RESEARCH ARTICLE

Clinicopathological Characteristics and Prognosis of Patients According to Recurrence Time After Curative Resection for Colorectal Cancer

Oktay Bozkurt[1]*, Mevlude Inanc[2], Esma Turkmen[3], Halit Karaca[1], Veli Berk[1], Ayse Ocak Duran[1], Ersin Ozaslan[1], Mahmut Ucar[1], Ilhan Hacıbekiroglu[3], Baki Eker[4], Osman Baspınar[4], Metin Ozkan[1]

Abstract

Purpose: To investigate clinicopathological features in patients with recurrent colorectal cancer within 1 year and more than 1 year after curative resection. Materials and Methods: We retrospectively evaluated 103 patients with disease recurrence before versus after 1 year of resection. Thirty-two patients (31%) were diagnosed with recurrence less than 1 year after curative resection for colorectal cancer (early recurrence) and 71 (69%) after more than 1 year (non-early recurrence). Results: The early recurrence group displayed a significantly lower overall survival rate for both colon cancer (p=0, 01) and rectal cancer (p<0.001). Inadequate lymph node dissection was a significant predictor for early relapse. There were no statistically significant differences in clinicopathological variables such as age, sex, primary tumor localization, stage, depth of invasion, lymphovascular invasion and perineural invasion between the early and non-early recurrence groups. However, a K-ras mutation subgroup was significantly associated with early recurrence (p<0.001). Conclusions: Poor survival is associated with early recurrence for patients undergoing resection for non-metastatic colorectal cancer, as well as K-ras mutation.

Keywords: Predictive and prognostic factors - early recurrence - colorectal cancer - K-ras

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Introduction

The incidence of colorectal cancers (CRC) has increased and the third most common neoplasm in Turkey and in the world in recent years (Jemal et al., 2010; Karaca et al., 2011). The main treatment for colorectal cancer is resection of the primary tumor. Adjuvant chemotherapy following curative resection is the most common approach to CRC treatment. However, it has been determined that the overall recurrence rate of colorectal cancer after curative surgery is between 26 and 43 % according to the duration of follow-up and the initial pathological stage of disease (Galandiuk et al., 1992; Moertel et al., 1993; Kjeldsen et al., 1997; Graham et al., 1998). The majority of these recurrences occur during the first 5 years after surgery (Galandiuk et al., 1992; Kjeldsen et al., 1997; Graham et al., 1998).

Hence, most follow-up programs currently include more intense testing throughout this period of maximum risk . In some studies, it was shown that the time from initial treatment to relapse was significantly linked to survival following relapse in patients who underwent curative surgery for colorectal cancer (Kaiser et al., 2006; O'Connell et al., 2008; Tsai et al., 2009). On the other hand, the exact prognostic importance of postoperative early recurrence in patients who underwent surgery for colorectal cancer remains uncertain (Kaiser et al., 2006; O'Connell et al., 2008, Kobayashi et al., 2009; Tsai et al., 2009). We investigated the clinicopathological features in patients with recurrent colorectal cancer within 1 years or more than 1 years after curative resection and determined the predictors of survival after recurrence.

Materials and Methods

Data were obtained from the chart reviews of colorectal cancer patients in two oncology departments in Turkey. We retrospectively evaluated 103 patients who were diagnosed with recurrence within 1 year and more than 1 year after potentially curative resection for non-metastatic colorectal cancer from January 1991 to January 2013.

All of the patients underwent standard colectomy and regional lymphadenectomy according to the tumor location. Curative resection was considered as when no

*[1]Department of Medical Oncology, Erciyes University Faculty of Medicine, [2] Medical Oncology Department, [4]Internal Medicine Department, Kayseri Training and Research Hospital, Kayseri, [3]Department of Medical Oncology, Trakya University Faculty of Medicine, Edirne, Turkey *For correspondence: bozkurt.oktay8@gmail.com*

gross residual tumor remained in the surgical bed and the proximal and distal resection margins were pathologically negative for tumor invasion. The pathologic stage was determined by the American Joint Committee on Cancer TNM staging system.

Follow-up evaluation consisted of history and physical examination, routine blood tests with serum metabolic panels, serum carcinoembryonic antigen assay and imaging every 3 months during the first 2 years, every 6 months from the 3rd to the 5th year, and annually thereafter. Additional imaging examinations were conducted when the patient presented with symptoms related to cancer recurrence. The sites and number of recurrence were determined according to radiological findings of disease on computerized tomography, ultrasound, positron emission tomography and computerized tomography (PET/CT) and colonoscopy . Disease recurrence was determined by radiographic evidence; suspect imaging findings were followed by biopsy of the doubted lesion and classified as disease recurrence after pathological confirmation.

High-risk stage II or III patients received either adjuvant chemotherapy or chemoradiation therapy. Patients with risk factors for relapse (such as poorly differentiated, tumor perforation or obstruction, lymphatic/vascular invasion and number of retrieved lymph nodes <12,) were determined as high-risk stage II cases.

Two chemotherapeutic regimens were used: (1) FUFA : 5-fluorouracil (5-FU) and leucovorin (6 cycles of monthly bolus intravenous 5-FU 400-425mg/m^2/day, days 1-5, and leucovorin 20mg/m^2/day, days 1-5), and (2) FOLFOX : oxaliplatin+5-FU+ leucovorin (12 cycles of oxaliplatin 85mg/m^2 on day 1 and leucovorin 200mg/m^2 as a 2-h infusion on day 1, 5-FU 400mg/m^2 as a bolus and 600mg/m^2 as a 22-h infusion on days 1 and 2 bimonthly). Postoperative radiotherapy consisted of 50.4 Gy in 28 fractions delivered to the pelvis using a four-field box technique.

Tumor specimens from 103 tumors were retrospectively analyzed for the presence of k-ras mutations in codons 12,13 and 61. DNA was extracted from 10% formalin-fixed paraffin-embedded tissue blocks that best represented each tumor, previously selected from hema-toxylin-eosin stained slides. The 5 μm-thick sections were cut from blocks that had been selected for maximal tumor content. These sections were deparaffinized with three baths of xylene for 10 minutes followed by three baths of 100% ethanol solution for 5 minutes. Following this, tissues were digested with Proteinase K and genomic DNA was isolated using the QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. After extraction, the quantity of DNA was measured with a NanoDrop spectrophotometer (Thermo Fisher Scientific, DE). Mutation studies were performed using polymerase chain reaction and direct nucleotide sequencing. The study covered codons 12, 13 and 61of k-ras.

For statistical analyses of the study data SPSS 18.0 software was used. The differences between the groups were tested with chi-square tests. Survival analysis was performed using the Kaplan- Meier method. For significance of area under the curves the log-rank test was used. Statistical significance was considered at a P-value<0.05

Results

A total of 103 patients who underwent curative resection for non-metastatic colorectal cancer were evaluated. Of these, 32 patients (31%) were diagnosed with recurrence within 1 year after surgery resection (early recurrence) and 71 patients (69%) were diagnosed with recurrence more than 1 year after surgery resection (non-early recurrence). K-ras somatic mutation was detected in 33 % (34/103) of the patients. Table 1 shows the demographic and histopathological characteristics of the two patient groups.

The median OS was 32 (95% CI 25.2-39.4) months in the early recurrence group, and 87 (95% CI 69.3-104.8) months in the non-early recurrence group in the whole patients (p<0.001, Figure 1). The median OS was 35 (95% CI 24-45.9) months in the early recurrence group, and 92 (95% CI 62-123) months in the non-early recurrence group in colon cancer (p=0.01, Figure 2). The median OS was 28 (95% CI 21.4-35.9) months in the early recurrence

Table 1. Correlation between Recurrence and the Clinicopathological Factors in the Patients with Postoperative Recurrence

		Early recurrence (n=32)	Non-early recurrence (n=71)	p-value
SAge (y)				
	<65	21(65.6)	56(78.9)	
	≥ 65	11(34.4)	15(21.1)	0.15
Gender				
	Male	22(31.3)	41(42.3)	
	Female	10(68.8)	30(57.7)	0.28
Tumor location				
	Colon	16(50)	35(49.3)	
	Rectum	16(50)	36(50.7)	0.94
Depth of invasion				
	T1+T2	5(15.6)	5(7)	
	T3+T4	27(84.4)	66(93)	0.17
Postoperative chemotherapy				
	No	6(18.8)	16(22.5)	
	Yes	26(81.3)	55(77.5)	0.66
N category				
	Negative	15(46.9)	27(38)	
	Positive	17(53.1)	44(62)	0.39
No. of lymph nodes retrieved				
	<12	18(56.3)	24(33.8)	
	≥12	14(43.8)	47(66.2)	0.03
Lymphovascular invasion				
	Negative	15(46.9)	45(63.4)	
	Positive	17(53.1)	26(36.6)	0.11
Perineural invasion				
	Negative	22(68.8)	50(70.4)	
	Positive	10(31.3)	21(29.6)	0.86
Type of postoperative recurrence				
	Local	4(12.5)	8(11.3)	
	Distant	28(87.5)	63(88.7)	0.85
Differentiation				
	Well and moderate	25(78.1)	64(90.1)	
	Poor	7(21.9)	7(9.9)	0.1
Stage				
	II	11(34.4)	24(33.8)	
	III	21(65.6)	47(66.1)	0.95
K-ras mutation status				
	K-ras mutation	19(59.4)	15(21.1)	
	Wild tip	13(40.6)	56(78.9)	<0.001

group, and 84 (95% CI 67.6-101.4) months in the non-early recurrence group in rectal cancer (p<0.001, Figure 3). The early recurrence group displayed a significantly lower overall survival rate than that of the non-early recurrence group for both stage II cancer (p<0.001) and stage III tumors (p<0.001).

K-ras mutations were significantly associated with early recurrence (p<0.001). An inadequate lymph node was a significant predictor for early relapse (p=0,03). There was no statistically significant difference in the other clinicopathological variables such as age, sex, primary tumor localization, stage, depth of invasion, lymphovascular invasion and perineural invasion between the early and non-early recurrence groups (respectively, p=0.15, p=0.28, p=0.95, p=0.17, p=0.11, p=0.86).

Eighty-two patients (70.9 %) received adjuvant therapy. Thirty-seven of these patients received adjuvant chemotherapy after chemoradiotherapy. Fifty-one and thirty of the patients received FOLFOX and FUFA chemotherapeutic regimens respectively. While twenty-two (62%) of the thirty-five patients with stage II cancer received adjuvant therapy, sixty(88%) of the eighty-two patients with stage III cancer received adjuvant therapy (p=0.002).

K-ras somatic mutation was detected in 39.2 % (21/52) of patients who received FOLFOX therapy. While early recurrence was observed in nine of these patients, twelve patients were observed to have non-early recurrence (p=0.69). K-ras somatic mutation was detected in 26.7 % (7/29) of patients who received FUFA therapy. While the early recurrence was observed in six of these patients, one patient was observed non-early recurrence (p=0.001).

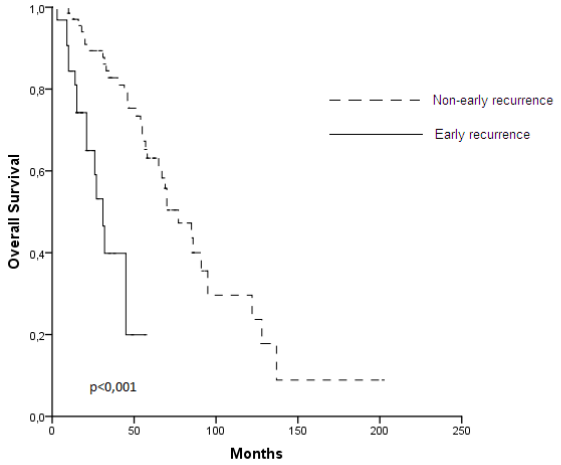

Figure 1. Kaplan-Meier Curves for OS According to Patients Recurrence Group in the Whole Patients

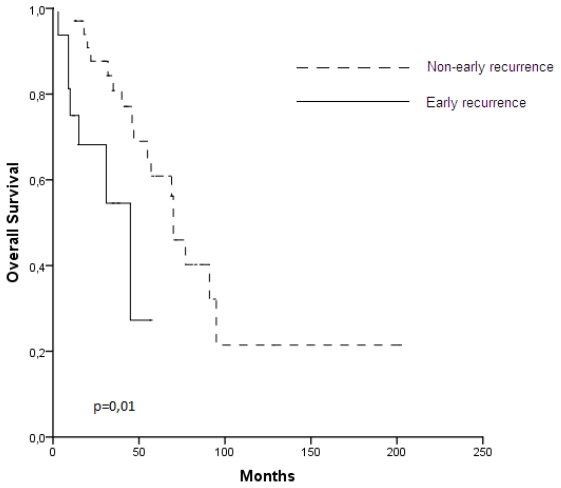

Figure 2. Kaplan-Meier Curves for OS According to Patients Recurrence Group in Colon Cancer

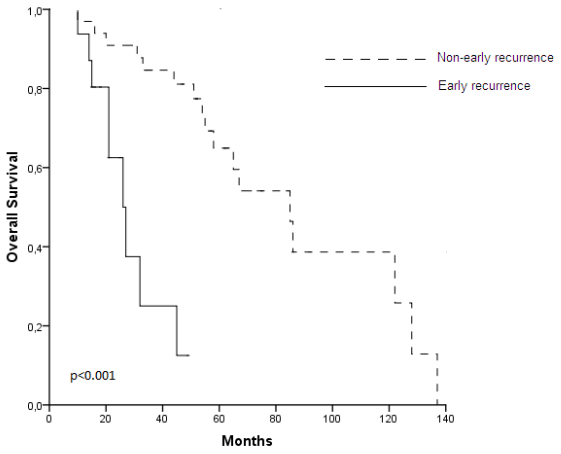

Figure 3. Kaplan-Meier Curves for OS According to Patients Recurrence Group in Rectal Cancer

Discussion

Although approximately two-thirds of patients with CRC undergo resection with curative surgery at the time of initial diagnosis, 30% - 50% of these patients relapse and die of their disease (Abulafi and Williams, 1994). Early recurrence after surgical resection for colorectal cancer may be associated with clinicopathological parameters such as lymphovascular invasion, an unfavorable genotype, deeper tumor invasion, advanced cancer stage, lymph node metastasis and chemotherapy resistance.

Tsai et al. observed that the overall survival rate in the early recurrence group was significantly lower than that of the non-early recurrence group (Tsai et al., 2009). Huh et al. showed that the overall survival rate in the early recurrence group was significantly lower than that of the non-early recurrence group irrespective of tumor location (Huh et al., 2013). In our study, we observed that the overall survival rate in the early recurrence group was significantly lower than that of the non-early recurrence group, which was consistent with the results of this study.

Recurrence rates after curative surgical resection of CRC are related to the International Union Against Cancer (UICC) stage of the disease, and the time to recurrence differs by UICC stage. In our study, there was not a statistically difference significant between stages III and II with regard to postoperative early relapse. This status can be explained by the fact that 88% of the eighty-two patients with stage III cancer received adjuvant therapy. However, 62% of the thirty-five patients with stage II cancer received adjuvant therapy.

Several investigators have shown that lymphovascular invasion is a prognostic factor for predicting recurrence in patients with colorectal cancer (Meguerditchian et al., 2005; Lim et al., 2010; Wang et al., 2014). Lymphovascular invasion was not a significant factor in predicting postoperative early recurrence of colorectal cancer in our study.

Some studies showed that the depth of tumor invasion was a significant prognostic factor of postoperative relapse and survival rate in CRC patients undergoing curative resection (Tsai et al., 2007; Tsai et al., 2008; Tsai et al., 2009; Wu et al., 2013). In this present study, depth of invasion was not significant for postoperative early relapse and survival in colorectal cancer.

The American Joint Committee on Cancer (AJCC) has recommended assessment of 12 or more lymph nodes for correct staging (Nelson et al., 2011). On the basis of these data, when we accepted a lower limit of >12 resected lymph nodes as a measure of adequate lymphadenectomy for colorectal cancer, an inadequate lymph node dissection was a significant predictor for early relapse according to our present observations (p=0.03).

Eisenberg et al. demonstrated that left-sided colon or rectal cancer is more likely to slowly progress more compared to colon cancers at other locations (Eisenberg et al., 1982). There was not a statistically significant difference between tumor location and the time interval to recurrence in our study.

The relationship between the time interval to recurrence and adjuvant therapy is substantial (Bentzen et al., 1992; Galandiuk et al., 1992; Wolmark et al., 1993; Kaiser et al., 2006; Huh et al., 2012). According to our present investigation, adjuvant therapy was not correlated with the postoperative early relapse in the colorectal cancer patients. This result can be explained by the majority of patients who not received treatment consisted of patients with stage II compared to stage III patients.

There was not a statistically significant difference between early recurrence and the clinicopathological parameters such as age, sex, primary tumor localization, stage, depth of invasion, lymphovascular invasion and perineural invasion. These data were contrary to some studies. The retrospective nature of their analysis, differences in inclusion criteria and different number of patients between studies may explain the differences in results.

Hence, molecular markers are needed as potential predictors for prognosis and facilitate treatment in colorectal cancer patients (Ding et al., 2012; Fang et al., 2012; Hu et al., 2013; Li et al., 2013). To our knowledge, this is the first report to compare the relationship between k-ras mutation status with early recurrence. In our study, the k-ras mutation subgroup were significantly associated with early recurrence compared to the wild k-ras subgroup.

Several subgroup analyses of randomized prospective clinical trials suggested that k-ras gene mutation status might predict the efficacy of cytotoxic chemotherapy, especially for oxaliplatin-based regimens in CRC. OPUS and PRIME studies, which were both planned for patients receiving first-line FOLFOX with/without EGFR monoclonal antibodies, are appropriate examples (Bokemeyer et al., 2009; Douillard et al., 2010). The chemotherapy-only group in the OPUS study showed that first-line progression-free survival (PFS) in the k-ras mutant group was longer than in the wild-type group, with 8.6 versus 7.2 months in the chemotherapy-only group, and 8.8 versus 8.0 months in the chemotherapy-only group in the PRIME study. On the other hand in the CRYSTAL study, which was planned for patients receiving first-line irinotecan/5FU/leucovorin with/without EGFR monoclonal antibody, a similar finding was not observed. The median first-line PFS in k-ras mutant and wild-type patients was 7.7 and 8.4 months, respectively (Van Cutsem et al., 2009). According to these findings, k-ras mutant CRC patients might benefit more from receiving first-line oxaliplatin- based regimens than wild-type patients. In the whole group, k-ras mutations were statistically associated with the early recurrence groups in our study. However, k-ras mutations were not significantly associated with the early recurrence groups in patients who received FOLFOX. According to our observation, the k-ras mutation may be a predictor of oxaliplatin sensitivity in CRC. Nevertheless, this finding is needs to be confirmed by large prospective clinical trials.

As a result, the k-ras mutation subgroup was significantly associated with early recurrence compared to the wild-type k-ras subgroup. An inadequate lymph node dissection was a significant predictor for early relapse. However, no statistically significant difference vas found between early recurrence and the other clinicopathological parameters such as age, sex, performance status, primary tumor localization, stage, depth of invasion, lymphovascular invasion and perineural invasion. In addition, we observed that the overall survival rate in the early recurrence group was significantly lower than that of the non-early recurrence in stage II and III colorectal cancer. K-ras mutation patients should be intensively followed up in terms of early recurrence and for these patients, oxaliplatin-based regimens may be preferred for adjuvant treatment. However, our results emphasize the need for further studies.

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