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 G1, S, G2, and M phases of the cell cycle. It cannot be detected in the G0 phase [2].

Cadherin adhesion molecules also play an important role in colorectal carcinogenesis [3]. Cadherins are a family of glycoproteins that perform calcium (Ca2+) 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 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)
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 mm² 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 mm². To test the density of Ki67+ cells per mm², 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” were determined 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” were determined 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” were determined 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” were determined successively. The absolute value of the density of positive cells in the “hot-spot” was determined stereometrically [8].

**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

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**Table 1. Clinicopathological features of colorectal carcinoma studied**

| Clinicopathological factors | Number of cases (%) | Age (min/max/X–) |
|----------------------------|---------------------|------------------|
| Males                      | 42 (58.33)          | 34/83/66.1 ± 10.6|
| Females                    | 30 (41.77)          | 27/83/61.4 ± 14.2|
| **Localization**           |                     |                  |
| 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** |                      |                  |
| Ulceroinfiltrative/Infiltrative/Vegetatively-infiltrative/Vegetative/Ulcerative | 27 (37.5) / 25 (34.7) / 16 (22.2) / 3 (4.2) / 1 (1.4) |                  |
| **Histological grade**     |                     |                  |
| Grade I / Grade II / Grade III | 4 (5.6) / 63 (87.5) / 5 (6.9) |                  |
| **Invasion of lymphatic vessels** |                     |                  |
| Not identified/Present     | 33 (45.8) / 39 (54.2)|                  |
| **Invasion of blood vessels** |                     |                  |
| Not identified / Present   | 61 (84.7) / 11 (15.3)|                  |
| **Nerve invasion**         |                     |                  |
| Not identified/Present     | 59 (81.9) / 13 (18.1)|                  |
| **Pathological stage of the tumor(pT)** |                     |                  |
| pT1, pT2, pT4               | 2 (2.8) / 54 (75.0) / 4 (5.6) |                  |
| **Lymph node metastases**  |                     |                  |
| 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**     |                     |                  |
| Not identified/Present     | 64 (88.9) / 8 (11.1)|                  |
| **Astler–Coller stage of tumor** |                     |                  |
| B1/B2/C1/C2/D               | 11 (15.3) / 21 (29.2) / 3 (4.2) / 30 (41.7) / 7 (9.7) |                  |

Clinical significance of proliferation index and E-cadherin expression in colorectal adenocarcinoma.
features, between and within the groups, \(\chi^2\)-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 mm\(^2\) of tissue. Ki67 expression reported by the number of immunopositive cells in mm\(^2\) (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).

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 proIDX 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. ProIDX 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–Collier 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 |
|---------------|----------------------|
|                | Ki67 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 (33.3) | 11 (36.7)  | 3 (10) |
| **Significance** | 0.237 |       |     |    |    |
| **Age of the subjects** | 0.096 |       |     |    |    |
| ≤ 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) |
| **Localization** | 0.369 |       |     |    |    |
| 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 | 1 (100) | 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** | 0.369 |       |     |    |    |
| **Tumor growth pattern** | 0.328 |       |     |    |    |
| 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** | 0.328 |       |     |    |    |
| **Histological grade of the tumor** | 0.124 |       |     |    |    |
| 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** | 0.245 |       |     |    |    |
| **Lymphatic invasion** | 0.285 |       |     |    |    |
| Not identified | 35 (57.4) | 26 (42.6) | 28 (45.9) | 30 (49.2) | 3 (4.9) |
| Lymphatic invasion present | 7 (17.9) | 32 (82.1) | 34 (87.2) | 5 (12.8) | 0 (0) |
| **Significance** | < 0.001* |       |     |    |    |
| **Blood vessels invasion** | < 0.001* |       |     |    |    |
| Not identified | 35 (59.3) | 24 (40.7) | 26 (44.1) | 31 (52.5) | 2 (3.4) |
| Blood vessels invasion present | 1 (7.7) | 12 (92.3) | 12 (92.3) | 0 (0) | 1 (7.7) |
| **Significance** | < 0.001 |       |     |    |    |
| **Nerve invasion** | 0.022* |       |     |    |    |
| 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** | < 0.001 |       |     |    |    |
| **Lymph node metastases** | 0.020* |       |     |    |    |
| 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** | < 0.001* |       |     |    |    |
| **Distant metastases** | < 0.001* |       |     |    |    |
| 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** | 0.003* |       |     |    |    |
| **Pathological stage of the tumor** | 0.018* |       |     |    |    |
| pT1 | 11 (91.7) | 1 (8.3) | 0 (0) | 10 (83.3) | 2 (16.7) |
| pT2 | 21 (38.9) | 33 (61.1) | 34 (63) | 19 (35.2) | 1 (1.9) |
| pT3 | 2 (50) | 2 (50) | 4 (100) | 0 (0) | 0 (0) |
| **Significance** | 0.005* |       |     |    |    |
| **Astler–Coller stage of tumor** | < 0.001* |       |     |    |    |
| B1 | 10 (90.9) | 1 (9.1) | 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** | < 0.001* |       |     |    |    |

*significant difference p < 0.05, χ2 test, Fisher’s exact test
The absence of E-cadherin expression was significantly associated with distant metastases. In a significant number of cases, the absence of E-cadherin expression was associated with lymph node metastases and distant metastases. 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 C2 and D Astler–Coller stage tumors in 100% of cases \( (p < 0.001) \). E-cadherin expression was associated with the Astler–Coller 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 G1 phase when levels are low, and then its expression increases through the S and G2 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 pT1 and pT2 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 pT1 and 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 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.

**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 pT1 and pT2 stages of the tumor \( (p > 0.05) \). There was also a strong 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 \( (r = -0.515) \).
|                | 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            | 1.000        | 0.188        | -0.058       | 0.102    | -0.913        | 0.034    | -0.204         | 0.076               | 0.555               | 0.043           | 0.024             | 1.000               | 0.077     |           |
| Age            | 0.188        | 1.000        | 0.099        | -0.191   | 0.012         | -0.205   | -0.202         | 0.071               | 0.088               | 0.722           | 0.840             | 0.803               | 0.519     |           |
| Localization   | 0.058        | 0.099        | 0.018        | 0.104    | 0.104         | 0.014    | 0.000          | 0.000               | 0.000               | 0.569           | 0.070             | 0.860               | 0.798     | -0.031    |
| pT stage       | 0.012        | 0.141        | 0.158        | 0.015    | 0.154         | 0.638    | -0.082         | 0.120               | 0.000               | 0.100           | 0.324             | 0.284               | 0.735     | 0.053     |
| Histol. Grade  | 0.113        | 0.012        | 0.088        | 0.154    | 0.100         | 0.115    | -0.104         | 0.011               | 0.084               | 0.142           | 0.001             | 0.117               | 0.020     | 0.002     |
| AC stage       | 0.034        | 0.025        | 0.014        | 0.000    | 0.000         | 0.120    | -0.082         | 0.224               | 0.000               | 0.000           | 0.000             | 0.000               | 0.000     |           |
| Growth pattern | 0.024        | 0.057        | -0.155       | -0.104   | -0.082        | 0.756    | 0.468          | 0.437               | 0.726               | 0.503           | 0.187             | 0.000               | 0.000     |           |
| Lymph vessel invasion | 0.722 | 0.046 | -0.079 | 0.142 | 0.000 | 0.437 | 0.061 | 0.359 | 0.403 | 0.000 | 0.279 | 0.638 | 0.397 | -0.293 |
| Neural invasion | 0.024        | 0.021        | 0.141        | 0.201    | 0.021         | 0.000    | 0.000          | 0.000               | 0.000               | 0.000           | 0.000             | 0.000               | 0.000     |           |
| Lymph node metastases | 0.021 | 0.007 | 0.037 | 0.000 | 0.016 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Distant metastases | 0.030 | 0.072 | 0.037 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Ki67           | 0.000        | 0.031        | 0.053        | 0.002    | 0.000         | 0.000    | 0.000          | 0.000               | 0.000               | 0.000           | 0.000             | 0.000               | 0.000     |           |
| E-cadherin     | 0.077        | 0.015        | -0.060       | -0.515   | -0.075        | -0.376   | -0.031         | -0.293              | -0.729              | -0.315          | -0.794            | 1.000               | -0.097    |           |

* 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.

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Клинички значај индекса пролиферације и експресије е-калдхерина у колоректалном аденоцарциному

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

Методе: Биопсиски материјал 72 болесника добијен рецензисаним колоректалним карциномом је у Институту за патолошку истраживања покретао две студијске групе: групу контроле (n=72) чињени су оперативне биопсисе колоректалног карцинома, а контрольну групу (n=72) чиније су биопсисе суседног нетуморског ткива. Примењени су рутински хематоксилин-еозин бојење и имунохистохемијски ABC (avidin-biotin-peroxidase complex) метода са анти-Ки67 и анти-е-калдхерин антителима. Након квалификације 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 рТ стадијумом, инвазијом лимфних и крвних судова, пери- неумалним инвазијом, метастазама у лимфним чворовима и удаљеним метастазама и стадијумом уморских боlesti Aстер-Колер. Такође је запажено да је одсуство експресије е-калдхерина значајним, високим али негативним коефицијентом корелације повезано са стадијумом, инвазијом лимфних и крвних судова, пери- неумалним инвазијом, метастазама у лимфним чворовима и удаљеним метастазама, а са стадијумом уморских боlesti C2 и D Aстер-Колер. Експресија е-калдхерина је значајан, високим али негативним коефицијентом корелације повезана са високим индексом пролиферације.

Закључак: Наши резултати указују да је на основу експресије Ки67 и е-калдхерина могуће идентификисати болеснике у групе већег, односно мањег ризика од појаве метастатске болести.