

The Significance Of Angiogenesis in the Biological Behavior of Colorectal Carcinoma and its Metastatic Lymph Nodes Using CD34 Antibody

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Abstract:

A retrospective histopathological study was carried on 31 cases of colorectal adenocarcinoma using the paraffin embedded tissue sections and 10 cases negative from malignancy (colitis). the aims of this study were to study the angiogenesis in colorectal carcinoma and in regional L.N with positive metastasis and assessing the microvascular density (MVD) as a marker of angiogenesis and estimate the correlation of angiogenesis with various clinic pathological parameters .Results MVD is significantly correlated with the type of lesion with a higher mean in malignant cases (19.73) in comparison to non malignant cases (6.4) mean MVD in metastatic L.N (19) while in non metastatic L.N(follicular hyperplasia) was 8.5. Significant correlation was found between MVD and site of the tumor in favor of left site , significant correlation was found between MVD and pathological grading,

Regarding staging system (modified Astler-coller) staging system significant correlation was also found. A higher mean MVD in CRC with metastasized L.N (24.8).while in non metastatic CRC it was (19.6). conclusions: Assessment of tumor vascularity (MVD) by immunohistochemical staining method using CD34 is a useful way to quantify angiogenesis in CRC. MVD is an important parameter to assess the malignant behavior of the lesion, as it is found to be higher in malignant cases than the non-malignant ones.

Introduction

Colorectal cancer is the third most common form of cancer and the second leading cause of death among cancers in the Western World (1). Adenocarcinoma represents 98% of colorectal cancer. In Iraq, It is the 7th most common malignant tumor according to the commonest ten cancers by site in 2005. the 7th commonest in male , while the 4th commonest in female (2).

The importance of CRC comes from the fact that in spite of its high incidence but it's a complete treatable disease if caught early .It represents a challenge to the medical profession because they almost always arise in adenomatous polyps that are generally curable by resection (3) Early in their growth, most human tumors do not induce angiogenesis. They remain small or in situ for years until the angiogenic switch terminates the stage of vascular quiescence so tumors can not enlarge beyond 1-2 mm in diameter or thickness ,unless they are vascularized (4).Through neovascularization (angiogenesis), cancer cells are perfused and as a result, cancer cells obtain more nutrients and dispose of more waste. Angiogenesis is required not only for continued tumor growth but also for metastasis (3). According to Nordic recommendations on the use of molecular targeted agents in combination with chemotherapy for metastatic colorectal cancer, bevacizumab(an anti-vascular endothelial growth factor monoclonal antibody), should be considered in the first-line treatment of patients with a good performance status and cetuximab (is a recently approved monoclonal antibody that

targets the epidermal growth factor receptor, a receptor tyrosine kinase involved in the development and progression of colorectal cancer (CRC) and other solid tumors), in third-line treatment (5)

Materials, and Methods

Thirty-one (31) 15 male and 16 female were selected randomly all were colonic adenocarcinomas. The ages of patients were ranging from 27 up to 75 years, 4 metastatic lymph nodes from these cases were included.. (15) cases were biopsies and the rest (16) were colectomies specimens, the cases collected were from the year 2004 to 2008, all from The Teaching laboratories of medical city hospital and private pathology laboratory. Clinicopathological data were taken from the pathological reports with reassessment of the grading and staging of cases by re-examining the H&E stained slides. Primary tumors and associated metastatic lymph node were immunostained for CD34 antigen. We used the streptavidin–peroxidase conjugate (SP) method to immunohistochemically stain the sections. All the micro vessels were highlighted by staining the endothelial cells with anti-CD34 monoclonal mouse primary antibody. The results of the staining procedure were compared to that of the appropriate controls.

Micro vessel density was assessed using Weidner's method .positive staining of CD34 showed brown particles located in cytoplasm and cell membranes. For the MVD quantization, the stained slides were examined at low-power magnification(X4 and X10 total magnification) to identify the areas of highest neovascularization (so-called "hot spots") of the section. For vessels counting a X20 field [i.e., X 20 objective lens and X10 ocular lens (counting lens)] was used .MVC was expressed as the mean value of highest five fields values examined by dividing it on the area of that field which is 0.785 mm², so the MVD (microvessel density) was obtained. The statistical significance of the difference in mean between two groups was assessed by using Student t-test and chi -square test .

Results:

The patients ages ranged from 27-75 years with a mean of 55.9±12.5 SD .Mean age of male was 57.1 ± 14.8 SD ,while the mean age of female was 54.9±10.2 SD. Female to male ratio was 1: 0.937. 22.6% of tumors were in the rectum. (54.84%) of all case were moderately differentiated adenocarcinoma. according to Astler_coller stage C1 and C2 which were included in one group (C) for statistical purposes were (50%). A total (56.25%) of the 16 colectomy specimens were with lymph node metastasis with out regards to the number of the involved lymph nodes or the specific site but all are regional lymph nodes.. from these 16;(43.75%) were with negative lymph node metastasis

Table(1) Correlation between MVD using CD34 antibody in malignant and normal cases studied.

Cases	No.	MVD using CD 34		
		Mean	SD	Range
CRC	31	19.73	0.493	11.8-37.4
Normal	10	6.4	0.160	4-11

t-test=18.682 ,p=0.000 p<0.001 *** (HS)*** (HS) :Highly significance

Table (2): Correlation between MVD using CD34 antibody in metastatic and non metastatic lymph node (F.H)

L. N.	No.	MVD using CD 34 antibody		
		Mean	SD	Rage
Metastatic L.N	4	19	0.475	14.2-27.2
Non-metastatic L.N (F.H)	2	8.5	0.212	7-10

T-test=3.28 P=0.047 P<0.05 ****(S)**

Table (3): Correlation between MVD using CD34 antibody with tumor location.

Tumor site	Patients		MVD using CD34 antibody		
	No.	%	mean	SD	Range
Caecum	2	9.52	17.4	0.435	17.4-17.4
Ascending colon	5	16.12	15.12	0.378	12.2-20.2
Transverse colon	5	16.12	18.1	0.452	11.8-24
Descending colon	4	12.90	18.75	0.468	16-25
Sigmoid & recto-sigmoid	4	12.90	22.45	2.464	14.4-19
Rectum	6	22.6	20.73	2.280	13-37
Colon (others,not specified))	5	16.12	19.86	2.184	16-24
Total	31	100	-	-	-

t-test =3.628 P-value=0.044 P<0.05 ****(S)** ****(S)** Significant

Table (4) Correlation between MVD using CD34antibody with tumor grading.

Tumor grading	Patients		MVD using CD 34			t-test	p- value
	No.	%	Mean	SD	Range		
Well differentiated(W)	9	29.03	17.31	3.07	14.2-22.0	2.48	0.049 **(S)
Moderately differentiated(M)	17	54.84	18.74	5.95	11.8-37.4	3.06	0.032 **(S)
Poorly differentiated(P)	5	16.13	27.49	0.687	24-37.0	9.46	0.003 **(S)

****(S)** Significant

Table (5)Correlation between MVD using CD34 antibody with tumor staging.

Stage grouping of the tumor	Patients		MVD using CD 34			Chi- Sq.	p- value
	No.	%	Mean	SD	Rage		
B	7	43.75	19.6	1.372	16-37.4	3.62	0.03 **(S)
C	8	50	21.2	1.484	11.8-27.2	3.83	0.039 **(S)
D	1	6.25	24	-	-	-	-
Total	16	100	-	-	-	-	-

****(S)** significant

Table (6)Correlation between MVD using CD34 antibody and L.N metastasis.

L.N metastasis	Patients		MVD using CD 34 antibody		
	No.	%	Mean	SD	Range
Negative	7	43.75	19.6	0.049	18-22
Positive	9	56.25	24.8	0.622	11.8-37.4
Total	16	100	-	-	-

T-test=8.22 p=0.036 p< 0.05***(S)** ***(S)** significant

Discussion:

Microvessel density (MVD) assessment is the most commonly used technique to quantify intratumoral angiogenesis in cancer. It was first developed by Weidner et al (1991) (6)

Table (1) shows that the higher mean MVD was in malignant cases as compared to the non malignant cases ,this is similar to many studies in this field such as in Goa Y et al (7)

Table(2);the mean MVD in regional metstatic L.N. obtained was higher in comparison to L.N with follicular hyperplasia)

As there is no similar studies to compare this result with them ;the explanation of this high MVD in metastatic LN may be due to the fact that tumor cells produce angiogenic factors that stimulate neoangiogenesis in the preexisting blood vessels in the trabeculae of L.N. In table (3) ; the highest mean MVD was in sigmoid and recto sigmoid tumors Similar to the study of Tanigawa N, et al (1997) who founded that significant correlation between MVD and tumor site ,also they considered the tumor location as an independent prognostic factor (8).any how ; this also may be due to the small sample size used or to the group of (not specific site) that may affect this result. No significant correlation was found between MVD and specimen type, which was either of biopsy or colecomy obtained after operation. The idea from this analysis to reach a result that we can estimate the tumor vascularity even pre-operatively from colonoscopic examination and to manage for anti-angiogenic therapy even before starting post-operative chemotherapy as anti-angiogenic drugs can limit the growth of tumor and to make the patient ready for post-operative adjuvant therapy for maximal benefit. Table(4) ,these finding agree with many studies in this field especially with CD34 antibody as a marker for MVD in CRC that correlate the MVD with tumor

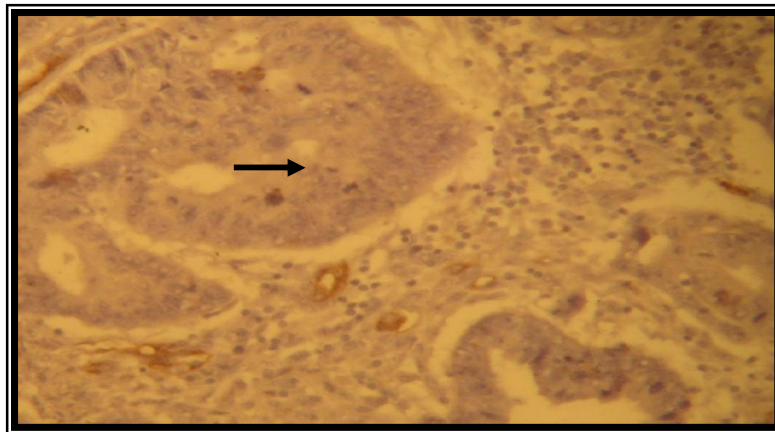
behavior. Zheng et al. (2003) founded that MVD was associated with an aggressive behavior (9) Regarding staging in table (5) ;With the progression of CRC ,the demand for oxygen and nutrient increases dramatically . As a result ,more blood vessels are needed with cancer development. Thus ,MVD was significantly increased in advanced disease. In our study due to the fact that we have only one case in stage D, this may be due to either insufficient information recorded in the pathological reports or due to the relatively small number of cases included in our study. so statistically we couldn't make a statistical analysis for it. stage A was not included in our study ,this may be due to either late diagnosis of the collected cases or to the small size of the study, that by chance due to randomly collecting of cases that which may missed this stage ,so we couldn't detect the correlation with MVD in this early stage .so we depended only on stage B and C .

Table (6) showed the higher mean MVD was in cases with positive L.N meatastasis; the newly formed capillaries have fragmented basement membranes and are leaky , making them more penetrable by the tumor cells than mature vessels, in addition to the fenestrated endothelium and open interendothelial junctions in tumor vessels.

The ability of neoplastic cells to disseminate from a primary tumor to lymphatic nodes and to adjacent and distant tissues and organs is a feature of malignant tumors and the main cause of failure in their treatment (3).

Conclusions:

1. Assessment of tumor vascularity (MVD) by immunohistochemical staining method using CD34 is a useful way to quantify tumor angiogenesis.
2. MVD is an important parameter to assess the malignant behavior of the lesion, as it is found to be higher in malignant cases than the non –malignant ones.



well differentiated CRC .endothelial cells immunostained with CD34 antibody, brown particles membranous and cytoplasmic. (X20)

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