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Original Article

Expression of *p16* Marker in Colorectal Cancer and its Association with Clinicopathological Parameters in Patients with Colectomy Surgery

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Abstract

Introduction: Colorectal Cancer (CRC) is a genetic disease with complex and diverse pathways. The *p16* is a tumor-inhibiting gene that acts as a regulator of the cell cycle. Therefore, the present study aimed to investigate the expression of the *p16* marker in CRC and its relationship with clinical and pathological parameters.

Materials and Methods: In this retrospective study, paraffin blocks of tumors of consecutive CRC patients registered in the histopathology laboratory of hospitals under the auspices of Ahvaz Jundishapur University of Medical Sciences were used. Clinicopathological information such as the degree of tumor differentiation, tumor depth of invasion, lymph node involvement status, etc. were extracted from the patient's files and pathology reports and using paraffin blocks, specific staining for *p16* factor was performed using immunohistochemistry. Data were analyzed by SPSS software.

Results: In the immunohistochemistry technique from 38 samples, the staining rate of *P16* marker: 13 samples (34.2%) scored 3, 12 samples (31.6%) scored 2, seven samples (18.4%) scored 1 and six samples (15.8%) scored zero. Also, the staining intensity was severe in 10 cases (26.3%), moderate in 14 cases (36.8%), mild in 8 cases (21.1%), and negative in 6 cases (15.8%). The amount and intensity of staining for the *p16* factor in the immunohistochemistry technique were not associated with sex, age, tumor location, tumor differentiation rate, tumor depth of invasion, lymph node involvement, lymph vascular invasion, and perineural invasion ($p>0.05$). Tumor size was not significantly associated with staining rate but was significantly associated with staining intensity ($p<0.05$), so that in cases with a larger tumor size, staining intensity was lower.

Conclusions: Despite the positive expression of *P16* in 84.2% of colorectal cancer cases, its expression was not associated with clinical and pathological parameters.

Keywords: Colorectal Cancer, *p16* Marker, Immunohistochemistry, Clinicopathological Parameter
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Introduction

Colorectal Cancer (CRC) is the third most common cancer in the world after lung cancer and breast cancer. In 2018, more than 10% of all new cancer cases was attributed to CRC, with approximately 1.8 million reported cases.[1,2] The mortality rate for this cancer in both sexes and all ages is 9.2% of all cancer deaths.[3] In 2018, CRC was responsible for 9864 new cases (in both men and women) out of 110115 new cases of all cancers (9%) in Iran, which was the third most common cancer after breast cancer (12.5%) and gastric cancer (10.6%).[4,5] CRC is a genetic disease which occurs in the normal mucosa of the large intestine through different molecular mechanisms.[6]

First, chromosomal instability occurs through cumulative genetic mutations in many tumor suppressor genes such as *K-ras*, *p53*, *c-Myc*, *and Cyclin-D*, which are responsible for approximately 80% of sporadic infections. Second, the microsatellite instability pathway, which is characterized by genetic changes in genes related to DNA repairing (responsible for repairing deletions, and incorrect bases during DNA replication and recombination by avoiding mutations in frame change and open replacement).[7-10] This event usually occurs sporadically in 10-15% of cases. Third is the lynch syndrome, which causes 3% of diseases. Finally, familial adenomatous polyposis syndrome accounts for 1% of CRC cases.[11]

P16 is a tumor suppressor gene which is now recognized as the second most common point of mutation in human cancer.[12,13] The *p16* tumor suppressor gene binds to CD4/6 and prevents its interaction with cyclin D. Finally, this reaction inhibits cell cycle progression from stage G1 to S.[14,15]

Thus, the *p16* is a key element in oncogenesis and cell aging processes. Decreased *p16* regulation by hypermethylation, point mutation, or gene deletion leads to cell cycle progression;[16,17] while the activation of this gene is associated with cell aging. As a tumor suppressor gene, *p16* is inactive in many tumors and is closely related to tumor formation and progression.[18,19]

Inactivation of *p16* has been reported in CRC, oropharyngeal cancer, pancreatic cancer, esophageal cancer, non-small cell lung cancer and mesothelioma.[20] Due to the contradictory results in terms of the relationship between *p16* expression with clinicopathological parameters in various studies in the world and also few studies conducted in Iran, therefore the aim of this study was to investigate the expression pattern of the *p16* marker in CRC and its relationship with clinical and pathological parameters in patients referred to hospitals affiliated to Ahvaz Jundishapur University of Medical Sciences.

Materials and Methods

Case Selection and Tissue Samples

The present cross sectional study used a descriptive-analytical method. The pathologic records of colorectal carcinoma were retrieved from the archive of the Pathology Department, Imam Khomeini Hospital, Ahvaz, Iran between the years 2018-2020. The hematoxylin-eosin stained slides were reviewed. Inclusion criteria were adequate tumoral mass, absence of necrosis/hemorrhage, presence of lymph node pathologic slides, and complete medical records. The total number of colorectal adenocarcinoma paraffin samples during the years 2018 to 2020 were considered as the sample size. Based on the inclusion criteria, 38 formalin-fixed, paraffin-embedded samples were enrolled. Clinical information including sex, age, tumor location, tumor size, tumor differentiation degree, depth of tumor invasion, lymph node status, vascular invasion (lymph vascular) and perineural invasion were extracted from patients' pathology report and were recorded in a checklist.

Immunohistochemical Assay

The 5-µm paraffinized sections were soaked in water-alcohol solution for 5 min. Slides were placed in a microwave oven for 30 min at 60 °C. Deparaffinization was performed by soaking the slides in xylene (Merck, Germany) and, then, alcohol (from 100% to 75% concentration) for 5 to 10 min. Sections were rinsed with 10% phosphate-buffered saline (PBS; Yekta Tajhiz Azma, Iran), followed by H_2O_2/ methanol (1:9) and 10% PBS for 10 min. Then, the slides were heated in a microwave oven for 10 min in ethylenediaminetetraacetic acid (EDTA; Yekta Tajhiz Azma, Iran). The samples were left to reach the room temperature; then, were rinsed with PBS. Sections were incubated with 1 µg/ml diluted *p16* primary anti-mouse monoclonal antibody for an hour at room temperature (clone E6H4, Ventana Medical Systems, Inc. Tucson, AZ) and, then, were reincubated with biotinylated antibody for 30 min and soaked in 10% PBS for 10 min. Sections were incubated with conjugated enzyme for 30 min and developed in 3, 3`-diaminobenzidinehydrochloride chromogen (Dako, code K3468, USA). The haematoxylin stain (Yekta Tajhiz Azma, Iran) was used to develop the ground contrast. Positive staining for *p16* was interpreted as the presence of brown spots on the nucleus/cytoplasm of cells.[21-23] The percentage of cells stained brown compared to cells stained blue was then determined in each tissue. Every tumor was given a score according to the intensity of the nuclear or cytoplasmic staining. Positive staining for *p16* was interpreted as the presence of brown spots on the nucleus/cytoplasm of cells. The percentage of cells stained brown compared to cells stained blue was then determined in each tissue. The staining intensity of *p16* marker was scored negatively (0), weak (+1), medium (+2) and strong (+3). Also, the extent of staining of *p16* marker in terms of percentage of stained cells was scored as follows:[24]

- Negative staining (score 0)
- Immune staining in <10% of cells (score 1)
- Immune staining in 10%-40% of cells (score 2)
- Immune staining in >40% of cells (score 3)

Statistical Analysis

Descriptive statistics including mean index and standard deviation for quantitative variables and frequency and percentage for qualitative variables were used. Normal distribution of the quantitative data was checked with the Kolmogorov–Smirnov test. Differences in staining degree and staining intensity in variables were analyzed using one-way analysis of variance (ANOVA), Chi-squared test and Kruskal-Wallis test. Significance level $p<0.05$ and all analyzes were performed using SPSS software version 22.

Results

In the present study, a colectomy sample of 38 patients with CRC with a mean age of 55 ± 13.34 was examined. Among these patients, 20 cases (52.6%) were female and 18 cases (47.4%) were male. The distribution of gender and clinical characteristics and pathology of patients are shown in Table 1. The mean age and size of the tumor are also presented in Table 2.

In terms of tumor grade, the frequency of G2 grade was the highest (52.6%). Regarding the depth of tumor invasion, the majority of patients (23 cases; 60.5%) were in PT3 stage. The tumor was located in 34.2% of the rectum, 36.8% of the sigmoid, 23.7% of the transverse and descending colon, and 5.3% of the cecum. In terms of lymph node involvement, the majority of patients (55.3%) had PN0 and the rest PN1a (2.6%), PN1b (15.8%), PN1c (7.9%), PN2a (7.9%), and PN2b (10.5%). Vascular invasion and perineural invasion

Table 1. Distribution of Gender, Clinical Characteristics and Pathology of Patients

Variable		Frequency (Percent)
Gender	Female	20 (52.6)
	Male	18 (47.4)
Grade tumor	G1	13 (34.2)
	G2	20 (52.6)
	G3	5 (13.2)
Depth of tumor	PT1	1 (2.6)
	PT2	4 (10.5)
	PT3	23 (60.5)
	PT4a	6 (15.8)
	PT4b	3 (7.9)
	PTis	1 (2.6)
Tumor site	Rectum	13 (34.2)
	Sigmoid	14 (36.8)
	Colon	9 (23.7)
	Cecum	2 (5.3)
Lymph node involvement	PN0	21 (55.3)
	PN1a	1 (2.6)
	PN1b	6 (15.8)
	PN1c	3 (7.9)
	PN2a	3 (7.9)
	PN2b	4 (10.5)
Vascular invasion	No	21 (55.3)
	Yes	17 (44.7)
Perineural invasion	No	25 (65.8)
	Yes	13 (34.2)

were observed in 44.7% and 34.2% of patients, respectively (Table 1). The minimum age of patients was 29 years and the maximum age was 81 years. The mean tumor size was 5.17 ± 2.45, the minimum size was 1.5 cm and the maximum size was 11 cm.

The results of immunohistochemical staining marker *p16* showed that out of the 38 studied samples, 13 samples (34.2%) had a score of 3, 12 samples (31.6%) had a score of 2, seven samples (18.4%) had scores 1 and six (15.8%) had a score of 0.

The results related to the staining intensity of the *p16* marker indicated that out of 38 samples studied, the staining intensity in 10 cases (26.3%) was severe, in 14 cases (36.8%) moderate, in eight cases (21.1%) was mild and six cases (15.8%) was negative (Figure 1).

The results of Chi-square test showed that there was no significant relationship between variables gender, tumor location, tumor grade (well, moderate and poorly differentiated), depth of tumor invasion, lymph node involvement, vascular invasion and perineural invasion with immunohistochemical

Table 2. Relationship of Age and Gender with Staining Degree and Staining Intensity

Variable		Age	Gender (%)		*p* value
		(Mean ± SD)	Female	Male	
Staining Degree	0	54.16 ± 10.10	4 (20)	2 (11)	*p*>0.05
	1	48.28 ± 14.34	4 (20)	3 (16)	
	2	58.75 ± 16.17	5 (25)	7 (38)	
	3	56.53 ± 12.28	7 (35)	6 (33)	
Staining Intensity	Intense	53.70 ± 11.12	6 (30)	4 (22)	*p*>0.05
	Medium	60.00 ± 15.99	6 (30)	8 (44)	
	Mild	50.12 ± 14.35	4 (20)	4 (22)	
	Negative	54.16 ± 10.10	4 (20)	2 (11)	

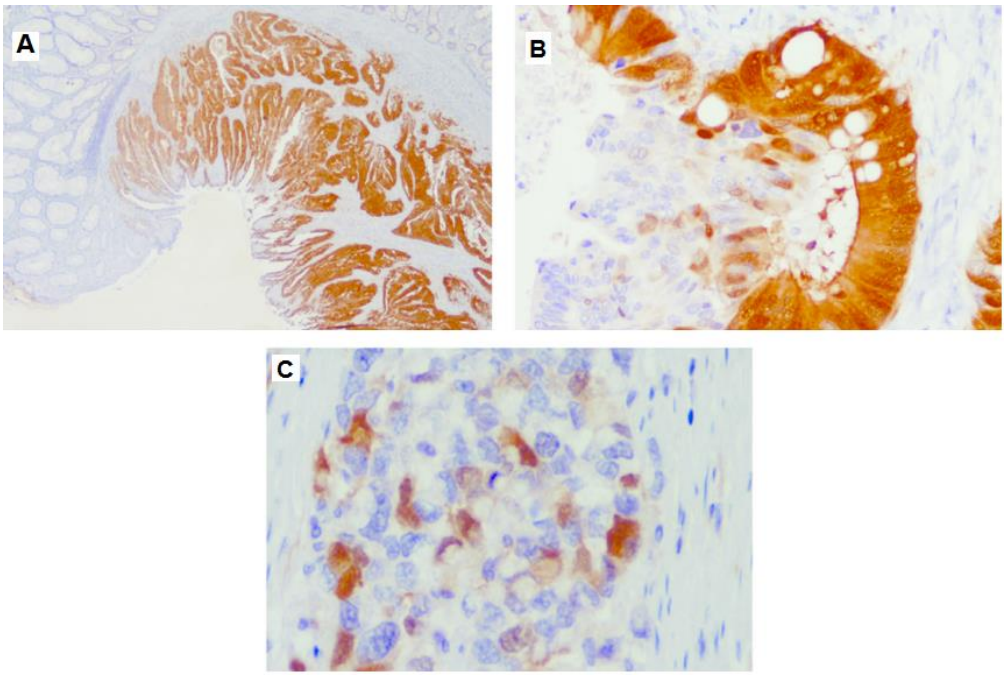

Figure 1. Representative of *p*16 Immunostaining in Colorectal Cancer. IHC staining for p16 expression of colorectal cancer was evaluated in different staining degree and staining intensity separately. From the above left: (A) Negative expression of P16 marker in normal colon compared with positive expression of this marker in colorectal cancer; (B) High expression (score 3) and strong intensity of P16 marker in colorectal cancer; (C) Low expression (score 1) and poor severity of P16 marker in colorectal cancer.

Table 3. Relationship between Tumor Site with Staining Degree and Staining Intensity

Variable		Tumor Site				*p* value
		Rectum (%)	Cecum (%)	Sigmoid (%)	Colon (%)	
Staining Degree	0	3 (23.1)	0 (0.0)	2 (14.3)	1 (11.1)	0.457
	1	0 (0.0)	1 (50)	3 (21.4)	3 (33.3)	
	2	3 (23.1)	1 (50)	5 (35.7)	3 (33.3)	
	3	7 (53.8)	0 (0.0)	4 (28.6)	2 (22.2)	
Staining Intensity	Intense	4 (30.8)	0 (0.0)	4 (28.6)	2 (22.2)	0.884
	Medium	5 (38.5)	1 (50)	4 (28.6)	4 (44.4)	
	Mild	1 (7.7)	1 (50)	4 (28.6)	2 (22.2)	
	Negative	3 (23.1)	0 (0.0)	2 (14.3)	1 (11.1)	

Table 4. Relationship between Tumor Differentiation and Tumor Size with Staining Degree and Staining Intensity

Variable		Tumor Size (Mean ± SD)	Differentiation		
			G1 (%)	G2 (%)	G3 (%)
Staining Degree	0	5.91 ± 2.59	1 (7.7)	4 (20)	1 (20)
	1	6.50 ± 2.39	1 (7.7)	3 (15)	3 (60)
	2	5.08 ± 2.36	5 (38.5)	6 (30)	1 (20)
	3	4.21 ± 2.34	6 (46.2)	7 (35)	0 (0.0)
Staining Intensity	Intense	4.18 ± 2.63	5 (38.85)	5 (25)	0 (0.0)
	Medium	4.21 ± 1.25	5 (38.5)	8 (40)	1 (20)
	Mild	7.56 ± 2.22	2 (15.4)	3 (15)	3 (60)
	Negative	5.91 ± 2.59	1 (7.7)	4 (20)	1 (20)

Table 5. Relationship between Depth of Tumor with Staining Degree and Staining Intensity

Variable		Depth of Tumor						*p* value
		PT1 (%)	PT2 (%)	PT3 (%)	PT4a (%)	PT4b (%)	PTis (%)	
Staining Degree	0	0 (0.0)	0 (0.0)	4 (17.4)	2 (33.3)	0 (0.0)	0 (0.0)	0.403
	1	0 (0.0)	0 (0.0)	5 (21.7)	2 (33.3)	0 (0.0)	0 (0.0)	
	2	0 (0.0)	2 (50.0)	6 (21.1)	1 (16.7)	3 (100)	0 (0.0)	
	3	1 (100)	2 (50.0)	8 (34.8)	1 (16.7)	0 (0.0)	1 (100)	
Staining Intensity	Intense	1 (100)	1 (25.0)	6 (26.1)	1 (16.7)	0 (0.0)	1 (100)	0.315
	Medium	0 (0.0)	3 (75.0)	9 (39.1)	0 (0.0)	2 (66.7)	0 (0.0)	
	Mild	0 (0.0)	0 (0.0)	4 (17.4)	3 (50.0)	1 (33.3)	0 (0.0)	
	Negative	0 (0.0)	0 (0.0)	4 (17.4)	2 (33.3)	0 (0.0)	0 (0.0)	

Table 6. Relationship between Lymph Node Involvement with Staining Degree and Staining Intensity

Variable		Lymph Node Involvement						*p* value
		PN0 (%)	PN1a (%)	PN1b (%)	PN1c (%)	PN2a (%)	PN2b (%)	
Staining Degree	0	4 (19)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	1 (25.0)	0.100
	1	1 (4.8)	1 (100)	1 (16.7)	1 (33.3)	0 (0.0)	3 (75.0)	
	2	8 (38.1)	0 (0.0)	3 (50.0)	1 (33.3)	0 (0.0)	0 (0.0)	
	3	8 (38.1)	0 (0.0)	2 (33.3)	1 (33.3)	2 (66.7)	0 (0.0)	
Staining Intensity	Intense	4 (19)	1 (100)	2 (33.3)	1 (33.3)	2 (66.7)	0 (0.0)	0.144
	Medium	11 (52.4)	0 (0.0)	2 (33.3)	1 (33.3)	0 (0.0)	0 (0.0)	
	Mild	2 (9.5)	0 (0.0)	2 (33.3)	1 (33.3)	0 (0.0)	3 (75.0)	
	Negative	4 (19)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	1 (25.0)	

Table 7. Relationship between Vascular and Perineural Invasion with Staining Degree and Staining Intensity

Variable		Perineural Invasion		Vascular Invasion	
		No (%)	Yes (%)	No (%)	Yes (%)
Staining Degree	0	5 (%20.0)	1 (%7.7)	3 (%14.3)	3 (%17.6)
	1	4 (%16.0)	3 (%23.1)	1 (%4.8)	6 (%35.3)
	2	7 (%28.0)	5 (%38.5)	8 (%38.1)	4 (%23.5)
	3	9 (%36.0)	4 (%30.8)	9 (%42.9)	4 (%23.5)
Staining Intensity	Intense	6 (%24.0)	4 (%30.8)	5 (%23.8)	5 (%29.4)
	Medium	10 (%40.0)	4 (%30.8)	11 (%52.4)	3 (%17.6)
	Mild	4 (%16.0)	4 (%30.8)	2 (%9.5)	6 (%35.5)
	Negative	5 (%20.0)	1 (%7.7)	4 (%14.3)	3 (%17.6)

staining marker *p16* ($p>0.05$). Also, the results of Chi-square test showed that there was no significant relationship between variables gender, tumor location, tumor grade (well, moderate and poorly differentiated), depth of tumor invasion, lymph node involvement, vascular invasion and perineural invasion with the staining intensity of the *p16* marker ($p>0.05$) (Tables 3-7). The results of Kruskal-Wallis test demonstrated that there was no significant relationship between tumor size and staining ($p>0.05$). The results of Kruskal-Wallis test indicated that there was a significant relationship between tumor size and staining intensity, so that in cases with a larger tumor size, staining intensity was lower ($p<0.05$) (Table 4).

The results of one-way ANOVA test presented that there was no significant relationship between age and staining rate ($p>0.05$). Also, there was no significant relationship between age and staining intensity ($p>0.05$) (Table 2).

Discussion

In the present study, the expression of *P16* marker in 38 colorectal cancer samples and its relationship with clinical and pathological parameters were studied. Staining of samples for *p16* marker in immunohistochemical technique showed, 32 positive samples (88.8%) and six negative samples (15.78%). Out of 32 positive samples, 13 samples (34.2%) had a score of 3, 12 samples (31.6%) had a score of 2 and seven samples (18.4%) had a score of 1. Staining intensity was severe in 10 cases (26.3%), moderate in 14 cases (36.8%), mild in eight cases (21.1%) and negative in six cases (15.8%). The findings of the present study did not show a significant relationship between *p16* expression and pathological findings of CRC.

In the study by Al-Ahwal et al. (2016), high expression of *p16* was observed in 4, 14.6, 30.4, and 28% of cases of normal mucosa, adenoma, CRC and nodal metastasis, respectively. In CRC samples, *p16* positive immunohistochemical staining was observed in 142/193 samples (73.6%), which was scored 1 in 84/142 (59.1%), 2 in 40/142 (28.2%) and finally 3 in 18/142 (7.12%). Negative staining of *p16* was also observed in 51/193 (26.4%) patients. The values reported in the above study are similar to the present study, however, the frequency of staining of score 1 in the above study and scores 2 and 3 in the present study were higher.[25] In the study of Stockmar-Von Wangenheim et al. (2008), the rate of positive staining of *p16* in 200 CRC samples was 94%.[26] In the study of Zhao et al. (2003), out of 74 CRC samples, 73 (98.6%) were positive for *p16* immunohistochemistry.[27] Positive expression of *p16* in the study of Cui et al. (2004) in Japan 38%, in the study of Tada et al. (2003) in Japan 98%, In the study of Norrie et al. (2003) in Australia 92% and in the study of McKay et al. (2002) in the UK was 74%. The findings of a number of the above studies in terms of positive expression of *p16* in the immunohistochemical technique are similar to the present study. However, differences in the expression values reported in different studies may be related to various factors, including the number of patients tested for *p16* staining, the *p16* antibody used, the method of incision, and the immunohistochemical method. Heidari et al.'s study (2017) demonstrated that the expression of P16 in adenocarcinoma, adenomatous, and normal colorectal tissues was 25.40%, 50.00%, and 69.50%, respectively.[28]

The results of the present study showed that the amount and intensity of staining in immunohistochemistry technique had no significant relationship with gender and age. In the study of Al-Ahwal et al. (2016),[25] on 191 CRC samples, *p16* expression in CRC samples had no significant relationship with age and gender, which is similar to the present study. Also in Stockmar-Von Wangenheim et al.'s study (2008)[26] on 200 CRC samples, *p16* expression in immunohistochemistry technique had no significant relationship with age and gender, which is consistent with the present study. However, in a study by Lam et al. (2008)[29] on 194 CRC samples, positive *p16* staining in immunohistochemistry was higher in men than women (86% vs. 73%) but had no significant relationship with age.

Of the *p16* positive samples, a strong expression pattern was observed in 53 cases and a weak expression pattern in 20 cases. Also, the results of the present study showed that the amount and intensity of staining in immunohistochemistry technique has no significant relationship with gender and age, which is similar to the above studies. The results of our study showed that the intensity of staining for *p16* factor in immunohistochemistry technique was not significantly related to tumor location. Also, the level of staining was not significantly related to tumor size, but the intensity of staining was significantly related to tumor size, so that in cases with a larger tumor size, the intensity of staining was lower.

In the study of Al-Ahwal et al. (2016),[25] *p16* expression in CRC samples had no significant relationship with tumor location and tumor size. Positive expression of *p16* in the study of Cui et al. (2004)[30] in Japan also had no significant relationship with tumor size, tumor location and tumor type. However, in the study of Lam et al. (2008),[29] positive staining of *p16* in immunohistochemistry technique had a significant relationship with tumor location but had no significant relationship with tumor size and tumor type.

The results of our study showed that the amount and intensity of staining in immunohistochemistry technique had no significant relationship with the degree of tumor differentiation, depth of tumor invasion and lymph node involvement.

In the study of Al-Ahwal et al. (2016), *p16* expression in CRC samples had no significant relationship with tumor location and tumor size.[25] Positive expression of *p16* in the study of Cui et al. (2004) in Japan also had no significant relationship with tumor size, tumor location and tumor type.[30] However, in the study of Lam et al. (2008), positive staining of *p16* in immunohistochemistry technique had a significant relationship with tumor location but had no significant relationship with tumor size and tumor type.[29] Due to the existing inconsistencies, more studies with larger sample sizes are needed in this regard.

The results of our study showed that the amount and intensity of staining in immunohistochemistry technique was not significantly related to tumor differentiation, depth of tumor invasion and lymph node involvement. In a study by Ayhan et al. (2010) on 44 cases of colon adenoma and 44 cases of adenocarcinoma, there was no association between *p16* expression and clinical features and prognostic data such as stage or lymph node/liver metastasis.[31] In the study of Al-Ahwal et al. (2016) on 191 CRC samples, *p16* expression in CRC samples was not significantly associated

with tumor grade, invasion depth (PT), nodal metastasis, distant metastasis and lymph vascular invasion.[32] In the study of Huh et al. (2010) on 356 patients with colorectal adenocarcinoma, the expression of *p16* immunohistochemistry was not significantly associated with clinical and pathological findings.[33] In the study of Zhao et al. (2003) on 74 CRC samples, the expression of *p16* was not significantly associated with the stage of cancer.[27] Positive expression of *P16* in the study of Cui et al. (2004) in Japan also had no significant relationship with the stage, degree of differentiation and involvement of lymph nodes.[30] The findings of the mentioned studies, which show that the expression of *p16* is not related to pathological and clinical findings, are consistent with the results of the present study.

Contrary to the findings of the present study, in a study by Stockmar-Von Wangenheim et al. (2008) which was done on 200 CRC samples, cytoplasmic expression of *p16* decreased in advanced pN stages (lymph node involvement) but was not significantly associated with growth pattern, stage, degree of differentiation and prognosis of CRC.[33] A study by Tada et al. (2003) in Japan also found that *p16* immune expression decreased in CRC in mucosal tumors, grade 3 tumor, advanced stage T, and lymphatic invasion.[34]

A meta-analysis study by Ning Zhou et al. (2018) showed that contrary to the results of the present study p16 protein expression was significantly associated with the Dukes stage, lymph node metastasis, tumor location, and tumor lymph node metastasis-stage of CRC.[35] Also, a similar study by HE Qian-qian (2015) demonstrated that *p16* expression was closely linked with Dukes' staging, lymph node metastasis and histological differentiation degrees.[36] Another study by Heidari et al. (2017) indicated that *p16* expression was significantly higher in non-neoplastic tissues compared to the adenomatous and colorectal tissues. There was a significant association between *p16* expression and differentiation grade and the primary location of the tumor.[28] The findings of the present study did not show a significant relationship between *p16* expression and pathological findings of CRC.

Conclusion

The results of the present study showed that the amount and severity of staining in immunohistochemistry technique had no significant relationship with sex, age, tumor location, tumor differentiation rate, tumor invasion depth, lymph node involvement, vascular invasion and perineural invasion. Tumor size was not significantly associated with staining rate, but was significantly associated with staining intensity, so that in cases with larger tumor size, staining intensity was lower.

Authors' Contributions

Study concept and design by PK.; Analysis and interpretation of data by NR and MT; Drafting of the manuscript by BB; Critical revision of the manuscript for important intellectual content by PK, NR, and MT; Statistical analysis by BB.

Ethics Approval

The study was accepted by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences. (IR.AJUMS.HGOLESTAN.REC.1399.126). Written, informed consent was obtained from each patient.

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

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