordance to other studies done, where they also did not find any significant association between microvessel density with age and sex. [5,7-9]

Other studies have demonstrated that moderately and poorly differentiated tumors present with higher malignant potential, through the higher incidence of lymphatic, venous invasion and lymph node and hepatic metastasis.[5,8,10] The data in the present study show a non-significant statistical difference in higher microvessel density of moderately and poorly differentiated tumors. This finding was in accordance to the study done by Tarta et al which also showed a non -significant difference with higher microvessel density in moderately and poorly differentiated tumors.[5]

The deeper tumor invasion significantly increase the rate of high microvessel count. Microvessel density is increased significantly in areas with tumoral invasion compared with adjacent non-neoplastic areas and proportionally to the progression of clinical stage. Further evaluation for angiogenesis in rectal carcinomas are done which showed correlation between microvessel density, with the depth of invasion within the bowel wall and shorter survival.[5,8,9,11] In our

study, no significant association is found between microvessel density and depth of invasion which is in accordance to the study done by Choi et al. [7]

It had been reported previously that angiogenesis plays an important role in metastasis and that MVD correlates significantly with lymph node metastasis of submucosal colorectal carcinoma. Lymph node metastasis in colorectal cancer is significantly high in cases with high MVD using CD34.[12] In this study, the lymph node invasion is seen higher in hypervascular group than in hypovascular. However significant correlation is not seen between microvessel density and lymph node metastasis which is concordant to the study done by Saclarides et al. In other studies, lymphatic vessel invasion is significantly higher in the hypervascular tumors. A strong statistical correlation between angiogenesis and lymph node metastasis, lymphatic and vascular invasion are seen in several studies.[7,8,11,13]

The microvessel density as calculated from CD34 increases with the stage and grade.[14] A significant correlation between the microvessel density and clinical stage is observed. MVD is significantly higher in more advanced tumor stages and may be used as a determinant of survival in patients with rectal cancers.[7,10] There is a significant correlation of MVD with Dukes′ stage and lymph node involvement.[7,10,15] However, in our study significant correlation was not observed between the microvessel density and clinical stage.

There is a significant increase in vascularity during the transition from normal tissue through the dysplastic state to early cancer in the study conducted by shieh et al. In our study, however a statistically significant correlation could not be established.[16]

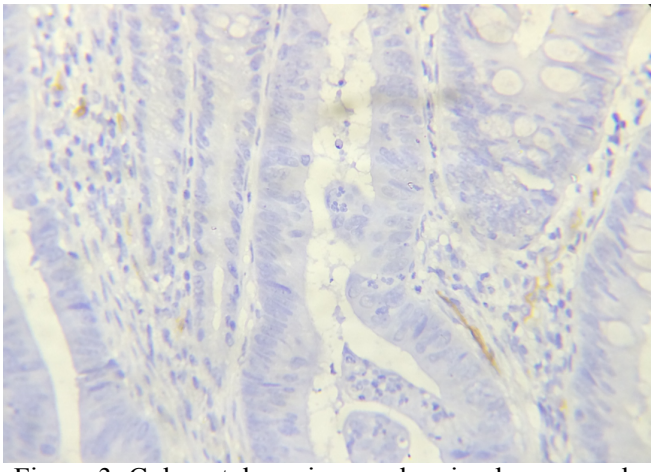

Figure 3. Colorectal carcinoma showing hypovascular area (Immunostaining, DAB chromogen-CD34 monoclonal antibody; 40x)

Some of the studies evaluated not only the number of new vessels, but also their diameter, observing that vessels with a lower cross-sectional area are found primarily in non-metastasizing lesions while those with a higher cross sectional area appears to be mostly localized in cases with nodal metastases which suggested that MVD is a useful marker to identify those patients with a more aggressive tumour, for whom a more therapeutic approach should be taken into consideration. Metastasis are related to overall tumoral vascular surface area : an increased vascular surface area would constitute an easier target for tumor cells in the process of escaping from the original site and entering the circulation.[13,17]

The onset of angiogenesis in colorectal tumorigenesis and the prognostic significance of microvessel quantitation were assessed. The neovascularization is invariably present in colorectal adenocarcinomas, irrespective of the different pathological disease stages.

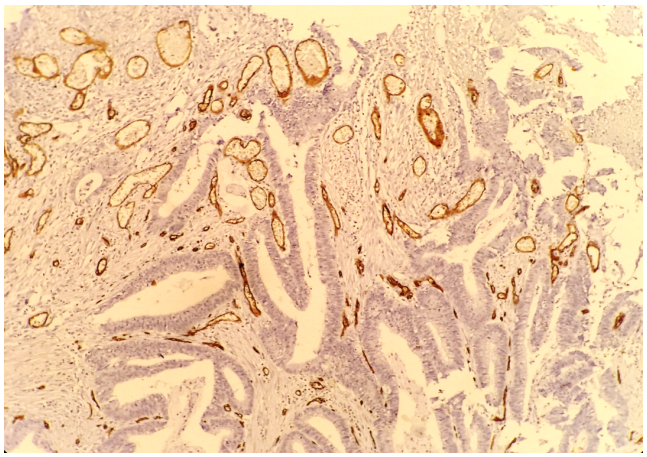

Figure 4. Colorectal carcinoma shows hypervascular area with endothelial cell lined capillaries around epithelial tumor islands (Immunostaining, DAB chromogen-CD34 monoclonal antibody; 40x)

Angiogenesis is stimulated early in colorectal tumorigenesis and reaches a maximum level in adenocarcinomas.[18] The vessel density was observed to be significantly higher in the parts of the tumors lacking basement membrane compared with normal mucosa and submucosa. The vessel density is a stable parameter throughout the entire colorectal tumor and might point to a biologic characteristic of a tumors well as a prognostic factor. The vessel density in normal mucosa was shown to exceed density in tumor tissue by a factor of 2.3 and 1.6 respectively. This probably reflects difference in counting methodology.[19,20]

In the study done by Hiroshi et al. demonstrated that MVD was almost equivalent between normal epithelium and hyperplastic polyp/ adenoma with low dysplasia; however, the transition from adenomas with low to high dysplasia and from adenoma with high dysplasia to carcinoma was not found in this study. This study showed no significant association of MVD and carcinoma correlation in colorectal tumor progression and tumor aggressiveness.[21] The variability in results could be due to different antibodies used to define endothelium and the different methodologies used in the assessment of parameters. Differences between immunohistochemical protocols; for instance selection of the paraffin block, level of sections within the tissue block (superficial or deep), and the issue of hot spot selection may have also contributed to variation in the results. The lack of reproducibility of measurements due to inadequate standardization and different methodology used also adds to it. Additionally quantifying the MVD involves selecting the most angiogenic areas, and this may not always be representative of the tumor.

A meta-analysis done reflecting angiogenesis, to relapse free and overall survival in colorectal cancer has suggested that high MVD significantly predicted poor relapse free and overall survival. The tumor vascularization is associated with the overall survival as well as the mode of metastasis of gastric carcinomas. The determination of tumor vascularization is not only useful as a prognostic marker but may also have the added potential of evaluating responses to anti-angiogenesis drugs.[8,22] A gradual increment of tumor angiogenesis during progression with an increase during transition from low dysplasia to high dysplasia and carcinomas as reflected by the significant rising of MVD and aberrant morphological changes of vessels as well are observed. One of the significant aspect of the study done by Aotake et al., was suggesting that the initiation of tumor

angiogenesis plays an additional role in inhibiting apoptosis.[23]

In our study, significant association between microvessel density with age, sex, tumor size, histological differentiation, tumor depth, lymph node metastasis and tumor stage were not found. Similarly, significant association between microvessel count with age, sex, tumor size and histological differentiation was not found in the study done by Tarta et al. but it was observed that deeper tumor invasion significantly increase the rate of high microvessel count.[5,24] This discrepancies in the clinicopathologic significance of MVD can be caused by methodologic differences between studies using different antibodies (factor VIII, CD31 or CD34). In addition, some authors have chosen patients with tumors in all Dukes' stages; some tumor-node-metastasis(TNM) and others have only studied certain stages. In addition, the limited number of cases and the subjectivity in the selection of areas with high vessel count (hot spots) should be accounted.[25]

Conclusion

In contrast to other studies, the present study has shown no significant association between angiogenesis and aggressiveness of colorectal tumor. One reason to put forward for the non-significant result could be the small size of sample used for our study. We studied microvessel density in 32 cases of colorectal carcinoma. A larger sample size could provide more information regarding the same.Hence, the current study has led us to conclude that further studies need to be undertaken which includes more sample size and the other factors like vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), tumor necrosis factor-alpha (TNF- α), transforming growth factor-beta (TGF- β), and the angiopoietins (Ang) involved in angiogenesis to determine their exact role. This would in turn help in the development of anti-cancer therapies utilizing angiogenesis as a target for the drugs.

Recommendation

Angiogenesis plays a crucial role in assessment of the severity of the disease. Therefore, other factors (like vascular endothelial growth factor, fibroblast growth factor, tumor necrosis factor-alpha, transforming growth factor-beta, and the angiopoietins) involved in angiogenesis should also be analyzed. More studies should be conducted for further determination of role of angiogenesis in colorectal carcinoma.

Reference:

1. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: Incidence, mortality, survival, and risk factors. Prz Gastroenterol. 2019;14(2):89–103. https://dx.doi.org/10.5114%2Fpg.2018.81072

2. Mingyang Song WSG and ATC. Nutrie. Gastroenterology. 2015;148(6):1244–60. https://doi.org/10.1053/j.gastro.2014.12.035

3. Sivapathasundharam B, Sharma B, Sriram G, Saraswathi T. Immunohistochemical evaluation of mast cells and angiogenesis in oral squamous cell carcinoma. Indian J Dent Res [Internet]. 2010 [cited 2021 Oct 28];21(2):260. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20657098

4. Ravi D, Ramadas K, Mathew BS, Nalinakumari KR, Nair MK, Pillai MR. Angiogenesis during tumor progression in the oral cavity is related to reduced apoptosis and high tumor cell proliferation. Oral Oncol. 1998;34(6):543–8. https://www.academia.edu/

1476791 5/Angiogenesis_during_tumor_progression_in_the_oral_cavity_is_related_to_reduced_apoptosis_and_high_tumor_cell_proliferation

5. Tarta C, Teixera CR, TANAKA S, HARUMA K, CHIELE-NETO C, SILVA e VD da. Angiogenesis in Advanced Colorectal Adenocarcinoma with Special Reference to Tumoral Invasion. Arq Gastroenterol. 2002;(1):32–8. https://doi.org/10.1590/S0004-28032002000100007

6. Noel Weidner, Joseph P. Semple WRW and JF. Tumor angiogenesis and Metastasis- Correlation in Invasive Breast Carcinoma. N Engl J Med. 1991;324. https://www.nejm.org/doi/pdf/10.1056/NEJM199101033240101

7. Choi HJ, Hyun MS, Jung GJ, Kim SS, Hong SH. Tumor angiogenesis as a prognostic predictor in colorectal carcinoma with special reference to mode of metastasis and recurrence. Oncology. 1998;55(6):575–81. https://doi.org/10.1159/000011915

8. Tanigawa N, Amaya H, Matsumura M, Lu C, Kitaoka A, Matsuyama K, et al. Tumor anglogenesis and mode of metastasis in patients with colorectal cancer. Cancer Res. 1997;57(6):1043–6. https://cancerres.aacrjournals.org/content/canres/57/6/1043.full.pdf

9. Nakasaki T, Wada H, Shigemori C, Miki C, Gabazza EC, Nobori T, et al. Expression of tissue factor and vascular endothelial growth factor is associated with angiogenesis in colorectal cancer. Am J Hematol. 2002;69(4):247–54. https://doi.org/10.1002/ajh.10061

10. Ludmila T, Kovatchki D, Stoilov G, Gegova A, Terziev I. Tumour Angiogenesis in Rectal Cancer-Computer-Assisted Endosonographic and Immunohistochemical Methods for Assessment. Rectal Cancer - A Multidiscip Approach to Manag. 2011. http://dx.doi.org/10.5772/25336

11. Saclarides TJ, Speziale NJ, Drab E, Szeluga DJ, Rubin DB. Tumor angiogenesis and rectal carcinoma. Dis Colon Rectum. 1994;37(9):921–6. https://doi.org/10.1007/bf02052599

12. Kaneko I, Tanaka S, Oka S, Yoshida S, Hiyama T, Arihiro K, et al. Immunohistochemical molecular markers as predictors of curability of endoscopically resected submucosal colorectal cancer. World J Gastroenterol. 2007;13(28):3829–35. https://dx.doi.org/10.3748%2Fwjg.v13.i28.3829

13. Liotta LA, Kleinerman J, Saidel GM. Quantitative Relationships of Intravascular Tumor Cells , Tumor Vessels , and Pulmonary Metastases following Tumor Implantation Quantitative Relationships of Intravascular Tumor Cells , Tumor Vessels , and Pulmonary MÃ © tastasesfollowing Tumor Implantati. Cancer Res. 1974;(May):997–1004. https://cancerres.aacrjournals.org/content/canres/34/5/997.full.pdf

14. Malik A, Mishra RN, Fanthome B, Rao R, Patrikar SR. Role of CD34, vascular endothelial growth factor, and p53 in neoangiogenesis as correlated with stage of disease in colorectal carcinoma. Med J Armed Forces India. 2011;67(4):320–5. https://dx.doi.org/10.1016%2FS0377-1237(11)60076-2

15. Fichera A. The angiogenic switch occurs at the adenoma stage of the adenoma-carcinoma sequence in colorectal cancer: Commentary. Dis Colon Rectum. 2008;51(2):268. https://dx.doi.org/10.1136%2Fgut.2007.125286

16. Shieh YS, Lee HS, Shiah SG, Chu YW, Wu CW, Chang LC. Role of angiogenic and non-angiogenic mechanisms in oral squamous cell carcinoma: Correlation with histologic differentiation and tumor progression. J Oral Pathol Med. 2004;33(10):601–6. https://doi.org/10.1111/j.1600-0714.2004.00252.x

17. Hannen EJM, Van Der Laak JAWM, Manni JJ, Freihofer HPM, Slootweg PJ, Koole R, et al. Computer assisted analysis of the microvasculature in metastasized and nonmetastasized squamous cell carcinomas of the tongue. Head Neck. 2002;24(7):643–50. https://doi.org/10.1002/hed.10100

18. Bossi P, Viale G, Lee AK, Alfano R, Coggi G, Bosari S. Angiogenesis in colorectal tumors: microvessel quantitation in adenomas and carcinomas with clinicopathological correlations. Cancer Res. 1995;55(21):5049–53. https://cancerres.aacrjournals.org/content/canres/55/21/5049.full.pdf

19. Vermeulen PB, Verhoeven D, Fierens H, Hubens G, Goovaerts G, Van Marck E, et al. Microvessel quantification in primary colorectal carcinoma: An immunohistochemical study. Br J Cancer. 1995;71(2):340–3. https://doi.org/10.1038/bjc.1995.68

20. Mlynek ML, Van Beunigen D, Leder LD, Streffer C. Measurement of the grade of vascularisation in histological tumour tissue sections. Br J Cancer. 1985;52(6):945–8. https://dx.doi.org/10.1038%2Fbjc.1985.282

21. Kawasaki H, Toyoda M, Shinohara H, Okuda J, Watanabe I, Yamamoto T, et al. Expression of survivin correlates with apoptosis, proliferation, and angiogenesis during human colorectal tumorigenesis. Cancer. 2001;91(11):2026–32. https://doi.org/10.1002/1097-0142(20010601)91:11%3C2026::aid-cncr1228%3E3.0.co;2-e

22. Des Guetz G, Uzzan B, Nicolas P, Cucherat M, Morere JF, Benamouzig R, et al. Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature. Br J Cancer. 2006;94(12):1823–32. https://dx.doi.org/10.1038%2Fsj.bjc.6603176

23. Aotake T, Lu CD, Chiba Y, Muraoka R, Tanigawa N. Changes of angiogenesis and tumor cell apoptosis during colorectal carcinogenesis. Clin Cancer Res. 1999;5(1):135–42. https://clincancerres.aacrjournals.org/content/clincanres/5/1/135.full.pdf

24. Sharifi N, Ghaffarzadegan K, Ayatollahi H, Shakeri MT, Sadeghian MH, Azari JB. Evaluation of angiogenesis in colorectal carcinoma by CD34 immunohistochemistry method and its correlation with clinicopathologic parameters. Acta Med Iran. 2009;47(3):161–4. https://acta.tums.ac.ir/index.php/acta/article/view/3567/3543

25. Qasim BJ, Hussein AG, Ali HH. Immunohistochemical expression of PCNA and CD34 in colorectal adenomas and carcinomas using specified automated cellular image analysis system: A clinicopathologic study. Saudi J Gastroenterol. 2012;18(4):268–76. https://doi.org/10.4103/1319-3767.98435

26.