Original Article

Investigating the Relation between Immunohistochemical Expression of Cytokeratin 20 and Clinicopathological Factors Determining Colorectal Adenocarcinoma Prognosis

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ABSTRACT

Background:
Cytokeratins, a family of intermediate filament proteins, are critical components of the cytoskeletal structure in epithelial cells. Among these, cytokeratin 20 (CK20) has garnered substantial attention as a potential biomarker in colorectal cancer (CRC) due to its role in distinguishing various subtypes of gastrointestinal epithelial cells. Therefore, we aimed to investigate the relationship between immunohistochemical expression of CK20 and colorectal adenocarcinoma prognostic factors.

Materials and Methods:
This cross-sectional study included 50 tissue blocks from patients with colorectal adenocarcinoma. Demographic and clinicopathological information was extracted from the patients' pathology report and recorded in a checklist. Then CK20 expression was examined using immunohistochemistry.

Results:
The present study included 50 patients with colorectal adenocarcinoma, including 23 (46%) men and 27 (54%) women, with a mean age of 50±11.2 years. Immunohistochemical expression of the CK20 marker was negative in 15 patients (30%), but 16 patients (32%) and 19 patients (38%) had focal and diffuse positive expression of this marker, respectively. In other words, the expression of this marker was positive in 40 patients (70%). Based on the results of the present study, a significant correlation between clinicopathological features such as tumor grade, lymphovascular invasion, perineural invasion, and lymph node involvement with the expression of the CK20 marker was reported ($P<0.05$).

Conclusion:
In general, based on the results of the present study, there is a significant association between the expression of CK20 and clinicopathological factors, so it is possible to use the CK20 marker in determining the prognosis of colorectal adenocarcinoma, however, more studies with a larger sample size will be required in the future.

Keywords: Colorectal adenocarcinoma; CK20; Immunohistochemistry, clinicopathological parameter

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INTRODUCTION

Colorectal cancer (CRC) is a significant global health challenge, ranking among the most prevalent malignancies and a leading cause of cancer-related morbidity and mortality. The intricate interplay of molecular and cellular factors contributes to the complex nature of CRC, which encompasses a wide spectrum of clinicopathological variations. In this context, the expression of specific biomarkers assumes a pivotal role in characterizing the disease's heterogeneity and guiding clinical decision-making (1, 2). Rectal cancer is the most common cancer in men and the third most common in women; hence, the prevalence in Iran is lower than in Western countries, and it is the fifth and third most common cancer in women and men, respectively (3, 4). Human CRC has various advanced stages, and the transformation of colorectal cryptepithelial cells into neoplastic cells is related to the cumulative changes of genes, proteins, and myriad molecular processes (5, 6). A metastatic lesion can be the first clinical manifestation of the neoplastic process, which probably accounts for more than 10% of all new tumor diagnoses. Although modern imaging technology has led to improved identification of primary tumors, their origin remains unknown in most patients (7, 8). Therefore, pathological evaluation has an important role in diagnosing the location (origin) of these tumors. Immunohistochemistry (IHC) has proven useful for identifying the primary tumor site (9). The immunohistochemical detection of tumor markers plays a crucial role in the prognosis (10). Cytokeratins (CKs), which are expressed as intermediate filaments consisting of 20 subtypes, primarily in epithelial cells, are an important part of the skeletal system, play a role in stabilizing the cell nucleus and maintaining cell morphology, and their first expression is primarily determined by the type of epithelial cell and the degree of differentiation (11). Different profiles of CKs are expressed in cancerous tissues (carcinomas) caused by epithelium. Today, researchers are focusing on the relationship between CKs and tumor malignancy, grade, and prognosis (12). Cytokeratin 20 (CK20) is a 46 kDa filamentous protein from mature enterocytes and goblet cells in the intestinal tract, urothelium, and Merkel cells in the skin (13, 14). CK20 immunostaining is strong in colorectal adenocarcinoma. Expression of CK20 is observed in adenocarcinomas of the colon, stomach, pancreas, and biliary system (15). CK20 expression is an independent prognostic factor of poorly differentiated adenocarcinoma (PDA) of the colon and rectum (16). Loss of CDX2 and CK20 overexpression are markers of tumor progression in mismatch repair-proficient CRC (17). Serum CK20 mRNA expression is significantly elevated in patients with CRC and could be a promising serum biomarker for CRC diagnosis with high specificity. CK20 is expressed in a higher percentage of CRC and nodal metastasis than CK7 (18). The CK7-/CK20+expression pattern is highly characteristic of CRCs, although not all CRCs show this pattern (19). The multi-marker phenotype CK20+/CK7− is observed in CRC in approximately 75–95% of cases, whereas CK7+/CK20− are observed in only 5–25% of cases (20-22). Early detection of Colorectal cancer and identification of factors affecting its prognosis play an important role in treatment strategy. One of the cases, which today emphasizes its role in the prognosis of gastrointestinal cancer is using the immunohistochemistry technique to identify various molecules, including tumor markers. Therefore, on this basis and following various reports on the level of CK20 expression and a few conflicting studies on the relationship between CK20 expression and various clinicopathological factors (22, 23), we aimed to determine the frequency of CK20 expression and its relationship to determine some of the prognostically effective factors including tumor grade, lymphovascular invasion, perineural invasion, and lymph node involvement.

MATERIAL AND METHODS

Case study, sample collection, and IHC test

In this cross-sectional study with descriptive and analytical aspects, 50 colorectal adenocarcinoma specimens were collected between 2019 and 2020 from the archives of the Department of Pathology, Imam Khomeini Hospital, Ahvaz, after approval by the Ethics Committee of Ahvaz University of Medical Sciences (IR.AJUMS.HGOLESTAN.REC.1400.039). Study eligibility criteria included pathologist confirmation of colorectal adenocarcinoma, completeness of medical records, adequate tissue volume, absence of necrosis

or bleeding, and availability of invasive tumor tissue and lymph nodes. The demographic and clinical characteristics of each sample, including the patient's age, sex, the tumor site, the number of involved lymph nodes, and the histological grade of the tumor, were extracted from the patients' chart and recorded in a checklist. The 5-µm paraffinized sections were soaked in a water-alcohol solution for 5 minutes. The slides were microwaved for 30 minutes at 60°C. Deparaffinization was accomplished by soaking the slides in xylene and alcohol (concentrations ranging from 100% to 75% for 5 to 10 minutes). The sections were rinsed with 10% phosphate-buffered saline (PBS) for 10 minutes, then with H_2O_2/methanol (1:9) and 10% PBS. The slides were then microwaved in ethylenediaminetetraacetic acid for 10 minutes. After allowing the samples to reach room temperature, they were rinsed with phosphate-buffered saline. Sections were incubated for 1 hour at room temperature with 1 g/mL diluted CK20 monoclonal antibody (Dako Cytomation Norden A/S, Glostrup, Denmark, dilution 1:100 each) and then reincubated with biotinylated CK20 monoclonal antibody.

CK20 expression was determined using a light microscope at 400X magnification. The total number of stained cells and the total number of malignant cells were counted, and the percentage obtained by dividing these two was regarded.

Less than 5% of the cells are stained (negative)

Between 5-50% of the cells are stained (focal positive)

More than 51% of the cells are stained (diffuse positive)

CK20 tumor marker staining value above 5% is considered positive and values below 5% are considered negative.

Statistical analysis

For quantitative variables, frequency, percentage, and descriptive statistics, such as mean index and standard deviation, were used. For qualitative variables, frequency, percentage, and descriptive statistics such as mean index and standard deviation were employed. The data were analyzed using Chi-Square, t-test, Spearman, and other related tests in the analytical section. The significance threshold was set at $P<0.05$, and all analyses were carried out using SPSS software version 25.

RESULTS

The present study included 50 patients with colorectal adenocarcinoma, including 23 (46%) men and 27 (54%) women, with a mean age of 50±11.2 years. Most patients (42%) were between 51 and 60 years old. Regarding clinicopathological characteristics such as tumor grade, most patients [28 (56%)] had a moderately differentiated grade (Figure 1). The tumor was located in the colon in 14 (28%) patients, in the sigmoid in 19 (38%) patients, and in the rectum in 17 (34%) patients. In addition, lymphovascular invasion, perineural invasion, and lymph node involvement were reported in 21 (42%), 19 (3%), and 28 (56%) patients, respectively. Immunohistochemical expression of the CK20 marker was negative in 15 patients (30%), but 16 patients (32%) and 19 patients (38%) had focal and diffuse positive expression of this marker, respectively. In other words, the expression of this marker was positive in 35 patients (70%, tables 1 & 2). Based on the results of the present

Table 1. Demographic and clinical information of the patients

		Frequency (percent)
Sex	Male	23 (46)
	Female	27 (54)
Tumor site	colon	14 (28)
	sigmoid	19 (38)
	rectum	17 (34)
Tumor grade	well-differentiated	14 (28)
	moderately differentiated	28 (56)
	poorly differentiated	8 (16)
Lymphovascular invasion	No	29 (58)
	Yes	21 (42)
Perineural invasion	No	31 (62)
	Yes	19 (38)
Lymph node involvement	No	22 (44)
	Yes	28 (56)

Table 2. Immunohistochemical expression of CK20 in samples

		Frequency (percent)
CK 20 expression status	Negative	15 (30)
	Focal positive	16 (32)
	Diffuse positive	19 (38)

study, there was no statistically significant association between demographic information, including age, sex, and tumor site, with CK20 immunohistochemical marker expression (P>0.05). On the other hand, a significant correlation between clinicopathological features such as tumor grade, lymphovascular invasion, perineural invasion, and lymph node involvement with the expression of the CK20 marker was reported (P<0.05, Table 3).

DISCUSSION

The current study delved into the relationship between CK20 expression and clinicopathological characteristics in a cohort of 50 patients diagnosed with colorectal adenocarcinoma. The results unveil a multifaceted landscape where CK20 expression intertwines with tumor characteristics, shedding light on potential implications for diagnosis and prognosis.

CK20 is a protein that is commonly used as a marker

Table 3. Correlation between demographic and clinical information patients with immunohistochemical expression of CK20

		CK 20 expression status			*P* value
		Negative	Focal positive	Diffuse positive	
Age	<= 50	3 (23.1%)	5 (38.5%)	5 (38.5%)	**0.495**
	51-60	9 (42.9%)	6 (28.6%)	6 (28.6%)	
	61-72	3 (18.8%)	5 (31.3%)	8 (50%)	
Sex	Female	9 (33.3%)	7 (26%)	11 (40.7%)	**0.603**
	Male	6 (26.1%)	9 (39.1%)	8 (34.8%)	
Tumor site	Colon	4 (28.5%)	5 (35.7%)	5 (35.7%)	**0.99**
	Sigmoid	5 (26.3%)	6 (31.5%)	8 (42.1%)	
	Rectum	6 (35.3%)	5 (29.4%)	6 (35.3%)	
Tumor grade	Well-differentiated	2 (14.3%)	0 (0%)	12 (85.7%)	**<0.001**
	Moderately differentiated	8 (28.6%)	13 (46.4%)	7 (25%)	
	Poorly differentiated	5 (62.5%)	3 (37.5%)	0 (0%)	
Lymphovascular invasion	No	3 (10.3%)	11 (37.9%)	15 (51.7%)	**0.001**
	Yes	12 (57.1%)	5 (23.8%)	4 (19%)	
Perineural invasion	No	5 (16.1%)	10 (32.3%)	16 (51.6%)	**0.010**
	Yes	10 (52.6%)	6 (31.6%)	3 (15.8%)	
Lymph node involvement	No	0 (0%)	10 (45.5%)	12 (54.5%)	**<0.001**
	Yes	15 (53.6%)	6 (21.4%)	7 (25%)	

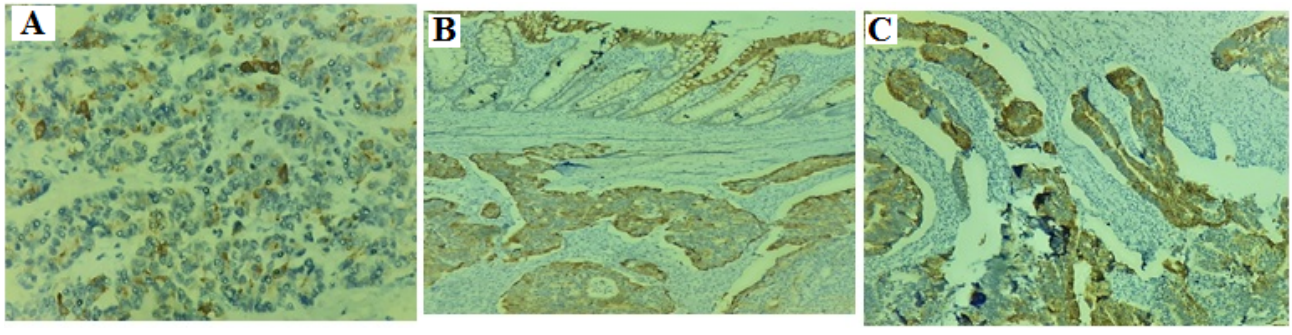

Figure 1. Representation of CK20 immunohistochemical in colorectal adenocarcinoma. From the above left: (A) poorly differentiated with negative staining for CK20 expression; (B) moderately differentiated with focal positive staining for CK20 expression; (C) well-differentiated with diffuse positive staining for CK20 expression

to identify certain types of epithelial cells, including those found in the gastrointestinal tract. It is especially relevant in the context of colorectal carcinoma, where its expression can provide insights into the clinicopathological characteristics of the cancer (24). CK expression as intermediate filaments in certain epithelial neoplasms has been used to identify the origin of metastatic tumors (25). Among the CKs used are CK7 and CK20. CK20 expression is mainly restricted to the colonic epithelium, urothelium, and Merkel cells and neoplasms resulting from these epithelia and cells (26), while expression of CK7 occurs in glandular epithelium and epithelial tumors of the lung, ovaries, endometrium, and breast. It is not observed in the gastrointestinal (GI) epithelium. The differential expression of two CKs 7 and 20 plays a role in the differentiation of primary and metastatic carcinomas. Colorectal epithelial tumors show the CK7-/CK20+pattern in 95% of cases in various studies. This percentage of CK20 positivity varies across studies, and few studies have discussed the relationship between CK20 expression and clinicopathological features (27). For this reason, the present study was performed to investigate the expression of CK20 and its relation to the clinicopathological features of colorectal adenocarcinoma. In this study, positive expression of CK20 was reported in 35 samples (70%) out of 50 samples. In the 2012 study by Bayrak and others, out of 118 colorectal adenocarcinoma samples, CK20 was positive in 99 samples (84%). Also, in another study by Bayrak and colleagues (28), CK20 positivity was observed in 159/196 (81.1%) of colorectal adenocarcinoma samples (15). The study by Gheini and colleagues showed that CK 20 expression was positive in (47/52) 90.38% of the samples. In two studies by Chu and Saad, CK20 positivity was reported in 95% and 89% of colorectal adenocarcinoma specimens (13, 29), respectively. In CRC, the expression of CK20 can provide valuable information about the tumor's origin and characteristics. The expression of CK20 in CRC can be influenced by several factors: such as tumor differentiation, tumor stage, and tumor origin. CK20 is typically expressed in cells of the intestinal epithelium. Therefore, tumors originating from the colon and rectum are more likely to express CK20 compared with tumors from other organs. In this study, the degree of tumor (poorly differentiated) had a significant effect on the expression of CK20 marker and it can be the reason for the decrease of CK20 expression in this study compared with other studies (30-32). However, in this study, most samples (19/50, 38%) showed diffuse positive expression of CK20. On the other hand, In the study by Reyhan Bayrak and colleagues, most samples (63/99, 64%) had (diffuse positive) CK20 expression staining, consistent with the results of the present study (28). The present study investigated the relationship between the expression pattern of CK20 and tumor grade, lymphovascular invasion, perineural invasion, and lymph node involvement in colorectal adenocarcinoma. Based on the results of the present study, the highest frequency of positive expression was at a well differentiated grade, followed by a moderately differentiated grade, and the lowest frequency was observed at a poorly differentiated grade, which was also statistically significant (Figure 1). Few studies have been conducted on the relationship between clinicopathological factors and CK20 expression. Consistent with the present study, the studies by Gheini and Bayrak showed that the highest frequency of positive expression of CK20 is found in low-grade tumors. In the present study, expression of CK20 is significantly lower in samples with lymph node invasion, perineural invasion, and lymph node involvement (15, 33). But in the study by Gheini and others, there was no difference in the expression pattern of CK20 between samples with and without lymph node involvement (33).

CONCLUSION

In general, based on the results of the present study, there is a significant association between the expression of CK20 and clinicopathological factors, so it is possible to use the CK20 marker in determining the prognosis of colorectal adenocarcinoma; however, more studies with a larger sample size will be required in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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