Original Article

Expression of D2-40 and CD34 in patient with colorectal carcinoma

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Summary:

Background: Colorectal is a worldwide health problem. Tumors stimulate the growth of host blood vessels, a process called angiogenesis, which is essential for supplying nutrients of the tumor, also stimulate the lymphatic vessels for metastasis.

Objective: This study design to investigate the distribution pattern of lymphatic vessels and blood vessels in patients with colorectal carcinoma and their relationship to metastasis and prognosis.

Materials and methods: A total of 40 cases of colorectal carcinomas were retrieved from the archives of the pathology department of teaching laboratories in Baghdad medical city, were included in this study. In addition 12 apparently normal colorectal autopsies use as control group. The lymphatic vessel and blood vessel in tumor tissue obtained from 40 patients with colorectal carcinoma, including 20 with metastases and 20 without metastases, were evaluated by immunohistochemistry using monoclonal antibodies directed against D2-40 and CD34.

Results: Significant correlation in the expression of D2-40 and CD34 showed in patients with colorectal carcinoma.

Conclusion: D2-40 is a new specific antibody for lymphatic endothelial cells and CD43 for blood vessels. Lymphogenesis and angiogenesis are commonly seen in patients with colorectal carcinoma.

Keywords: Lymphangiogenesis, Angiogenesis, Colorectal carcinoma, Metastasis.

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Introduction:

Colorectal cancer (CRC) is the third leading cause of cancer-related death in the world. Human colorectal carcinogenesis is a complex, multistep and multigenetic process [1]. Lymphangiogenesis (lymph vessel growth) and angiogenesis (blood vessel growth) are critical processes for tumor growth, invasion, and metastasis. Angiogenesis has established its role in the development and progression of a variety of malignancies, playing a crucial role in the dissemination of tumor cells [2][3]. However, lymphatic spread of cancer cells to lymph nodes is an important early event in the metastasis of carcinoma [4]. Previous studies have been limited by the lack of specific lymphatic endothelial makers that allow to discriminate between lymphatics and blood vessels. Recently, the monoclonal antibody D2-40, which is directed against the oncofetal membrane antigen M2A that has been identified in ovarian carcinoma cell lines and germ-cell tumors [5]. The CD34 is an endothelial antigen that has been used to highlight the microvessel density (MVD) as a direct marker of degree of neoangiogenesis, however, it can react not only with newly forming vessels but also with normal vessels just trapped within the tumor tissue [6]. Since 1991, when Weidner N [7]. Showed that assessing tumor MVD could be useful in evaluating the aggressiveness of breast carcinoma, several studies have found similar results for other of malignancies [8]. There is no Iraqi study have been reported on the role of D2-40 and CD34 in patients with colorectal adenocarcinoma in order that this study will try to take the first step of the work.

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Materials and methods:

Patients and specimens. A total of 40 colorectal carcinoma samples were obtained from the archives of the Department of Pathology, teaching laboratories in Baghdad medical city, from February 2008 to October 2010. Samples were derived from patients who were solely surgically treated. There were 21 men (52.5%) and 19 women (47.5%) with a range 25-89 years (58.0±10.5), 22 patients with colon and 18 patients with rectal tumors. Twenty seven patients (67.5%) had histologically confirmed lymph node metastases, whereas the remaining 13 patients (32.5%) were found to have no clinical or histopathologic evidence of lymph node involvement. According to the World Health Organization classification, tumors of 10 (25%) patients were well differentiated adenocarcinoma, that of 26 patients (65%) were moderately differentiated adenocarcinoma and that of 4 (10%) patients were poorly differentiated adenocarcinoma.

Immunohistochemistry. Tissues were fixed in 10% buffered formalin, processed, and stained with Hematoxylin and Eosin (H and E). Hematoxylin and Eosin-stained slides of

all samples were reviewed to confirm the diagnosis. One paraffin block with the bulk of tumor tissue was used for immunohistochemical studies. Sections, 4 microns-thick, of formalin-fixed paraffin embedded tissues were cut and mounted on positive charched slides. The sections were deparaffinized in Xylene and rehydrated in a descending Ethanol series. Heat induced epitope retrieval techniques were used for antigen retrieval as follows: citrate buffer (pH 6.0) and a water bath at 95°C-98°C for 30 minutes. Sections were incubated for 10 minutes in 3% hydrogen peroxide to quench endogenous tissue peroxidase. Then drop of protein block were added at 37°C for 5 minutes. The sections were immunostained with a monoclonal antibody (Clone D2-40, ab 77854-100, Ab.com. UK) at a 1:50 dilution directed against D2-40 and (clone CD34, ab 8536, Ab.com.UK) at a dilution of 1:50 directed against CD34.as recommended by the manufacturer instruction (Cambridge Science Park, England, cat. number ab 64259, kit contents). Tissue sections were incubated with the primary antibody over night at 4°C. After washing with phosphate-buffered saline, a Super picture secondary antibody were used 10 minutes at room temperature. Followed by the addition of the streptavidin-HRP for 10 minutes at room temperature. After washing with phosphate-buffer saline, the samples were treated with substrate chromogen solution for 10 minutes at room temperature, and then counterstained with hematoxylin for 30 second. Slides were washed well in running tap water for 5 minutes, then dehydrated by serial 70%, 80%, 95%, 100% ethanol and xylene then mounted with permanent-mounting medium (DPX) and the examining and scoring were done under light microscope by a pathologist at power 400 according to the scoring system of [9] [Score 1, less than 10 %, Score 2, more than 10 % and less than 50% and Score 3, more than 50 %].

Statistical analysis: Was performed using the Chi-square with Fischer exact test. P value < 0.05 was regarded as significant.

Results:

This is the first time that D2-40 has been detected in colorectal adenocarcinoma in Iraq. The result is limited due to the limitation of materials. The results of normal colorectal were negative for the control group. D2-40 was positive in 36 patients. From those positive cases, 25 patients had presence of lymphatic invasion (92.6%). While CD34 has positive in 28 patients. Regarding the statistical analysis of these results. Only age and gender of patients showed a significant correlation in the D2-40 positive cases while CD34 showed a significant correlation only with gender of patients as shown in table(2) (3). The results of frequency distribution of D2-40 and CD34 scores showed no significant correlation between each score and any of patients criteria like age of patients, tumor grade, presence or absence of lymphatic invasion.

Table (1): The Expression of D2-40 and CD34 in Patients with Colorectal Adenocarcinoma.

Result of Immunohistochemistry			D2-40 Expression	CD34 Expression	Comparison of Significance	
					p- value	Sig.
Patients	Positive	N %	36 (90%)	28 (70%)	0.00	Highly Sig. P<0.01
	Negative	N %	4 (10%)	12 (30%)		
	Total	N %	40 (100%)	40 (100%)		
Control	Positive	N %	0	0		
	Negative	N %	12(100%)	12 (100%)		
	Total	N %	12(100%)	12 (100%)		

Table (2): Immunohistochemistry expression of positive and negative D2-40 as related to clinicpathological profile of patients with colorectal adenocarcinoma.

Variables		D2-40 positive	D2-40 negative	Comparison of Significance	
				p-value	Sig.
Age	<= 50	8 (66.7%)	4 (33.3%)	0.00	Sig. (P<0.05)
	> 50	28(100%)	0		
Gender	Male	0	0	0.00	Sig. (P<0.05)
	Female	15 (78.9%)	4 (21.1 %)		
Tumor grade	I	8 (80%)	2 (20%)	0.42	Non Sig. (P>0.05)
	II	24 (92.3%)	2 (7.7%)		
	III	4 (100%)	0		
Lymphatic invasion	Invasive	25 (92.6%)	2 (7.4%)	0.43	Non Sig. (P>0.05)
	Non invasive	11(84.65%)	2 (15.4%)		

Table (3): Immunohistochemistry expression of positive and negative CD34 as related to clinicpathological profile of patients with colorectal adenocarcinoma.

Variables		CD34 positive	CD34 negative	Comparison of Significance	
				p-value	Sig.
Age	<= 50	10 (50%)	10 (50%)	0.26	Non Sig. (P>0.05)
	> 50	21(65.6%)	11 (34.4%)		
Gender	Male	0	5 (100%)	0.01	Sig. (P<0.05)
	Female	15 (57.7%)	11 (42.3%)		
Tumor grade	I	8 (80%)	2(20%)	0.38	Non Sig. (P>0.05)
	II	21(80.8%)	5(19.2%)		
	III	2(50%)	2(50%)		
Muscle invasion	Invasive	21 (77.8%)	6(22.2%)	0.95	Non Sig. (P>0.05)
	Non invasive	10(76.9%)	3(21.3%)		

Table (4): Correlation of D2-40 scores as related to different parameters.

Parameters		D2-40 scores			Comparison of Significance	
		Low	Intermediate	High	P-value	Sig.
Age	<= 50	2(25%)	2(20%)	4(22.2%)	0.96	Non Sig. (P>0.05)
	> 50	6(75%)	8 (80%)	14(77.8%)		
Gender	Male	2(25%)	6(60%)	13(72.2%)	0.07	Non Sig. (P>0.05)
	Female	6(75%)	4 (40%)	5(27.8%)		
Tumor grade	I	0	2(20%)	6(33.3%)	0.18	Non Sig. (P>0.05)
	II	8 (100%)	6(60%)	10(55.6%)		
	III	0	2 (20%)	2(11.1%)		
Invasion	Invasive	8(100%)	6(60%)	11(61.1%)	0.68	Non Sig. (P>0.05)
	Non invasive	0	4 (40%)	7(38.9%)		

Table (5): Correlation of CD34 scores as related to different parameters.

		CD34 scores			Comparison of Significance	
		Low	Intermediate	High	P-value	Sig.
Age	<= 50	2(50%)	1(12.5%)	3(18.8%)	0.30	Non Sig. (P>0.05)
	> 50	2(580%)	7 (87.5%)	13(81.3%)		
Gender	Male	4(100%)	3(37.5%)	8(50%)	0.11	Non Sig. (P>0.05)
	Female	0	5 (62.5%)	8(50%)		
Tumor grade	I	0	4(50%)	2(12.5%)	0.13	Non Sig. (P>0.05)
	II	4 (100%)	3(37.5%)	11(68.8%)		
	III	0	1 (12.5%)	3(18.8%)		
Invasion	Invasive	4(100%)	6 (75%)	9(56.3%)	0.21	Non Sig. (P>0.05)
	Non invasive	0	2 (25%)	7(43.7%)		

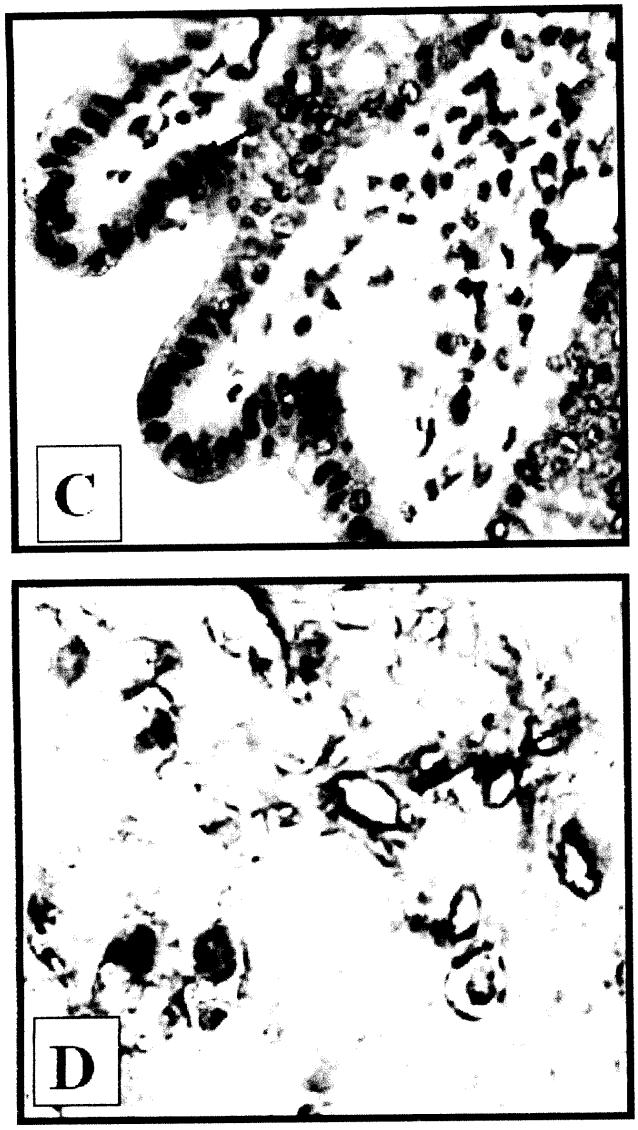

Figure (2):Immunohistochemistry for D2-40 and CD34 in colorectal adenocarcinoma tumor section, stained by DAB chromogen and counter stained with heamatoxylin is shown as radish brown in positive cases (magnification power, 400X). A-Negative expression for D2-40, B- D2-40 positive expression, C-Negative expression for CD34, D-CD34 positive expression.

Discussion:

This study is one of the first attempts to quantify colorectal carcinoma lymphangiogenesis and angiogenesis in the same sample by using the novel lymphatic marker D2-40 and CD34. We compared the distribution of lymphatic vessels and blood vessels, and related the results to clinicopathologic parameters. The lymphatic system is the primary pathway of metastasis for most human cancers. Lymphangiogenesis refers to the development and proliferation of new lymphatics from host vessels. Recently, antibodies specific for lymphatic endothelium have become available, providing important new insights into the process of tumor-associated lymphangiogenesis and its possible clinical relevance. Many studies have reported that tumors are able not only to induce lymphangiogenesis, but also to enhance lymphatic metastasis [10][11]. There are some antibodies for lymphatic endothelium now, including LYVE-1 (lymphatic endothelial hyaluronan receptor)[12], Prox-1[13], CD31[14], and podoplanin [15]. VEGFR-3, the receptor for vascular endothelial growth factors (VEGFs) C and D, is expressed on lymphatic endothelium and may play a role in lymphangiogenesis [16]. But, some studies indicated that VEGFR-3 was also involved in blood vessel angiogenesis in the adult and it was not a specific antibody for lymphatic endothelium [17]. CD31 also stained both in blood vessel and in lymphatic vessel. But, the monoclonal antibody D2-40 is a highly selective marker of lymphatic endothelium in sections of both frozen and formalin-fixed paraffin-embedded normal and neoplastic tissues. In a direct comparison of D2-40 and CD31 on paraffin sections of a series of tumors derived from lymphatic endothelium (lymphangiomas) and blood vessel endothelium (hemangiomas). The current study had demonstrated that D2-40 was over expressed in colorectal adenocarcinoma. These results might possible reflect the association between cellular expression of D2-40 and lymphogenesis. This was in agreement with the findings of Fogt ,Walgenbach-bruenage and Naik. Since they found over expression of D2-40 in colorectal adenocarcinoma. In comparison with other studies this marker is increased in several malignant tissues [21], gastric cancer [22], lung [23] might contribute to the lymphogiogenic process and metastasis in colorectal cancer. Our study, D2-40 results showed that was not associated with gender of patient, tumor grade and invasion, while significant correlation occur with age of patient. The rising incidence with age may be explained by the accumulation of somatic mutations associated with the emergence of malignant neoplasms. In addition, the observed impairment in the immune system in such ages, due to senescent decline in the immune surveillance, might lead to accumulation of cellular DNA mutation that could be regarded as an additional significant factor in the development of such malignancies [24]. We used CD34 staining to identify angiogenesis in the present study as shown in table (1) this results agreement with results of several authors have demonstrated that CD34 is expressed in colorectal adenocarcinoma [25][26] [27]. Regarding the correlation between expression of CD34 and clinicopathologic criteria like age of patients, gender, tumor grade, presence or absence of lymphatic invasion. The results not shown any significant correlation between CD34 expression and cilincopathologic, this result agreement with findings of Sharifi et al., found significant correlation only with grade while other criteria not found any relation [6]. In conclusion, over expression of D2-40 and CD34 are seemed to be associated with increased lymphogenic and angiogenic potential of colorectal adenocarcinoma. Therefore, elevated D2-40 and CD34 expression might possibly associated with tumor progression and could be used as targets for therapeutic management patients with primary colorectal adenocarcinoma.

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