DOI: https://doi.org/10.2298/SARH200929055Z
UDC: 616.348/.35-006.6

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Clinical significance of proliferation index and E-cadherin expression in colorectal adenocarcinoma

Janko Žujović[1], Milena Vuletić[2], Miroslav Stojanović[3], Ranko Lazović[4], Nebojša Đorđević[3], Miodrag Radunović[4], Snežana Jančić[2], Velimir Milošević[5]

[1]Clinical Centre of Montenegro, Centre for Abdominal Surgery, Podgorica, Montenegro;
[2]University of Kragujevac, Faculty of Medical Sciences, Department of Pathology, Kragujevac, Serbia;
[3]University of Niš, Faculty of Medicine, Niš, Serbia;
[4]University of Montenegro, Faculty of Medicine, Podgorica, Montenegro;
[5]Codra Hospital, Podgorica, Montenegro

SUMMARY

Introduction/Objective The aim of this study is to examine the association of E-cadherin expression and high proliferation index (proIDX) with clinical and pathological indicators of colorectal cancer progression.

Methods The biopsy of 72 patients, obtained by resection of colorectal cancer, was routinely processed at the Institute of Pathology of the Clinical Centre of Montenegro, embedded in paraffin and archived. Based on the archived pathohistological reports, two study groups were formed: the first group (n = 72) consisted of operative biopsies of colorectal cancer, and the control group (n = 72) consisted of biopsies of adjacent non-tumor tissue. Routine hematoxylin-eosin and immunohistochemical avidin-biotin-peroxidase complex method with anti-Ki67 and anti-E-cadherin antibodies was applied on. After quantification of the results for statistical tests, the software package SPSS for Windows (19.0) was used.

Results In colorectal carcinoma, we observed a significant association of proIDX with pT stage, lymph and blood vessel invasion, perineural invasion, lymph node metastases and distant metastases, and Astler–Coller stage tumor disease. We also observed that the absence of E-cadherin was significantly associated with pT stage, lymph and blood vessel invasion, perineural invasion with lymph node metastases, distant metastases, with C2 and D Astler–Coller tumor stage. E-cadherin expression is associated with proIDX with a significantly high, negative correlation coefficient.

Conclusion Our results indicate that it is possible to differentiate patients into groups with a higher or lower risk of developing metastatic disease, based on the expression of Ki67 and E-cadherin.

Keywords: colorectal cancer; E-cadherin expression; proliferative index; clinical significance

INTRODUCTION

Colorectal cancer is a complex disease caused by the interaction of genetic, epigenetic and environmental factors. Colorectal carcinogenesis is a consequence of the "interplay" between environmental factors on the one side and different oncogenes, suppressor genes and their products on the other, where cell proliferation is one of the most significant biological events in that process [1].

A nuclear antigen is isolated from the proliferating cells, which was used to produce a monoclonal Ki67 antibody of IgG1 class. The antigen detected by the Ki67 antibody is present in the cell nuclei during the G_1, S, G_2, and M phases on the cell cycle. It cannot be detected in the G_0 phase [2].

Cadherin adhesion molecules also play an important role in colorectal carcinogenesis [3]. Cadherins are a family of glycoproteins that perform calcium (Ca_2+) dependent intercellular adhesion at intercellular junctions [4]. Cadherins participate in the processes of embryogenesis and they are involved in the malignant transformation of cells, where a significant reduction of their expression occurs. It has been proved that cadherins inhibit invasion and it is hypothesized that they activate the process of malignant cell dissemination [5, 6]. E-cadherin plays a key role in establishing epithelial architecture, in differentiation and in maintaining cell polarity [4]. Numerous studies have shown that E-cadherin is a suppressor of tumor invasion and metastasis [6, 7].

Considering the fact that the loss of E-cadherin molecules results in a disruption of cytoskeletal structure, which allows cell separation from tumor and increased cell migration [6], this study aims is to examine the correlation between E-cadherin expression and proliferation index (proIDX) on the one side, and clinical and pathological indicators of colorectal cancer progression on the other.

METHODS

Patients and samples

The biopsies and the operative material of 72 patients were obtained by resection of colorectal tumor at the Centre for Abdominal Surgery of the Clinical Centre of Montenegro (CCM)

Received • Примљено: September 29, 2020
Revised • Ревизија: June 15, 2021
Accepted • Прихваћено: June 16, 2021
Online first: June 30, 2021

Correspondence to:
Janko ŽUJOVIĆ
Clinical Centre of Montenegro,
Centre for Abdominal Surgery,
Podgorica, Montenegro,
Ljubljanska bb, Podgorica 81101,
Montenegro
janko.zujovic@kccg.me

Table 1. Clinicopathological features of colorectal carcinoma studied

Clinicopathological factors	Number of cases (%)	Age (min/max/$\bar{X}$)
Males Females	42 (58.33) 30 (52.77)	34/83/66.1 ± 10.6 27/83/61.4 ± 14.2
Localization	**Number of cases (%)**	
Cecum (+ileocecum) / Ascendant colon / Transverse colon / Sigma (+descendant colon) / Rectum (+anorectum + rectosigma)	3 (4.2) / 11 (15.3) / 1 (1.4) / 9 (12.5) / 48 (66.7)	
Macroscopic appearance of the tumor	**Number of cases (%)**	
Ulceroinfiltrative/Infiltrative/Vegetatively-infiltrative/Vegetative/Ulcerative	27 (37.5) / 25 (34.7) / 16 (22.2) / 3 (4.2) / 1 (1.4)	
Histological grade	**Number of cases (%)**	
Grade I / Grade II / Grade III	4 (5.6) / 63 (87.5) / 5 (6.9)	
Invasion of lymphatic vessels	**Number of cases (%)**	
Not identified/Present	33 (45.8) / 39 (54.2)	
Invasion of blood vessels	**Number of cases (%)**	
Not identified / Present	61 (84.7) / 11 (15.3)	
Nerve invasion	**Number of cases (%)**	
Not identified/Present	59 (81.9) / 13 (18.1)	
Pathological stage of the tumor(pT)	**Number of cases (%)**	
pT_1 / pT_3 / pT_4	2 (2.8) /54 (75.0) / 4 (5.6)	
Lymph node metastases	**Number of cases (%)**	
No deposits/Deposits in 1–3 lymph nodes/Deposits in 4–6 lymph nodes/ Deposits in seven and more lymph nodes	35 (48.6) / 19 (26.4) / 8 (11.1) / 10 (13.9)	
Distant metastases	**Number of cases (%)**	
Not identified/Present	64 (88.9) / 8 (11.1)	
Astler–Coller stage of tumor	**Number of cases (%)**	
B1/B2/C1/C2/D	11 (15.3) / 21 (29.2) / 3 (4.2) / 30 (41.7) / 7 (9.7)	

between January 2010 and December 2012. At the Institute of Pathology CCM, 5–15 tissue samples were obtained from each surgery, according to the established protocol, depending on the size of the tumor, including 2–3 biopsies of the adjacent, non-tumorous colorectal tissue. After fixation the bioptic material was routinely processed, embedded in paraffin and archived. Based on the standard pathological reports from that period, an experimental group was formed that consisted of operative biopsies of colorectal adenocarcinoma (n = 72). The control group (n = 72) consisted of operative biopsies of the adjacent non-tumorous colorectal tissue (epithelial cells), from the same patients in the experimental group. The study protocol was approved by the local ethics committee.

Histopathology and immunohistochemistry

On paraffin blocks, where samples of tumor tissue and regional lymph nodes were embedded, 3–5 μm thick cuts were made. For histopathological verification of lesions, the routine hematoxylin-eosin (HE) method was applied.

Representative samples of tumor and adjacent non-tumor tissue were used for immunohistochemical analysis. Immunostaining was performed using the avidin-biotin-peroxidase complex (ABC) method (Vectastain ABC Elite-kit, Vector Laboratories, Burlingame, CA, USA), with mouse monoclonal anti-E-cadherin antibody (DAKO, Denmark, clone NCH-38, 1:50) and rabbit monoclonal anti-Ki67 antibody (Abcam, Burlingame, CA, USA, clone SP6, 1:100).

Expression of E-cadherin in carcinoma cells was measured in 10 fields of microscopic magnification 400× (mean value represents the final result for the case) and classified in the following manner: Intramembranous immunohistochemical reaction with < 10% of immunoreactive cells was considered a negative immunoreaction (−), and the presence of intramembrane expression in ≥ 10% was evaluated as a positive reaction; an immunohistochemical reaction present in < 50% of cells was classified as moderate expression (1+); a positive immunohistochemical reaction present in > 50% of cells was classified as pronounced expression (2+) of E-cadherin.

To evaluate the expression of Ki67+ cells per mm^2 by area, test system M42 according to Weibel was used. Objective micrometer calibrated the test system on a Nikon Eclipse Ni MBP 99 400 microscope at a magnification of 400, with a measurement field of 0.016 mm^2. To test the density of Ki67+ cells per mm^2, 10 "hot-spots" were counted successively. The absolute value of the density of positive cells in the "hot-spot" was determined stereometrically [8]. The means of the obtained values of the "hot-spots" represents the final number of Ki67+ cells in mm^2 per case. The median was subsequently determined and the absolute values of the density of positive cells were divided into two groups: those with the low level of expression (the value ≤ the median value) and those with the high level of expression (values>the median value). From absolute determined values of Ki67 regarding deviation from median Ki67 index (proIDX) was obtained.

Statistical analysis

The statistical software package SPSS for Windows, Version 19.0 (IBM Corp., Armonk, NY, USA) was used. To analyze the statistical significance of parametric and nonparametric

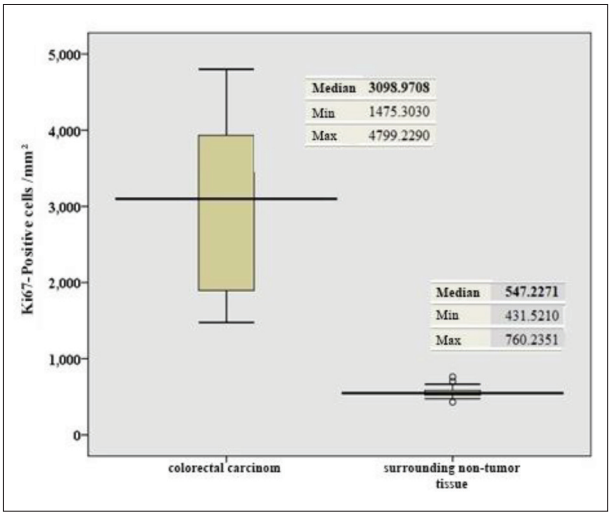

Figure 1. Distribution of Ki67–immunopositive cells in mm² of examined tissue

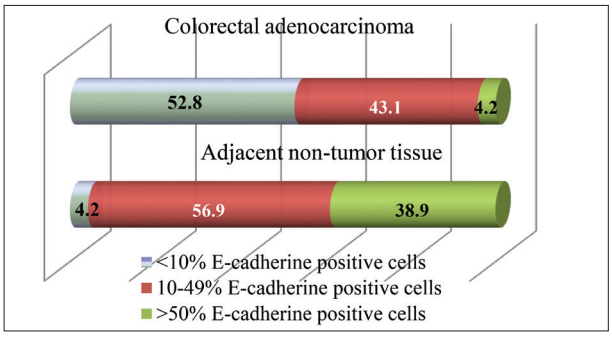

Figure 2. E-cadherin expression in colorectal cancer and adjacent non-tumor tissue

features, between and within the groups, χ^2-test, the Kruskal–Wallis test, Fisher exact test and Student's t-test were used. Afterwards, the Kolmogorov–Smirnov normality test, correlation analysis (Spearman's rank correlation coefficient).

P values less than 0.05 were considered statistically significant.

RESULTS

This study included 72 patients with colorectal adenocarcinoma (42 men, mean age 66.1 ± 10.6 years, range 34–83, and 30 women, mean age 61.4 ± 14.2 years, range 27–83). The main clinical and pathological characteristics of colorectal adenocarcinoma are shown in Table 1.

Immunohistochemical expression of Ki67 and E-cadherin in colorectal adenocarcinoma and adjacent non-tumor tissue

Immunohistochemical analysis of the Ki67 expression, gave absolute values of the number of immunopositive cells in mm² of tissue. Ki67 expression reported by the number of immunopositive cells in mm² (median = 3098.9708; min = 1475.3, max = 4799.2) in colorectal carcinoma tissue, was significantly higher compared to the control group-adjacent, non-tumor tissue (median = 547.227; min = 431.5, max = 760.2; $p < 0.001$) (Figure 1).

Immunohistochemical analysis of E-cadherin expression showed that there is a statistically significant difference in the expression of this marker between colorectal carcinoma tissue and adjacent non-tumor tissue. Namely, in colorectal adenocarcinoma, the absence of E-cadherin expression was found in a significant 52.8% of cases ($p < 0.001$). It is also observed that the expression of E-cadherin is significantly increased (38.9%, $p < 0.001$) in the adjacent non-tumor tissue compared to the colorectal carcinoma, where only 4.2% of cases expressed E-cadherin (Figure 2).

Association and correlation analysis between Ki67 expression/proliferative index (proIDX) and clinical and pathological characteristics of colorectal cancer (Table 2)

There was a significant correlation of proliferation index (proIDX) with the pT stage of the tumor because in 100% of cases there was the correlation between low pro IDX and pT1 and in 91.7% of cases between low proIDX and the pT2 stage of the tumor. This correlation is defined by a significant, positive correlation coefficient ($r = 0.352$, $p = 0.002$).

High proIDX was associated with lymphatic invasion in 82.1% of cases, while in 87.9% of cases low proIDX was associated with tumors in which lymphatic vessel invasion was not identified. Pro IDX was associated with lymph vessel invasion with a significant, positive and high correlation coefficient ($r = 0.697$, $p = 0.000$).

High proIDX was significantly associated with vascular invasion in 90.9% of cases, while in 92.3% of cases with neural invasion, there were lower but significant and positive correlation coefficients ($r = 0.347$ and $r = 0.397$, $p = 0.003$ and $p = 0.001$)

There was a significant correlation between high proIDX and lymph node metastases. High proIDX was significantly associated with metastases in 1–3 lymph nodes in 84.2%, with metastases of 4–6 lymph nodes in 100% and with metastases of seven and more lymph nodes in 100% of cases. It is also noteworthy that low proIDX was associated with the absence of lymph node metastases in 94.3% of cases. Lymph node metastases were highly, positively, and significantly correlated with proIDX ($r = 0.766$, $p = 0.000$).

High proliferation index (Figure 3) was in 100% of cases associated with distant metastases with a significant and positive correlation coefficient ($r = 0.354$, $p = 0.002$).

ProIDX was significantly associated with Astler–Coller stage of tumor disease. Low proIDX was significantly associated with stage B1 in 90.9% and stage B2 in 100% of cases. At the same time, high proIDX was significantly associated with stage C2 in 90% and with stage D in 100% of cases. This correlation was defined by a highly significant and high correlation coefficient ($r = 0.818$, $p = 0.000$).

In this study, no significant correlation was found between the proIDX/Ki67 expression and subjects' gender,

Table 2. Association of Ki67 and E-cadherin expression with clinico-pathological parameters

Type of tumor		Colorectal carcinoma				
Markers		Ki67		E-cadherin		
Tested parameters		**low**	**high**	**-**	**+**	**++**
		n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Sex	Male	21 (50)	21 (50)	22 (52.4)	20 (47.6)	0 (0)
	Female	15 (50)	15 (50)	16 (53.3)	11 (36.7)	3 (10)
Significance	p	/		**0.096**		
Age of the subjects	≤ 65	14 (42.4)	19 (57.6)	18 (54.5)	14 (42.4)	1 (3)
	> 65	22 (56.4)	17 (43.6)	20 (51.3)	14 (43.6)	2 (5.1)
Significance	p	**0.237**		**0.891**		
Localization	Rectum (anorectum + rectosygma)	23 (47.9)	25 (52.1)	26 (54.2)	19 (39.6)	3 (6.2)
	Sygma (+sin. colon)	7 (77.8)	2 (22.2)	4 (44.4)	5 (55.6)	0 (0)
	Colon ascendens	5 (45.5)	6 (54.5)	5 (45.5)	6 (54.5)	0 (0)
	Transverse colon	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)
	Cecum(ileocecum)	1 (33.3)	2 (66.6)	2 (66.7)	1 (33.3)	0 (0)
Significance	p	**0.369**		**0.893**		
Tumor growth pattern	Ulceroinfiltrative	12 (44.4)	15 (55.6)	16 (59.3)	10 (37)	1 (3.7)
	Infiltrative	13 (52)	12 (48)	11 (44)	13 (52)	1 (4)
	Vegetative	3 (100)	0 (0)	0 (0)	2 (66.7)	1 (33.3)
	Vegetatively-infiltrative	7 (43.8)	9 (56.2)	11 (68.8)	5 (31.2)	0 (0)
	Ulcerovegetative	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)
Significance	p	**0.328**		**0.124**		
Histological grade of the tumor	Grade I	3 (75)	1 (25)	2 (50)	2 (50)	0 (0)
	Grade II	32 (50.8)	31 (49.2)	31 (49.2)	29 (46)	3 (4.8)
	Grade III	1 (20)	4 (80)	5 (100)	0 (0)	0 (0)
Significance	p	**0.245**		**0.285**		
Lymphatic invasion	Not identified	**29 (87.9)**	4 (12.1)	4 (12.1)	**26 (78.8)**	3 (9.1)
	Lymphatic invasion present	7 (17.9)	**32 (82.1)**	**34 (87.2)**	5 (12.8)	0 (0)
Significance	p	**< 0.001***		**< 0.001***		
Blood vessels invasion	Not identified	35 (57.4)	26 (42.6)	28 (45.9)	30 (49.2)	3 (4.9)
	Blood vessels invasion present	1 (9.1)	**10 (90.9)**	**10 (90.9)**	1 (9.1)	0 (0)
Significance	p	**0.003***		**0.022***		
Nerve invasion	Not identified	35 (59.3)	24 (40.7)	26 (44.1)	31 (52.5)	2 (3.4)
	Nerve invasion present	1 (7.7)	**12 (92.3)**	**12 (92.3)**	0 (0)	1 (7.7)
Significance	p	**< 0.001**		**0.020***		
Lymph node metastases	No deposit	**33 (94.3)**	2 (5.7)	2 (5.7)	**30 (85.7)**	3 (8.6)
	Deposits in 1–3 LN	3 (15.8)	**16 (84.2)**	**18 (94.7)**	1 (5.3)	0 (0)
	Deposits in 4–6 LN	0 (0)	**8 (100)**	**8 (100)**	0 (0)	0 (0)
	Deposits in > 7 LN	0 (0)	**10 (100)**	**10 (100)**	0 (0)	0 (0)
Significance	p	**< 0.001***		**< 0.001***		
Distant metastases	Not identified	36 (56.2)	28 (43.8)	30 (46.9)	31 (48.4)	3 (4.7)
	Metastases present	0 (0)	**8 (100)**	**8 (100)**	0 (0)	0 (0)
Significance	p	**0.003***		**0.018***		
Pathological stage of the tumor	pT_1	**2 (100)**	0 (0)	0 (0)	**2 (100)**	0 (0)
	pT_2	**11 (91.7)**	1 (8.3)	0 (0)	**10 (83.3)**	2 (16.7)
	pT_3	21 (38.9)	33 (61.1)	**34 (63)**	19 (35.2)	1 (1.9)
	pT_4	2 (50)	2 (50)	4 (100)	0 (0)	0 (0)
Significance	p	**0.005***		**< 0.001***		
Astler–Coller stage of tumor	B1	**10 (90.9)**	1 (9.10)	**0 (0)**	**9 (81.8)**	2 (18.2)
	B2	**21 (100)**	0 (0)	1 (4.8)	**19 (90.5)**	1 (4.8)
	C1	2 (66.7)	1 (33.3)	0 (0)	3 (100)	0 (0)
	C2	3 (10)	**27 (90)**	**30 (100)**	0 (0)	0 (0)
	D	0 (0)	**7 (100)**	7 (100)	0 (0)	0 (0)
Significance	p	**< 0.001***		**< 0.001***		

*significant difference $p < 0.05$, χ^2 test, Fisher's exact test

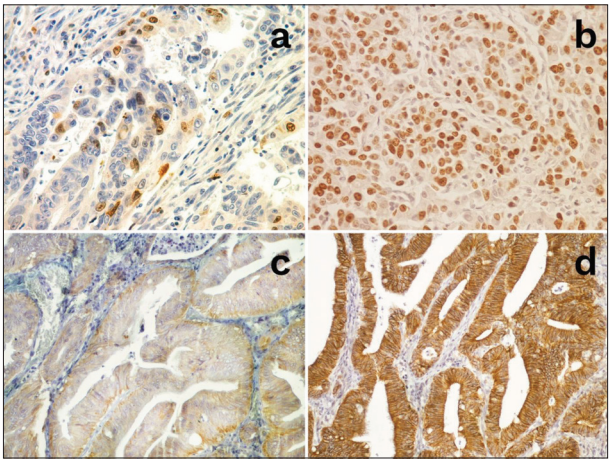

Figure 3. Immunohistochemical expression of Ki67 and E-cadherin in colorectal adenocarcinoma; a – low Ki67 expression index; b – high Ki67 expression index; c – lack of expression and expression of trace E-cadherin; d – pronounced E-catherin expression (ABC × 400)

age, localization of the tumor in the colorectum and the histological grade of the tumor ($p > 0.05$).

Association and correlation analysis between E-cadherin expression and clinical and pathological characteristics of colorectal cancer (Table 2)

A statistically significant correlation between E-cadherin expression and pT stage of the tumor was observed. In 63% of cases, there was a correlation between absent E-cadherin expression and pT3 stage of the tumor ($p = 0.039$). There was also a significant correlation between moderate E-cadherin expression and pT1 and pT2 stages ($p = 0.021$). This correlation was defined by a significant, inverse, moderate correlation coefficient ($r = -0.515$).

There was a statistically significant correlation between E-cadherin expression and lymphatic vessel invasion in colorectal cancer. Namely, the absence of E-cadherin expression was associated with lymphatic vessel invasion in 87.2% of cases. In cases where lymph vessel invasion was not identified, there was a moderate expression of E-cadherin in 78.8% of cases. E-cadherin expression was also associated with lymph vessel invasion with a high negative and significant correlation coefficient ($r = -0.726$, $p = 0.000$).

The expression of E-cadherin was associated with the invasion of blood vessels with a significant, negative and weak correlation coefficient ($r = -0.311$, $p = 0.008$), so that in 90.9% of cases, the absence of E-cadherin expression was associated with vascular invasion.

The absence of E-cadherin expression was significantly associated with nerve invasion in 92.3% of cases. Neural invasion and E-cadherin expression were associated with weak, significant negative correlation coefficient ($r = -0.293$, $p = 0.013$).

In a significant number of cases, the absence of E-cadherin expression was associated with lymph node metastases. In 94.7% of cases, there was an absence of E-cadherin expression associated with metastases in 1–3 lymph nodes, in 100% of cases with metastases in 4–6 lymph nodes, as well as in 100% of cases with metastases in seven or more lymph nodes. In lymph nodes, where no metastatic deposits were identified, moderate expression of E-cadherin was present in a significant number of cases (85.7%, $p < 0.001$). Lymph node metastases and E-cadherin expression were associated with a significant, high but negative correlation coefficient ($r = -0.729$, $p = 0.000$).

In 100% of cases, there was a significant correlation between the absence of E-cadherin expression and distant metastases.

There was a statistically significant correlation between E-cadherin expression and the Astler–Coller stage of tumor disease. Moderate expression of E-cadherin was associated with B1 and B2 Astler–Coller stage in a significantly higher number of cases (81.2% and 90.5%, $p = 0.021$). Absence of E-cadherin expression was significantly associated with C2 and D Astler–Coller stage tumors in 100% of cases ($p < 0.001$). E-cadherin expression was associated with the Astler–Colller stage of tumor disease with a very high, negative correlation coefficient ($r = -0.875$, $p = 0.000$).

In this study, there was also a highly significant correlation between E-cadherin expression and proIDX defined by a high, negative correlation coefficient ($r = -0.794$, $p = 0.000$).

No statistical significance was observed between E-cadherin expression and subjects' sex, age, tumor localization and histological grade of colorectal cancer ($p > 0.05$).

DISCUSSION

Numerous studies have observed the association of cell proliferation with the aggressive biological behavior of tumors of different localization [9–12], while the expression of Ki67 protein has been identified as a good predictor of recurrence and as an independent prognostic factor of survival rate [12, 13].

The expression of Ki67 protein during the cell cycle is strictly regulated by the balance between synthesis and degradation, which means that its half-life is short and is only 60–90 minutes long. During the cell cycle Ki67 becomes detectable in the mid-late G_1 phase when levels are low, and then its expression increases through the S and G_2 phases, and peaks in the early M phase. In the late stages of mitosis, the expression of Ki67 falls sharply and then disappears [14].

In this study, we observed that low proIDX has a good correlation coefficient significantly associated with pT_1 and pT_2 stages, while high proIDX is associated with significant and high correlation coefficients to lymph vessel invasion, blood vessel invasion, neural invasion, lymph node metastases and distant metastases. In a recent study by Tong et al. [15], a sample of 1,090 subjects with colorectal cancer observed a significant association of Ki67 expression with pT stage, histological grade, AJCC stage, and lymph node metastases.

The proliferation index calculated in our study indicates the existence of a very strong relationship between Ki67

Table 3. Correlation matrix of clinical-pathological parameters and expression of colorectal adenocarcinoma markers

		Sex	Age	Localization.	pT stage	Histol. Grade	AC Stage	Growth pattern	Lymph vessel invas.	Blood vessel invas.	Neural invasion	Lymph node metast.	Distant metastases	Ki67	E-cadherin
Sex	r	1.000	0.188	-0.058	0.102	-0.113	0.034	-0.204	-0.071	-0.202	0.043	0.024	-0.030	0.000	0.077
	p	/	0.113	0.630	0.394	0.345	0.776	0.086	0.555	0.088	0.722	0.840	0.803	1.000	0.519
Age	r	0.188	1.000	0.099	-0.141	0.012	-0.025	-0.057	-0.029	0.068	-0.046	0.021	0.072	-0.031	0.015
	p	0.113		0.408	0.238	0.917	0.833	0.635	0.807	0.569	0.700	0.860	0.546	0.798	0.904
Localization	r	0.058	0.099	1.000	0.158	0.088	0.104	-0.155	0.160	-0.037	-0.079	0.141	0.037	0.053	-0.060
	p	0.630	0.408		0.184	0.464	0.384	0.193	0.179	0.754	0.511	0.238	0.757	0.661	0.617
pT stage	r	0.102	0.141	0.158	1.000	0.154	**0.638**	-0.131	**0.429**	0.128	0.142	**0.372**	0.187	**0.352**	**-0.515**
	p	0.394	0.238	0.184		0.196	**0.000**	0.274	**0.000**	0.284	0.236	**0.001**	0.117	**0.002**	**0.000**
Histol. Grade	r	0.113	0.012	0.088	0.154	1.000	0.120	-0.104	0.115	-0.017	0.084	0.224	-0.014	0.197	-0.171
	p	0.345	0.917	0.464	0.196		0.316	0.382	0.336	0.889	0.484	0.058	0.908	0.098	0.150
AC stage	r	0.034	0.025	0.104	**0.638**	0.120	1.000	-0.082	**0.756**	**0.468**	**0.437**	**0.726**	**0.503**	**0.818**	**-0.875**
	p	0.776	0.833	0.384	**0.000**	0.316		0.494	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Growth pattern	r	0.204	0.057	-0.155	-0.131	-0.104	-0.082	1.000	0.071	-0.120	0.061	-0.153	-0.083	0.059	-0.013
	p	0.086	0.635	0.193	0.274	0.382	0.494		0.551	0.316	0.609	0.201	0.489	0.625	0.912
Lymph vessel invasion	r	0.071	0.029	0.160	**0.429**	0.115	**0.756**	0.071	1.000	**0.391**	**0.359**	**0.698**	**0.325**	**0.697**	**-0.726**
	p	0.555	0.807	0.179	**0.000**	0.336	**0.000**	0.551		**0.001**	**0.002**	**0.000**	**0.005**	**0.000**	**0.000**
Blood vessel invasion	r	0.202	0.068	-0.037	0.128	-0.017	**0.468**	-0.120	**0.391**	1.000	**0.403**	**0.255**	**0.710**	**0.347**	**-0.311**
	p	0.088	0.569	0.754	0.284	0.889	**0.000**	0.316	**0.001**		**0.000**	**0.030**	**0.000**	**0.003**	**0.008**
Neural invasion	r	0.043	0.046	-0.079	0.142	0.084	**0.437**	0.061	**0.359**	**0.403**	1.000	**0.279**	**0.638**	**0.397**	**-0.293**
	p	0.722	0.700	0.511	0.236	0.484	**0.000**	0.609	**0.002**	**0.000**		**0.018**	**0.000**	**0.001**	**0.013**
Lymph node metastases	r	0.024	0.021	0.141	**0.372**	0.224	**0.726**	-0.153	**0.698**	**0.255**	**0.279**	1.000	0.156	**0.766**	**-0.729**
	p	0.840	0.860	0.238	**0.001**	0.058	**0.000**	0.201	**0.000**	**0.030**	**0.018**		0.190	**0.000**	**0.000**
Distant metastases	r	0.030	0.072	0.037	0.187	-0.014	**0.503**	-0.083	**0.325**	**0.710**	**0.638**	0.156	1.000	**0.354**	**-0.315**
	p	0.803	0.546	0.757	0.117	0.908	**0.000**	0.489	**0.005**	**0.000**	**0.000**	0.190		**0.002**	**0.007**
Ki67	r	0.000	0.031	0.053	**0.352**	0.197	**0.818**	-0.059	**0.697**	**0.347**	**0.397**	**0.766**	**0.354**	1.000	**-0.794**
	p	1.000	0.798	0.661	**0.002**	0.098	**0.000**	0.625	**0.000**	**0.003**	**0.001**	**0.000**	**0.002**		**0.000**
E-cadherin	r	0.077	0.015	-0.060	**-0.515**	-0.171	**-0.875**	-0.013	**-0.726**	**-0.311**	**-0.293**	**-0.729**	**-0.315**	**-0.794**	1.000
	p	0.519	0.904	0.617	**0.000**	0.150	**0.000**	0.912	**0.000**	**0.008**	**0.013**	**0.000**	**0.007**	**0.000**	

*statistically significant difference $p < 0,05$; Spearman correlation coefficient

expression and Astler–Coller stage, supported by the high correlation coefficient that defined this relationship (Spearman rho = 0.818). High proIDX is significantly associated with C2 and D stages, which is consistent with the suggestion of Li et al. [16], that a high proliferative index may be a useful biomarker to aid in the assessment of disease outcome in patients with stage III and IV colorectal cancer.

In this study, no significant association was found between Ki67/proIDX expression with sex, age of the subjects, localization of the tumor in the colon and with the histological grade of colorectal cancer. However, there is an observation in the literature about a significant correlation the high expression of Ki67 with an older age of patients [17].

Invasion and metastasis of malignant tumors, in addition to the kinetics and proliferative capacity of tumor cells, include the interaction between tumor cells themselves and between tumor cells and their microenvironment in which changes in cell adhesion play an important role [3, 6].

The expression of E-cadherin on epithelial cell membranes maintains cell connections and suppresses cell invasion. Mutation of the *CDH1* gene which encodes E-cadherin results in the formation of a mutated, dysfunctional protein, which further results in decreased cell adhesion and uncontrolled growth of malignant cells [18]. Many studies have shown an association between reduced /absent E cadherin expression and progression of tumors [3, 6].

We identified a loss of E-cadherin expression in 52.8% of colorectal cancers, which is in complete agreement with the results of Kim et al. [6], who examined 689 colorectal cancers in 52% of cases to verify the loss of E-cadherin expression. Also, in agreement with other reports [3, 19], a significant difference in E-cadherin expression between tumor and adjacent non-tumor tissue was observed in our study.

As expected, the data on the expression of E-cadherin in relation to the clinical-pathological characteristics of colorectal cancer are heterogeneous and contradictory [5, 6]. In this study, we identified a significant association between reduced and/or absent E-cadherin expression with pT tumor stage, lymph and blood vessel invasion, perineural invasion, lymph node metastases, distant metastases, and Astler–Coller stage tumor disease. Other authors have observed that loss of E-cadherin expression is associated with lymph node metastases, but not with other parameters of colorectal cancer progression [6]. Kim et al. [6] found significantly lower E-cadherin expression in cases with infiltrative tumor growth, lymph node metastasis and distant metastasis.

Our results indicate the existence of a highly significant relationship between the loss of E-cadherin expression and proIDX/Ki67 expression defined by a high, negative correlation coefficient. Also, a significant inverse correlation between E-cadherin expression and proIDX was observed in other tumors as such esophageal squamous cell carcinoma [20].

In this study, we did not observe a significant association of E-cadherin expression with sex, age of the subjects, tumor localization in the colorectum, the mode of tumor growth or with the histological grade of the tumor. Kim et al. [6] reported a significant association between the loss of E-cadherin expression with infiltrative tumor growth, while Palaghia et al. [3], reported an association of negative expression of this marker with the age of subjects, with a higher prevalence in younger patients.

It has been observed that loss of E-cadherin expression stimulates Wnt, Rho GTPs and PI3K/AKT signaling ,which are thought to play an active role in the epithelial-mesenchymal transition (EMT) process [21, 22]. E-cadherin dysregulation promotes dysfunctions of these signaling pathways and affects the polarity, survival, invasion, and migration of cancer cells [4, 5, 21]. E-cadherin dysregulation occurs mainly due to somatic alterations of the *CDH1* gene, which is often an early stage in colorectal carcinogenesis. In addition to colorectal cancer, somatic mutations in the *CDH1* gene have also been identified in sporadic diffuse gastric cancers, lobular breast cancer, ovarian cancer, and prostate cancer [23].

CONCLUSION

High proliferative index/high levels of Ki67 expression and loss/reduced expression of E-cadherin are both mutually and strongly correlated with indicators of colorectal cancer progression. The proliferation index and expression of E-cadherin do not depend on sex, age, histological grade, localization and growth pattern of colorectal cancer.

Finally, this study supports the view that it is possible to differentiate patients into groups with a higher or lower risk of developing metastatic disease, based on the expression of these two biomarkers.

Conflict of interest: None declared.

REFERENCES

1. Nguyen LH, Goel A, Chung DC. Pathways of Colorectal Carcinogenesis. Gastroenterology. 2020;158(2):291–302.
2. Stojanovic D, Milenkovic SM, Mitrovic N, Stevanovic D, Jasarovic D, Matic S, et al. Clinical significance of neoangiogenesis and index of proliferation in the signet ring type of gastric adenocarcinoma. J BUON. 2018;23(4):992–1003.
3. Palaghia M, Mihai C, Lozneanu L, Ciobanu D, Trofin AM, Rotariu A, et al. E-cadherin expression in primary colorectal cancer and metastatic lymph nodes. Rom J Morphol Embryol. 2016;57(1):205–9.
4. Dobrescu AE, Basa SN, Cornianu M, Lazăr DC, Suciu SC, Tudor A, et al. Prognostic significance of E-cadherin expression in hepatocellular carcinoma: correlations with clinical features. Rom J Morphol Embryol. 2019;60(4):1243–51.
5. Mendonsa AM, Na TY, Gumbiner BM. E-cadherin in contact inhibition and cancer. Oncogene. 2018;37(35):4769–80.
6. Kim SA, Inamura K, Yamauchi M, Nishihara R, Mima K, Sukawa Y, et al. Loss of CDH1 (E-cadherin) expression is associated with infiltrative tumor growth and lymph node metastasis. Br J Cancer. 2016;114(2):199–206.

7. Na TY, Schecterson L, Mendonsa AM, Gumbiner BM. The functional activity of E-cadherin controls tumor cell metastasis at multiple steps. Proc Natl Acad Sci U S A. 2020;117(11):5931–7.
8. Slomianka L. Basic quantitative morphological methods applied to the central nervous system. J Comp Neurol. 2021;529(4):694–756.
9. Sun G, Wang S, Wang Y. Expressions of Topo IIα and Ki67 in breast cancer and its clinicopathologic features and prognosis. Pak J Med Sci. 2019;35(3):715–20.
10. Budak E, Solakoglu Kahraman D, Budak A, Yanarateş A, Inan AH, Kanmaz AG, et al. The prognostic significance of serum CA125 levels with ER, PR, P53 and Ki-67 expression in endometrial carcinomas. Ginekol Pol. 2019;90(12):675–83.
11. Imaizumi K, Suzuki T, Kojima M, Shimomura M, Sakuyama N, Tsukada Y, et al. Ki67 expression and localization of T cells after neoadjuvant therapies as reliable predictive markers in rectal cancer. Cancer Sci. 2020;111(1):23–35.
12. Pergolini I, Crippa S, Pagnanelli M, Belfiori G, Pucci A, Partelli S, et al. Prognostic impact of Ki-67 proliferative index in resectable pancreatic ductal adenocarcinoma. BJS Open. 2019;3(5):646–55.
13. Luo ZW, Zhu MG, Zhang ZQ, Ye FJ, Huang WH, Luo XZ. Increased expression of Ki-67 is a poor prognostic marker for colorectal cancer patients: a meta analysis. BMC Cancer. 2019;19(1):123.
14. Sun X, Kaufman PD. Ki-67 more than a proliferation marker. Chromosoma. 2018;127(2):175–86.
15. Tong G, Zhang G, Liu J, Zheng Z, Chen Y, Niu P, et al. Cut off of 25% for Ki67 expression is a good classification tool for prognosis in colorectal cancer in the AJCC-8stratification. Oncol Rep. 2020;43(4):1187–98.
16. Li P, Xiao ZT, Braciak TA, Ou QJ, Chen G, Oduncu FS. Association between Ki67 Index and Clinicopathological Features in Colorectal Cancer. Oncol Res Treat. 2016;39(11):696–702.
17. El Otmani I, El Agy F, El Baradai S, Bouguenouch L, Lahmidani N, El Abkari M, et al. Analysis of Molecular Pretreated Tumor Profiles as Predictive Biomarkers of Therapeutic Response and Survival Outcomes after Neoadjuvant Therapy for Rectal Cancer in Moroccan Population. Dis Markers. 2020:8459303.
18. Bogdanovic A, Despotovic J, Galun D, Bidzic N, Nikolic A, Rosic J, et al. Prognostic Significance of CDH1, FN1 and VIM for Early Recurrence in Patients with Colorectal Liver Metastasis After Liver Resection. Cancer Manag Res. 2021;13:163–71.
19. Gao M, Zhang X, Li D, He P, Tian W, Zeng B. Expression analysis and clinical significance of eIF4E, VEGF-C, E-cadherin and MMP-2 in colorectal adenocarcinoma. Oncotarget. 2016;7(51):85502–14.
20. Gu S, Qian L, Liu Y, Miao J, Shen H, Zhang S, et al. Upregulation of long non-coding RNA MYU promotes proliferation, migration and invasion of esophageal squamous cell carcinoma cells. Exp Ther Med. 2021;21(6):644.
21. Wong SHM, Fang CM, Chuah LH, Leong CO, Ngai SC. E-cadherin: Its dysregulation in carcinogenesis and clinical implications. Crit Rev Oncol Hematol. 2018;121:11–22.
22. Kourtidis A, Lu R, Pence LJ, Anastasiadis PZ. A central role for cadherin signaling in cancer. Exp Cell Res. 2017;358(1):78–85.
23. Bustos-Carpinteyro AR, Oliveira C, Sousa A, Oliveira P, Pinheiro R, Carvalho J, et al. CDH1 somatic alterations in Mexican patients with diffuse and mixed sporadic gastric cancer. BMC Cancer. 2019;19(1):69.

Клинички значај индекса пролиферације и експресије е-кадхерина у колоректалном аденокарциному

Јанко Жујовић[1], Милена Вулетић[2], Мирослав Стојановић[3], Ранко Лазовић[4], Небојша Ђорђевић[3], Миодраг Радуновић[4], Снежана Јанчић[2], Велимир Милошевић[5]

[1]Клинички центар Црне Горе, Центар за абдоминалну хирургију, Подгорица, Црна Гора;
[2]Универзитет у Крагујевцу, Факултет медицинских наука, Институт за патологију, Крагујевац, Србија;
[3]Универзитет у Нишу, Медицински факултет, Ниш, Србија;
[4]Универзитет Црне Горе, Медицински факултет, Подгорица, Црна Гора;
[5]Болница *Codra*, Подгорица, Црна Гора

САЖЕТАК

Увод/Циљ Циљ студије је испитивање повезаности експресије е-кадхерина и високог индекса пролиферације са клиничко-патолошким показатељима прогресије колоректалног карцинома.

Методе Биопсијски материјал 72 болесника добијен ресекцијом колоректалног карцинома је у Институту за патологију Клиничког центра Црне Горе рутински обрађиван, калупљен у парафин и архивиран. На основу архивираних патохистолошких извештаја формиране су две студијске групе: прву групу ($n = 72$) чиниле су оперативне биопсије колоректалног карцинома, а контролну групу ($n = 72$) чиниле су биопсије суседног нетуморског ткива. Примењени су рутински хематоксилин-еозин бојење и имунохистохемијска *ABC* (*avidin-biotin-peroxidase complex*) метода са анти-*Ki67* и анти-е-кадхерин антителима. Након квантификације резултата за статистичка тестирања је коришћен програмски пакет *SPSS* за *Windows* (19.0).

Резултати У ткиву колоректалног карцинома је запажена значајна повезаност високог индекса пролиферације са *pT* стадијумом, инвазијом лимфних и крвних судова, перинеуралном инвазијом, метастазама у лимфним чворовима и удаљеним метастазама и стадијумом туморске болести Астлер–Колер. Такође је запажено да је одсуство експресије е-кадхерина значајно повезано са *pT* стадијумом, инвазијом лимфних и крвних судова, перинеуралном инвазијом, са метастазама у лимфним чворовима, удаљеним метастазама, и са стадијумом тумора *C2* и *D* Астлер–Колер. Експресија е-кадхерина је значајним, високим али негативним коефицијентом корелације повезана са високим индексом пролиферације.

Закључак: Наши резултати указују да је на основу експресије *Ki67* и е-кадхерина могуће издиференцирати болеснике у групе већег, односно мањег ризика од појаве метастатске болести.

Кључне речи: колоректални карцином; експресија е-кадхерина; пролиферативни индекс; клинички значај