Evaluation of Vimentin Expression in Colorectal Cancer and Its Association With Prognostic Factors

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Abstract- Vimentin is a cellular marker that has recently been considered in the prognosis of colorectal cancer (CRC), and its expression appears to indicate increased malignancy. The aim of this study was to investigate the expression of vimentin in CRC patients and its association with prognostic factors. This retrospective study was performed on CRC patients who had undergone colectomy at Imam Khomeini Hospital, Ahvaz, Iran, in 2019 and 2020. Data around the microscopic degree of the tumor differentiation and the status of lymph node involvement were extracted from the patients' pathology reports. Immunohistochemistry staining for vimentin was performed on biopsy specimens, and its expression was assessed and compared in both CRC specimens and normal colon tissues. Appropriate statistics were used with $P<0.05$ considered as statistically significant. Out of 31 CRC patients, vimentin expression was moderate-positive in 20 (64.5%) and strong-positive in 11 patients (35.5%). Mean percentage of stained cells, the intensity of staining, and vimentin expression in the immunohistochemistry evaluations had no significant relationship with tumor grade and tumor invasion rate ($P>0.05$), but they showed a significant relationship with lymph node involvement ($P<0.05$) and mean percentage of stained cells, the intensity of staining, and expression of vimentin marker increased with increasing lymph node involvement. In the normal tissue samples, 5 out of 30 samples showed weak-to-moderate vimentin expression. Vimentin expression was significantly associated with lymph node involvement; however, further studies with larger sample sizes are required to determine its probable association with other prognostic variables.

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Keywords: Colorectal cancer; Vimentin; Cancer grade; Cancer stage; Prognosis

Introduction

Despite advancements in medicine and the control of infectious diseases, non-communicable diseases, including cancer, remain a major challenge in the health system (1). In recent years, the prevalence of cancer has increased worldwide, so that in 2018, about 18.1 million new cases of cancer and 9.6 million deaths due to cancer (1 death out of 6 deaths) has occurred (2). This increasing burden of cancer puts enormous physical, emotional, and financial pressures on individuals, families, communities, and health systems (3).

Colorectal cancer (CRC) is among the most incident human malignancies, ranked third after lung and prostate cancer in men and second in women worldwide (4). In 2020, about 1 million new cases of CRC had been diagnosed worldwide, resulting in 700,000 deaths (5). Following surgical treatment, about 30% of patients eventually show local tumor recurrence or distant metastasis, leading to a poor overall prognosis (6). Patients with local recurrence or distant metastases usually receive chemotherapy in combination with targeted monoclonal antibody therapy, which responds 50% at the best condition (7). This limitation is exacerbated by the fact that almost half of these patients frequently experience treatment-related side effects

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without any therapeutic benefit (8). Therefore, identifying patients with CRC who are at a higher risk for metastatic disease helps to better classify and select candidate patients for standard or intensive adjuvant chemotherapy (9).

Recent research has focused on the role of epithelial-mesenchymal transmission (EMT) in cancer attack and metastasis. In CRC, tumor cells undergoing EMT are shown histologically on the invasive front despite the presence of tumor buds (10). Tumor germination predicts lymph node metastasis, vascular and lymphatic attack, distant metastasis, local recurrence, and poor survival (11). Therefore, research on molecular genomic targets and biomarkers of EMT regulation has tremendous potential in identifying a subset of patients at highest risk for metastasis. One of the distinguishing features of establishing the EMT phenotype is the regulated expression of mesenchymal markers, such as vimentin and fibronectin and the reduced expression of structural adhesion proteins such as E-cadherin (6). Although the association between increased vimentin expression and decreased E-cadherin expression in various cancers with tumor progression and metastasis has been well established, studies on colorectal cancer are limited (8). In this study, we aimed to investigate vimentin expression in colon samples of patients with CRC underwent colectomy and to find any possible correlation between vimentin expression and factors associated with colorectal cancer prognosis.

Materials and Methods

This retrospective study was conducted at the clinical oncology department of Imam Khomeini Hospital in Ahvaz, Iran, and medical records of all patients with CRC undergone colectomy in the years 2019 and 2020 were collected. Randomly paired normal tissues were also collected from colon tissues of patients underwent a resection surgery. Demographic characteristics, including age, sex, and family history of cancer were recorded for all patients. Information about the degree of tumor and the status of lymph node involvement was extracted from the patients' pathology report. Before the surgeries and performing this study, written informed consent was obtained from each patient. Patients not willing to participate in this study were excluded. Besides, in the normal group, samples with pathologic findings were excluded from the study process to include only normal tissues. All the study protocols were approved by the Ethics in Medical Research Committee of the Ahvaz Jondishapur University of Medical Sciences (NO. IR.AJUMS.HGOLESTAN.REC.1399.094)

To determine the expression of the vimentin marker, specific staining for the vimentin factor was performed using the immunohistochemistry method as follows. First, deparaffinization was performed by immersion of biopsy specimens in xylene. Irrigation was then performed by descending ethanols (99, 95, and 70%). In the next step, the samples were incubated for 5 minutes in the presence of 5% hydrogen peroxide and then washed in running water. The samples were then placed at 0.01 M sodium citrate buffer for one minute at 98° C in a microwave for antigen retrieval. In the next step, the slices were transferred to Tris-buffered saline (TBS) for 5 minutes. Endogenous peroxide was stopped by 10% natural serum (goat) for 5 minutes, and excess serum was removed. The initial antibody was added at a dilution of 1:200 and placed at 4° C for one hour. Washing with TBS was repeated twice (each for 5 minutes). The samples were then incubated with secondary antibody at a ratio of 1:500 for 45 minutes and washed again twice with TBS for 5 minutes each time. The chromogenic reaction was performed by diaminobenzidine, and contrast staining was carried out with hematoxylin. Finally, the samples were examined under a light microscope. In the negative control groups, PBS was used instead of specific antibodies. After the preparation of immunohistochemical slides, microscopic examination of antibody-labeled sections was performed. For this purpose, antibody-labeled sections for microscopic examination, at least 1000 cells per slide, were counted, and the percentage of stained cells in the tumor stroma was determined. The percentage of brown-stained cells to blue-stained cells was then determined in each tissue. Staining intensity was scored negative (0), weak (+1), moderate (+2), or strong (+3). Also, the extent of staining in terms of the percentage of stained cells was considered as 0 (score 0), less than 1% (score 1), 1-10% (score 2), 11-33% (score 3), 34-66% (score 4), or 67-100% (score 5). Vimentin marker expression was determined based on the information provided in Table 1.

Statistical Analysis

Data was eventually analyzed using SPSS software version 22 by independent t-test (or Mann-Whitney), Chi-square (or Fisher's exact), and one-way Analysis of variance (ANOVA) (or Kruskal-Wallis) tests. The significance level was considered 0.05.

Table 1. Vimentin marker expression based on immunohistochemical staining (modified Allred scoring system)

Proportion score		Intensity score		Allred score	
0	**No cell**	0	Negative	0-1	Negative
1	**≤ 1 % of cells**	1	Weak	2-3	Weak positive
2	**1-10 % of cells**	2	Intermediate	4-6	Moderate positive
3	**11-33 % of cells**	3	Strong	7-8	Strong positive
4	**34-66 % of cells**	--		--	
5	**67-100 % of cells**	--		--	

Results

In this study, 31 patients with CRC (mean age 65.6±15.4 years) and 30 patients having a normal colon sample (mean age 59.4±12.4 years) were investigated. Frequency distribution of tumor grade, tumor invasion, lymph node involvement, and expression of the vimentin marker are shown in Table 2. Out of 31 CRC patients, vimentin marker expression was moderate-positive in 20 cases (64.5%), and strong-positive in 11 cases (35.5%); negative and weak-positive expression was not observed in any of these patients (Figure 1). However, in the normal group, weak-to-moderate vimentin expression was observed in 5 patients out of 30 included participants (16.7%). These investigations showed remarkably more positive samples regarding vimentin expression in the CRC group compared to the normal group (P<0.001).

Table 2. Frequency distribution of tumor grade, tumor invasion, lymph node involvement, and expression of vimentin marker in colorectal cancer patients

Variable	Level	Frequency (%)
Tumor Grade	G1	13 (41.9%)
	G2	17 (54.8%)
	G3	1 (3.4%)
Tumor Invasion	PT1	1 (3.2%)
	PT2	6 (19.4%)
	PT3	21 (67.7%)
	PT4	2 (6.5%)
	PT4b	1 (3.2%)
Lymph Node Involvement	PN0	21 (67.7%)
	PN1a	2 (6.5%)
	PN1b	4 (12.9%)
	PN1c	1 (3.2%)
	PN2a	1 (3.2%)
	PN2b	2 (6.5%)
Vimentin Marker Expression	Moderate-positive	20 (64.5%)
	Strong-positive	11 (35.5%)

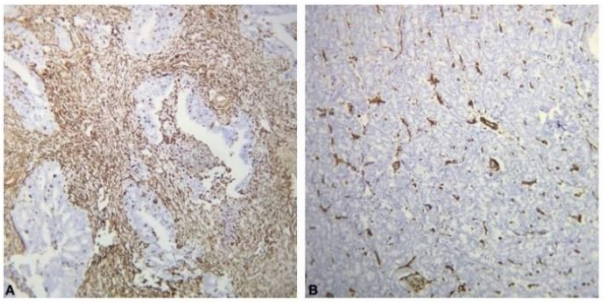

Figure 1. A: The high expression level of vimentin marker (strong-positive) B: The low expression level of vimentin marker (moderate-positive)

We found a significant negative relationship between age and the percentage of stained cells in CRC patients, and with aging, the mean percentage of stained cells on immunohistochemistry studies decreased significantly (r= -4.00, P=0.026). In addition, the age of CRC patients was significantly associated with the expression of vimentin marker in immunohistochemistry (P<0.05), but no significant relationship was observed between age and intensity of staining (P>0.05) (Table 3).

Table 3. The relationship between staining intensity and expression of vimentin marker in immunohistochemistry technique with colorectal cancer patients' age

Variable		Frequency	Age (Mean±SD)	*P*
Staining Intensity	**Intermediate**	23	67.6 ± 15.5	0.207
	Strong	8	59.6 ± 14.4	
Expression of Vimentin	**Morerate**	20	71.1 ± 14.6	0.002
	Strong	11	55.6 ± 11.8	

According to the results of Table 4, stained cells and staining intensity on immunohistochemistry did not show a significant relationship with disease grade and tumor invasion (P>0.05), but a significant relationship was observed between these factors and lymph node involvement (P<0.05). In addition, vimentin marker expression had no significant relationship with disease grade and tumor invasion (P<0.05), but it showed a significant relationship with lymph node involvement (P<0.05, Table 5).

Table 4. Relationship between the percentage of stained cells in immunohistochemistry technique with disease grade, tumor invasion, and lymph node involvement

Variable		Percentage of Stained Cells	*P*	Intensity of Staining		*P*
				Intermediate	Strong	
Grade	**G1**	46.9 ± 18.2	0.510	10 (43.5%)	3 (37.5%)	0.226
	G2	45.5 ± 22.1		13 (56.5%)	4 (50.0%)	
	G3	60.0 ± 1.0		0 (0.0%)	1 (12.5%)	
Tumor Invasion	**PT1**	15.0 ± 1.0	0.093	1 (4.3%)	0 (0.0%)	0.685
	PT2	43.3 ± 24.2		5 (21.7%)	1 (12.5%)	
	PT3	48.9 ± 19.5		14 (60.9%)	7 (87.7%)	
	PT4	50.0 ± 14.1		2 (8.7%)	0 (0.0%)	
	PT4b	40.0 ± 1.0		1 (4.3%)	0 (0.0%)	
Lymph Node Involvement	**PN0**	40.8 ± 18.9	0.004	19 (82.6%)	2 (25.0%)	0.010
	PN1a	75.0 ± 7.1		0 (0.0%)	2 (25.0%)	
	PN1b	56.2 ± 12.5		3 (13.0%)	1 (12.5%)	
	PN1c	60.0 ± 1.0		0 (0.0%)	1 (12.5%)	
	PN2a	20.0 ± 1.0		0 (0.0%)	1 (12.5%)	
	PN2b	65.0 ± 7.1		1 (4.3%)	1 (12.5%)	

Table 5. The relationship between vimentin marker expression and disease grade, tumor invasion, and lymph node involvement

Variable		Expression of Vimentin		*P*
		Moderate	Strong	
Grade	**G1**	9 (45.0%)	4 (36.4%)	0.378
	G2	11 (55.0%)	6 (54.5%)	
	G3	0 (0.0%)	1 (9.1%)	
Tumor Invasion	**PT1**	1 (5.0%)	0 (0.0%)	0.607
	PT2	4 (20.0%)	2 (18.2%)	
	PT3	12 (60.0%)	9 (81.8%)	
	PT4	2 (10.0%)	0 (0.0%)	
	PT4b	1 (5.0%)	0 (0.0%)	
Lymph Node Involvement	**PN0**	17 (85.0%)	4 (36.4%)	0.029
	PN1a	0 (0.0%)	2 (18.2%)	
	PN1b	2 (10.0%)	2 (18.2%)	
	PN1c	0 (0.0%)	1 (9.1%)	
	PN2a	1 (5.0%)	0 (0.0%)	
	PN2b	0 (0.0%)	2 (18.2%)	

Discussion

In the present study, all the CRC patients had positive staining for vimentin marker—the majority of the cases were moderate-positive; no case with negative and weak-positive expression was observed. While, the positive

expression of vimentin marker in immunohistochemical staining of CRC in previous studies was reported 56.4% (12), 56% (13), 29% (14), 24% (15), 14% (16), and 17.3% (8). Extensive variation in the identification of vimentin expression can be due to several factors, including different tumor phenotypes, different scoring systems, and different ethnic groups of patients (13-16). Besides, in the present study, 16.7% of normal tissues were reported to have a weak-to-moderate positive vimentin expression.

The results of the present study also showed that there was a significant and inverse relationship between vimentin marker expression and the age of CRC patients, so that the mean age in the group with moderate-positive expression status was significantly higher than that of the strong-positive group. While, in studies of Toiyama *et al*., (6), Al-maghrabi *et al*., (8), Niknami (12) and Liu *et al*., (9) the expression of vimentin marker had no significant relationship with age of patients. However, Ngan *et al*., reported that the mean age of patients with low and high vimentin expression was 61.1 and 64.4 years, respectively, which the difference was statistically significant (17).

In the present study, the mean percentage of stained cells, staining intensity and expression of the vimentin marker in the biopsy specimen had no significant relationship with tumor grade and tumor invasion rate, but significant relationship was observed with lymph node involvement and all the three parameters increased with increasing lymph node involvement. In a study on 181 patients with CRC, the expression of vimentin in cancerous tissues was significantly associated with tumor size, higher tumor stage, lymph node involvement, and liver metastasis (6), which was consistent with the present study in terms of lymph node involvement. In our study, in patients who were strong-positive in terms of vimentin marker expression, the frequency of tumor grade 1, 2, and 3 was 36.4%, 54.5% and 9.1%, respectively, while it was respectively 45%, 55%, and 0% in morerate-positive patients, which indicates the higher prevalence of grade 1 in morerate-positive patients.

In a study by Niknami *et al*., on 39 CRC specimens, it was observed that vimentin expression with metastasis in 1 to 3 regional lymph nodes (pN1) significantly increased compared to non-metastatic lymph node samples (pN0) and it was the most in tumors with involvement of four or more lymph nodes. In addition, they showed that with increasing the grade of the tumor, the expression of vimentin increased and its value in grade III tumors was significantly higher than that of grade I and II. However, in the evaluation of the PT group of cancers, it was observed that with the increase of tumor invasion from under the mucosa (pT1) to the invasion of the tumor to other organs such as the peritoneum, the expression of vimentin did not increase significantly (12). In the present study, similarly, vimentin expression was significantly associated with lymph node involvement.

In a study by Ngan *et al*., on 142 colorectal cancer specimens, it was observed that the vimentin marker has prognostic power for recurrence in both stages II and III and is a better prognostic indicator for disease recurrence than the widely used lymph node condition (17). However, in a study by Liu *et al*., on 203 tissue samples from patients with colorectal cancer, it was found that vimentin expression was associated only with tumor stage and had no significant relationship with tumor size, tumor differentiation, lymphatic and vascular invasion (9). In a study by Al-Maghrabi *et al*., in 2020 on 202 samples of primary colorectal cancer (41 specimens of adenoma and 37 specimens of normal colonic mucosa), vimentin expression was positive in 35 specimens (3.17%). Their study showed that there was a significant relationship between positive vimentin expression and high tumor grade, distant metastasis, and survival, but no relationship was observed between vimentin expression and tumor location, tumor size, tumor stage, lymph node involvement, lymphovascular attack, tumor margin status and tumor recurrence (8). In the present study, although vimentin expression was significantly associated with lymph node involvement, it did not show any association with the degree of invasion and tumor grade. The difference in study results is likely due to differences in the studied populations. For example, in the study of Al-maghrabi *et al*., the patients were investigated in the early stages, but in the present study, the majority of patients had advanced disease.

In a meta-analysis study by Du *et al*., (18) which evaluated 11 studies involving 1969 patients, it was found that positive vimentin expression predicted poor overall survival and disease-free survival in patients with colorectal cancer. This analysis also showed that vitamin expression was associated with lymph node metastasis, TNM stage, and N stage. Finally, it was stated that the immunohistochemical expression of vimentin could be used as a valuable biomarker indicating EMT and disease prognosis as well as designing therapeutic goals (18). The disadvantage of this meta-analysis was that the entire population were included in studies in Southeast Asia, so further studies worldwide can lead to a better overall conclusion.

In the present study, the expression of vimentin marker on immunohistochemistry did not show any

significant relationship with tumor grade and tumor invasion rate, but its expression significantly increased by increasing lymph node involvement. However, before introducing vimentin as a potential biomarker in determining the prognosis of the disease and a goal to provide treatment strategies for colorectal cancer, further studies are needed with larger sample sizes to evaluate the association of vimentin marker with tumor size, tumor location, tumor invasion, lymph node involvement, disease grade/stage, survival rate and mortality, and response to treatment.

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