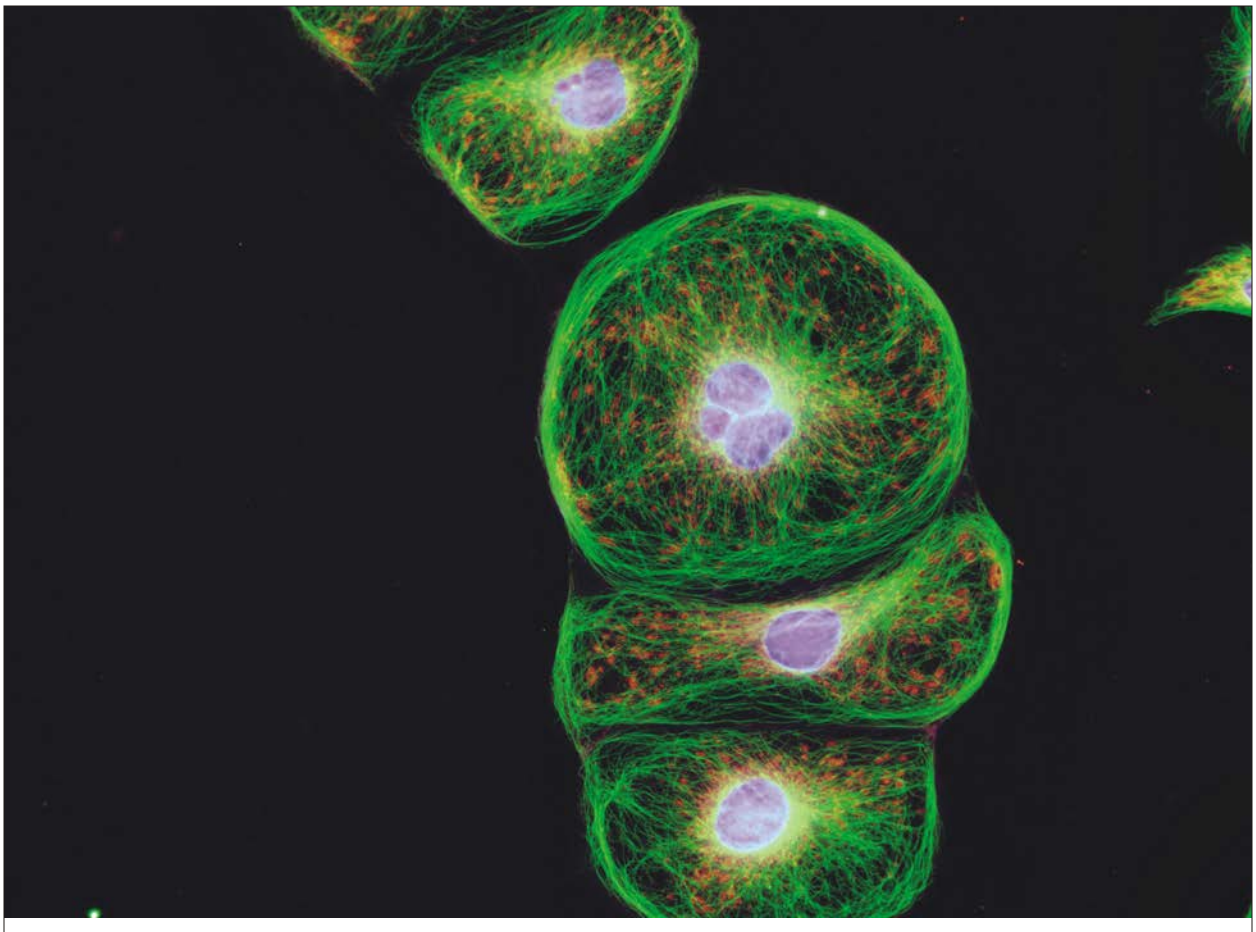

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Evaluating the Expression of CD34 Marker in Colorectal Adenocarcinoma and its Relationship with Clinicopathologic Factors

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ABSTRACT

Colorectal cancer is known as one of the most common types of cancer which the prevalence is increasing. According to reports, malignancies of the large intestine have highly broken out in Iran. Several studies have shown the correlation between the CD34 cell surface markers and some cancers. In the present study, the relationship between the protein expression and clinico-pathologic characteristics was evaluated. In this study, 40 paraffin blocks belonged to patients with colorectal cancer referring to Imam Khomeini Hospital in Ahvaz during 2003-2013 were studied by immunohistochemistry method. From 40 samples, 22 were males and 18 females. Their mean age was 56±17.9 years and ranging from 22-87 years. The mean tumor size was 5.1 cm ranging 11-1.5 cm. The most common site of involvement was in the right colon (45%) and the rarest site in the transverse colon (10%) and 75% was in poorly-differentiated state in terms of tumor grade. Generally, marker expression was positive in 13 cases and negative in 27 cases. The CD34 expression was significantly correlated with tumor size, tumor depth, degree of differentiation as well as lymphatic, vascular and neural invasions. A positive correlation was observed between the marker expression and pathological characteristics. Therefore, CD34 marker expression can be effective as a marker in the diagnosis of pathological characteristics of colorectal cancer. However, further studies with a large population are recommended.

Key words: Colorectal cancer, stromal cells

INTRODUCTION

Colorectal cancer is the third most common cancer in the world which is considered as one of the leading causes of death in developed countries. More than half a million people in the world annually are victims of it (Oladipo *et al*., 2011; Peddareddigari *et al*., 2010). This is also the third most common cancer among Asian men and women and its prevalence is increasing (Pourhoseingholi, 2012). Types of cancer include adenocarcinoma, carcinoid tumor, lymphoma, colon stromal tumors (Rosai, 2011). About 98% of all malignancies of the large intestine are adenocarcinoma (Kumar *et al*., 2005). This is the most common malignancy in the United States and is the most curable cancer of the gastrointestinal tract (Rosai, 2004). According to statistics released by cancer centers in Iran, malignancies of the large intestine are the third most common cancer in men and fourth most common in women. Its standardized prevalence is 8.6 cases per 10 thousand people based on the age (Sadjadi *et al*., 2003).

Currently, the results of treatment of colon cancer have improved with early diagnosis and removal of precancerous polyps. However, it reduced the mortality rate within 5 years in the past three decades (Goldman and Fisher, 2006) but the clinical strategy is further reinforced by understanding the molecular pathology of carcinogenesis (Worthley and Leggett, 2010).

The CD34+stromal cells (have human cell precursor antigen), also known as dendritic interstitial cells (Tardio, 2009), are dispersed in many human organs such as breast (Yamazaki and Eyden, 1995), thyroid (Yamazaki and Eyden, 1996), the dermis (Nickoloff, 1991), uterine (endometrial) stroma (Lindenmayer and Miettinen, 1995) and large intestine (Vanderwinden *et al*., 1999). CD34+stromal cells (dendritic interstitial cells) not only have a protective role in the maturation and proliferation of epithelial and mesenchymal cells but also play this role in responses related to immunity (Nakayama *et al*., 1999). The importance of the cells containing CD34 is that their presence and diffusion in cancer can be considered as a marker for the diagnosis and study of tumors.

The CD34 is a highly glycosylated molecule made of single-chain glycoprotein with a weight of 110 kDa which is known as a cell surface marker in blood precursor cells as well as endothelial cells (Sidney *et al*., 2014). CD34 is activated as a receptor or ligand in the cell membrane through phosphorylating by kinases and transfers messages and the cells interaction (Sidney *et al*., 2014). Expression of CD34 in colon stromal tumor is increased compared to normal tissue, so that it was significantly more than benign tumors in colon malignant tumors (Sidney *et al*., 2014). The CD34 expression is significantly associated with adenomas containing severe dysplasia and large adenomas (more than 1 cm) (Qasim *et al*., 2012; Sidney *et al*., 2014). In many other immunohistochemical studies, the expression of this protein is used as a reliable marker to evaluate cancer tumors including: colon (Moreira *et al*., 2011; Sidney *et al*., 2014), blood (Borowitz *et al*., 1990), liver (Borowitz *et al*., 1990), penile (Martins *et al*., 2002), lung (Van de Rijn *et al*., 1994) and trichilemmal (Chaichamnan *et al*., 2010) cancers. The studies have shown the correlation between CD34 with many clinicopathologic factors as well as the ability of this marker as a prognostic factor. Recently, reports have been published about the presence of CD34+cells at the margins of major salivary glands pleomorphic adenoma (Moreira *et al*., 2011).

However, the relationship between CD34+stromal cells and neoplasms desmoplastic stromal have not been widely and sufficiently studied, particularly in colon carcinoma. However, in recent limited studies that examines the CD34 immunohistochemical analysis in the colorectal cancer, it is attempted to obtain a full understanding of the role of CD34 expression cells in this cancer and association of CD34 marker with clinico-pathologic features and survival of patients; no final and reliable results have been achieved in this field.

The purpose of this study is to determine the frequency distribution of CD34 expression in colon adenocarcinoma to identify prognostic factors and contribute to the treatment goals of this disease.

MATERIALS AND METHODS

Study design and population: This is a descriptive study. The study population was selected from a sample of colon pathology of patients with colon adenocarcinoma in Imam Khomeini hospital of Ahvaz. Overall 40 patients with an early diagnosis of colon cancer, who were treated by colon adenocarcinoma surgery in Imam Khomeini hospital during the years 2003-2014, were studied.

Methods: Colon samples were fixed in formaldehyde 10% and embedded in paraffin. In the next step, 3 shears with a thickness of 4 μm were prepared from each paraffin blocks and were placed

on slides. Then, each sample was stained by conventional Haematoxylin and Eosin (HE). The tissue slides were deparaffinized using xylene and then were dehydrated by alcohol and immunohistochemical staining with CD34 marker was done using the kit on slides and finally, the discoloration caused by light microscopy was evaluated.

Statistical analysis: For each sample, tumor size, malignancy grade, depth of tumor invasion, nerve and vascular invasion and lymph node status were determined and examined. Demographic characteristics (age and gender) were also determined based on the patient's record. Data was analyzed using the SPSS (IMP SPSS Statistics version 20) and chi-square and Fisher's exact tests.

RESULTS

Out of 40 patients with colon cancer, 22 patients (55%) were males and 18 (45%) female and all patients were adenocarcinoma pathologically. No significant difference was observed in terms of sex ($p = 0.52$).

The mean age of patients was 56±17.9 years in the range of 22-87. 9 cases (22.5%) were under 40 years old, 6 patients (15%) in 40-50 years, 6 (15%) in 50-60 years, 8 (20%) in the range of 60-70 and 11 (27.5%) over 70 years of age. The mean age of men and women was 58.1±16.47 and 53.4±19.66, respectively.

The mean tumor size in all patients was 5.1 cm in a range of 11-1.5 cm. In 6 (15%), the tumor size was uncertain. The mean tumor size in patients with CD34-negative expression was 2.4±4.6 and in positive people 1.5±6.2 mm and a significant difference was observed between tumor size and CD34 expression ($p = 0.03$).

The site of tumor in 18 patients (45%) was in the right-side colon, in 10 patients (25%) in the rectum and/or sigmoid, in 8 (20%) in the left-side colon and in 4 (10%) in the transverse colon, which difference was significant ($p = 0.01$). No significant difference exists between CD34 marker expression based on tumor site ($p = 0.61$).

In terms of tumor grade, 20 cases (50%) were well-differentiated, 16 patients (40%) were moderately-differentiated and 4 (10%) were poorly differentiated which difference was not significant ($p = 0.06$). Also, a significant correlation was found between the marker expression and grade of differentiation ($p = 0.03$).

In terms of depth of conflict, 11 patients (27.5%) were in T1, 20 (50%) in T2 and 9 patients (22.5%) in T4, which difference was not significant ($p = 0.07$). In terms of the depth of the tumor, CD34 expression showed a significant difference ($p = 0.03$).

The mean age of patients who were negative CD34 marker was56.7±17.5 years old and in patients who are positive CD34 was 54.9±19.3 years old, which was not significant ($p = 0.78$). CD34 marker expression was not significant based on the age of the patients ($p = 0.20$).

The invasion of the lymph nodes was observed in 25 patients (62.5%) and it was not observed in 15 patients (37.5%) and the difference between them was not significant ($p = 0.11$). The incidence of invasion was significantly different based on the marker expression ($p = 0.03$).

In terms of vascular invasion, 31 patients (77.5%) had the invasion and 9 (22.5%) lacked it and the difference between them was significant ($p = 0.01$). CD34 expression of these variables showed a significant difference ($p = 0.01$).

In the next phase, patients were evaluated in terms of the invasion of nerve, of which 35 (87.5%) had the invasion and 5 (12.5%) had no lymph node invasion, that the difference was significant ($p = 0.000$). A significant difference was observed between them in terms of CD34 marker expression ($p = 0.03$) (Table 1).

Table 1: CD34 frequency and expression based on clinicopathologic characteristics of cancer patients

		CD34 expression (%)			
Variables	Subgroups	Positive	Negative	Total frequency (%)	Significance level
Gender	Man	7 (53.8)	15 (55.5)	22 (55)	0.92
	Woman	6 (46.2)	12 (44.5)	18 (45)	
Patient age (year)	Under50	5 (55.5)	10 (32.3)	15 (37.5)	0.78
	Above 50	4 (44.5)	21 (67.7)	25 (62.5)	
Tumor size (cm)	< 5	3 (37.5)	12 (46.1)	15 (37.5)	0.03
	5-7	4 (50)	7 (26.9)	11 (27.5)	
	7	1 (12.5)	7 (26.9)	8 (20)	
Tumor site	Right-side colon	7 (38.9)	11 (61.1)	18 (45)	0.61
	Transverse colon	2 (50)	2 (50)	4 (10)	
	Left-side colon	2 (25)	6 (75)	8 (20)	
	Rectum and/or sigmoid	2 (20)	8 (80)	10 (25)	
Tumor differentiation grade	Good	3 (15)	17 (85)	20 (50)	0.03
	Average	0 (43.8)	9 (56.3)	16 (40)	
	Poor	3 (75)	1 (25)	4 (10)	
Depth of tumor invasion	T2	1 (90.1)	10 (90.9)	11 (27.5)	0.02
	T3	6 (30)	14 (70)	20 (50)	
	T4	6 (66.7)	3 (33.3)	9 (22.5)	
Lymphatic invasion	Yes	5 (20)	20 (80)	25 (62.5)	0.03
	No	8 (53.3)	7 (46.7)	15 (37.5)	
Vascular invasion	Yes	7 (22.6)	24 (31)	31 (77.5)	0.01
	No	6 (66.7)	3 (33.3)	9 (22.5)	
Neural invasion	Yes	9 (12.5)	26 (15.4)	35 (87.5)	0.03
	No	4 (87.5)	1 (84.6)	5 (12.5)	

DISCUSSION

The study was performed on 40 samples from patients with colon adenocarcinoma, CD34 expression was only observed in 32.5% of them and a positive correlation was observed between the expressions of immunohistochemical marker with most of studied pathological parameters. Given the positive correlation between the marker expression and most of pathological features of the hypothesis, the relationship between CD34 expression and colon carcinoma is further strengthened. The presence of a significant correlation between the CD34 expression and other tumor characteristics were generally consistent with what was already expected and with a review of other studies in this area, it was found that similar results exist in many cases which confirm the findings of this study completely. Although, a number of studies on the relationship between CD34 marker and colon cancer are not so much. Qasim *et al*. (2012) compared the CD34 expression in the adenomas, adenocarcinomas and normal colon tissues and concluded that the expression of this marker in carcinoma was significantly higher than in the adenoma group and in adenoma was more than the control group. The CD34 expression was correlated with adenomas containing severe dysplasia and large adenomas (more than 1 cm) and the significant association of this marker with tumor grade, lymphovascular invasion and lymph node involvement in colorectal carcinoma were also approved (Qasim *et al*., 2012). Similarly, in a retrospective study, Moreira *et al*. (2011) evaluated the vascular density through immunohistochemical analysis in colorectal carcinoma and showed that the CD34 expression in the central area, cancer is associated with relapse, metastasis and survival rate. The study showed that the incidence of CD34 in the inner area of colorectal carcinoma tumors has the prognostic value in this cancer. Another study examined the immunohistochemical analysis of CD34 in colorectal cancer, colorectal adenomas and colorectal non-neoplastic lesions. In this study, the expression of CD34 was associated with recurrence, metastasis and survival of colorectal malignancies and this indicated the prognostic role of CD34 in colorectal cancer (Nakayama *et al*., 2000). Liang *et al*. (2004) used the

immunohistochemical expression of CD34 as a marker for the diagnosis and assessment of Micro-Vessel Density (MVD) and its association with the capillary development in colon cancer cells. In another study, Kaneko *et al*. (2007) have also used the CD34 expression for examining the correlation between MVD and colon adenocarcinoma tumor characteristics.

The positive expression of CD34 marker in gastric cancer and its correlation with tumor characteristics has been approved. Tenderenda *et al*. (2001) conducted a lab trial and evaluated the expression of CD34 in 58 cases of gastric cancer and showed a significant correlation exists between the CD34 expression and malignancy grade of tumor and histological type of tumor. Also, the increased presence of CD34 marker was positively correlated with the tumor, infiltration and lymph node and thus it was concluded that this marker in gastric cancer is correlated with disease development (Chen *et al*., 2008).

On the other hand, studies with results other than those mentioned above can challenge the above studies. By examining the distribution of CD34+stromal cells and myofibroblasts in colorectal carcinoid tumor, Kuroda *et al*. (2005) reported that stromal cells expressing CD34 which were dispersed in the sub-mucosal and sub-serous area of normal tissues, were disappeared after the invasion this tumor in this area and were transferred to the deeper layers of the tumor. In other words, no expression of this marker was found in stromal carcinoid. They added that using CD34 immunohistochemistry may be useful in detecting large neoplasms. In another study, it was shown that most stromal cells are CD34+in the sub-mucosal, muscularis propria and suborosa as well as normal perirectal tissues, while the inflamed tissues around tumor and tumor stroma of stromal cells lacked CD34 (Nakayama *et al*., 2000). The study findings showed that lack of CD34 expression of stromal cells in colorectal adenocarcinoma is involved in creation of a desmoplastic reaction. Increasing CD34 expression in liver (hepatocellular) cancer is also reported in sinusoids endothelial cells that likely shows the phenotypic changes of these cells in carcinoma and correlated with hepatocarcinogen process. Overexpression of CD34 has been identified as a risk factor for hepatocellular carcinoma (Cui *et al*., 1996). Martins *et al*. (2002) reported that no link exists between CD34 expression and tumor grade and stage factors. They also confirmed the lack of correlation between the marker and prognosis. In another study, the absence of CD34 in non-neoplastic fibroblasts has also been reported around epithelial carcinomas (Kirchmann *et al*., 1995). Similarly, malignant phyllodes tumors of the breast have lower levels of CD34 expression than benign phyllodes (Chen *et al*., 2000; Moore and Lee, 2001; Silverman and Tamsen, 1996). The expression of this marker is lost in invasive breast carcinoma. This loss is attributed to the malignant phenotype and although the role of expression change is not clarified but it shows the regional messaging mechanisms with epithelial origin (Chauhan *et al*., 2003).

Therefore, since the results of examining CD34 in different studies was a bit contradictory and since there is no thorough understanding of the mechanism of CD34 expressing cells, the use of expressing this protein as a marker for colorectal cancer is still controversial and more studies are necessary to reach firm and reliable conclusions.

CONCLUSION

According to the survey, it was concluded that initially, a significant relationship exists between the CD34 expression and examined factors and secondly, it seems that CD34 expression (CD34+cells) can be considered as a marker for colon carcinoma and the diagnosis of tumor characteristics. However, further studies in this area can be helpful with a large statistical population. Another important point that can greatly help is checking the status of marker by genetic methods.

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