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RESEARCH

Effect of microsatellite instability on histopathological parameters and prognosis in colon cancers

Kolon kanserlerinde mikrosatellite instabilitenin histopatolojik parametreler ve prognoz üzerine etkisi

Tuğba Toyran[1], Kıvılcım Eren Erdoğan[1], Emine Kılıç Bağır[1], Arbil Açıkalın[1], Merve Aksın[1], Semra Paydaş[2], Burak Mete[3], Şeyda Erdoğan[1]

[1]Cukurova University Faculty of Medicine, [2]Department of Pathology, [2]Department of Oncology, [3]Department of Public Health, Adana, Turkey

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Abstract

Purpose: The aim of this study was to compare the clinicopathological features, while evaluating the frequency of MSI, and the survival rates in these patients in our patient group with MSI and microsatellite stabil (MSS) colorectal carcinomas (CRCs).

Materials and Methods: We retrospectively enrolled 146 patients who underwent colon resection between the years of 2014-2022. The expression of MSI status was evaluated by immunohistochemistry. The association of MSI status, presense of tumor-infiltrating lymphocytes (TILs), and tumor budding score with a patient's survival was assessed by the Kaplan–Meier method and Cox regression analysis.

Results: There were 104 (71.2%) MSS cases and, 42 (28.8%) MSI cases. 15 (10.3%) MSI tumors were found to be MSI-low, and 27 (18.5%) to be MSI-high. MSI tumors were significantly associated with younger patients (<50), earlier stage (T1-T2), right localization, lower rate of lymph node metastasis, presence of mucinous component and TILs response. The Cox-regression model revealed TILs, tumor budding score, and MSI are variables that significantly affect survival. The presence of TILs exhibited a protective effect (Hazard ratio (HR)=0.446), which decreased the mortality risk by 2.24 times for the follow-up period, while the presence of high TBS increased the risk of mortality by HR=3.22.

Conclusion: This study revealed that patients with MSI CRCs may show unique clinicopathological features and should be evaluated using some guiding parameters that will improve survival.

Keywords:. Colorectal carcinoma, microsatellite instability, tumor-infiltrating lymphocytes, tumor budding score.

Öz

Amaç: Bu çalışmada, MSİ ile mikrosatellit stabil (MSS) kolorektal karsinomlu (KRK) hasta grubumuzda, klinikopatolojik özellikleri karşılaştırmayı, MSİ sıklığını ve bu hastalarda sağkalım oranlarını değerlendirmeyi amaçladık

Gereç ve Yöntem: Çalışmaya retrospektif olarak 2014-2022 yılları arasında kolon rezeksiyonu yapılan 146 hasta dahil edildi. MSI durumu, immünohistokimyal belirteçlerle değerlendirildi. MSI durumu, tümörü infiltre eden lenfositlerin (TIL'ler) varlığı ve tümör tomurcuklanma skorunun sağkalım ile olan ilişkisi, Kaplan-Meier yöntemi ve Cox regresyon analizi ile değerlendirildi.

Bulgular: Olgularımızın 104'ü (%71,2) MSS, 42'si (%28,8) MSİ'dir. MSI tümörlerin 15'i (%10,3) MSI-düşük, 27'si (%18,5) MSİ-yüksek olarak bulundu. MSİ tümörler, genç yaş (<50 yaş), erken evre (T1-T2), sağ kolon lokalizasyonu, düşük lenf nodu metastazı oranı, müsinöz komponent ve TİL varlığı ile istatistiksel olarak anlamlı şekilde ilişkiliydi. Cox-regresyon modeli, TIL'lerin, tümör tomurcuklanma skorunun ve MSİ'nin sağkalımı önemli ölçüde etkileyen değişkenler olduğunu ortaya çıkardı. TIL'lerin varlığı, takip süresince ölüm riskini 2,24 kat azaltan koruyucu bir etki (Tehlike oranı (HR)=0,446) sergilerken, yüksek tümör tomurcuklanma skorunun ölüm riskini (HR=3,22) artırdığı bulundu.

Sonuç: Çalışmamızda, MSİ KRK'li hastaların, kendilerine özgü klinikopatolojik özellikler gösterebileceği ve sağkalımda yol gösterici bazı parametrelerle birlikte değerlendirilmesi gerektiği vurgulanmaktadır.

Anahtar kelimeler: Kolorektal karsinom, mikrosatellite instabilite, tümörü infiltre eden lenfositler, tümör tomurcuklanma skoru.

Address for Correspondence: Dr. Tuğba Toyran, Cukurova University Faculty of Medicine, Department of Pathology, Adana, Turkiye E-mail: tugbaolcan@hotmail.com
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INTRODUCTION

Colorectal carcinomas (CRCs) rank third in cancer-related mortality worldwide[1]. CRCs are heterogeneous diseases with various molecular subtypes presenting different pathological and clinical features. In the pathogenesis of CRCs, 3 molecular pathways are mentioned, which are chromosomal instability, microsatellite instability (MSI), and epigenetic instability[2].

MSI or mismatch repair deficiency (dMMR) pathway is responsible for the development of 15-20% of colorectal cancers[3]. The MMR system consists of the proteins MLH1, MLH3, MSH2, MSH3, MSH6, PMS1, and PMS2. The system is also responsible for repairing single nucleotide base pairings and slippage errors during DNA replication. Once there is a dysfunction in MMR genes, MSI develops. The MSI condition results in the accumulation of genetic mutations affecting the function of numerous genes. This, in turn, ends up with an increased risk of developing neoplasia[4].

MSI is primarily caused by mutational inactivation of at least one of the four main MMR genes (MLH1, MSH2, MSH6 or PMS2)[5]. Lynch syndrome, which manifests itself as hereditary non-polyposis CRCs (HNPCC) in the gastrointestinal tract, is the most characteristic hereditary disease associated with DNA MMR defect, accounting for 1-5% of all CRC cases. In Lynch syndrome, the most commonly affected proteins are MSH2 and MLH1. Inactivation of these proteins results in complete loss of MMR function. MLH1 is the main protein most frequently affected in sporadic MSI CRCs[6].

The incidence of MSI tends to vary based on the locus investigated, as there are excessive number of microsatellite regions throughout the human genome. A panel of 5 microsatellite loci, 2 mononucleotide repeats (Bat25, Bat26) and 3 dinucleotide repeats (D2S123, D5S346, D17S250) were selected at the National Cancer Institute (NCI) conference to be examined in all CRCs investigated for MSI[7]. According to this panel, colorectal cancers are divided into three groups in terms of MSI. While the tumors presenting MSI in >30% of the investigated loci are defined as MSI-high (MSI-H), the microsatellite instability in <30% of loci is defined as MSI-low (MSI-L). The tumors in which MSI is not detected at any locus are called microsatellite stable (MSS)[8].

MSI colon cancers have clinical and pathological features which are different from MSS ones. Several studies have demonstrated that MSI tumors have some specific clinicopathological features such as being poorly differentiated, high-grade, located in the right colon, multiple presence of tumor-infiltrating lymphocytes (TILs), Crohn-like lymphocytic infiltrate, pushing border, and mucinous/signet ring/medullary histology[9]. MSI plays a key role in the pathogenesis of CRCs, as well as it responds differently to conventional chemotherapeutic treatment protocols, which requires appropriate personalized treatment protocols for patients in MSI CRCs[10]. In this study, we aimed to compare the clinicopathological features, while evaluating the frequency of MSI and the survival rates in these patients in our patient group with MSI and MSS CRCs. Because we think that specifying this situation is necessary to guide oncologists in finding treatment options related to conventional chemotherapy as well as new immunotherapy.

MATERIALS AND METHODS

Sample

We retrospectively enrolled 146 patients who underwent colon resection between the years of 2014-2022 in Cukurova University Faculty of Medicine (CUFM), Department of Pathology Adana, Turkey. For the study, the necessary permission was obtained from the Ethics Committee of CUFM, with session number 28, dated 3/06/2022 and numbered 123.

Power=80%, confidence interval=95%, d=0.5 was taken as the two-tail test, and the minimum number to be reached in the sample size analysis was found to be 128. Patients with histologically confirmed primary adenocarcinoma and pathological stage I-IV colon cancer were selected for the study. All patients for whom immunohistochemical antibodies were studied for microsatellite instability were included.

Procedure

The age, gender, tumor size, localization, distant metastasis and survival information of the cases were obtained using the hospital information system. In hematoxylin-eosin (H&E) stained sections prepared from the materials taken from all cases, histological type and grade, tumor diameter, presence of

lymphovascular and perineural invasion, TILs, tumor budding score (TBS), presence of metastatic lymph node and pathological TNM stage were evaluated by at least two pathologists based on the 8th edition of the American Joint Committee on Cancer staging guidelines.

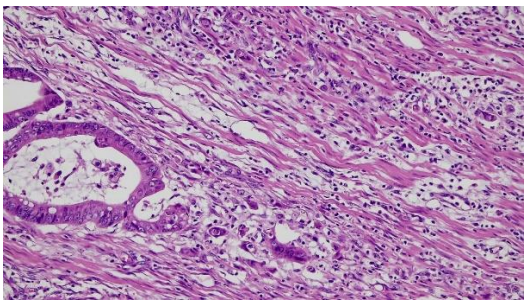

Figure 1. Tumor budding is defined as the presence of single cells or small clusters of cancer cells in the tumor stroma with a desmoplastic stromal response. Budding scor:high (H&E stain, high-power image ×200).

To evaluate TBS, criteria set at The International Tumor Budding Consensus Conference[11] in 2016 were employed and in accordance with these criteria, the entire tumor area was scanned in H&E stained preparations. Infiltrative tumor cells were counted at a magnification of 20 either singly or in groups of less than five cells around the tumor. The number of buds observed in these areas was scored in line with the low (0-4 buds), medium (5-9 buds) and high (≥10 buds) grading system (Figure 1).

TILs status was first evaluated at x4 objective magnification to determine the most TILs-abundant area in H&E slices. After the hotspot area was determined, the number of lymphocytes in the tumor epithelium was counted in 5 consecutive high magnification fields (HMF) (x40 objective). Once an average of ≥2 lymphocytes/ HMF was detected in the tumor, it was considered TILs-positive (Figure 2).

Immunohistochemistry (IHC)

MSS or MSI status was evaluated using IHC staining method. Paraffin blocks that best exhibited tumor characteristics were selected for MLH1, MSH2, MSH6 and PMS2 markers applied with the IHC method. The sections 4 µm in thickness from paraffin blocks were taken on positively charged slides. These sections were then deparaffinized with xylol for 15 minutes after waiting for one hour in an oven at 60°C. Following this, they were hydrated by passing through gradually decreasing alcohols and washed in distilled water. Prepared sections were stained with MLH-1 (Mouse monoclonal antibody, ES05, Dako), MSH-2 (Mouse monoclonal antibody, G219-1129, Cell Marque), MSH-6 (Rabbit Monoclonal Antibody, EP49, Epitomics), PMS-2 (Rabbit Monoclonal antibody, EP51, Dako) antibodies in Ventana BenchMark XT brand IHC device. Preparations stained in the automatic staining device were covered with a liquid-based sealant. The prepared sections were then evaluated by two pathologists at different magnifications using an Olympus microscope. All 4 markers were scored based on their percentage of staining. Colon crypt epithelium or nuclear staining in lymphocytes was considered for positive control. The average percentage values were obtained by counting at least 4 HMF (x40 objective) for tumor cells. The level of expression was accepted as the threshold value of 1% in the tumor. < 1% was considered negative, while ≥ 1% as positive.

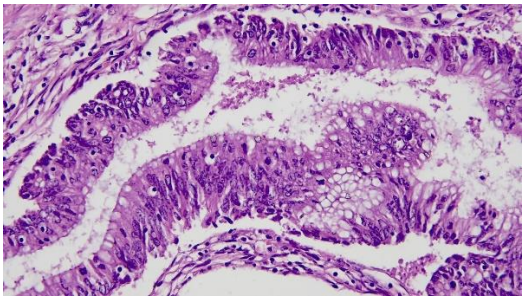

Figure 2. The presence of TILs was determined by H&E stain. (High-power image ×400)

Microsatellite was reported stable if nuclear expression was present in all MSH2, MSH6, PMS2, MSH1 proteins, while it was reported as MSI-L if one of them had loss in nuclear expression, MSI-H if there was loss of nuclear expression of two or more MMR proteins.

Statistical analysis

SPSS 22 program was used in the analysis of the data. Kolmogorov Smirnov test was used as the normal distribution test. Parametric tests were preferred in the analysis of normally distributed data, and non-parametric tests were preferred in the analysis of non-distributed data. Chi-square tests were used to compare categorical (nominal) data. Fisher chi-square test was used in cases where the expected value was below 5, Yates chi-square test was used between 5-

25, and Pearson chi-square test was used in cases of greater than 25 or mxn matrix. Cox regression analysis and Kaplan Meier analysis were used for survival analysis. The independent variables included in the model in the Cox regression analysis are TILs (reference category: no TILs), TBS (reference category: low budding), microsatellite instability (reference category: stable). Kaplan-Meier survival analysis was used to examine the mean survival times according to the presence of inflammation around the tumor. A value of $p<0.05$ was considered statistically significant.

Table 1. Comparison of clinicopathological features by stability status

	Microsatellite characteristic n(colon%)			
Sex	Stable	Instable-low	Instable-High	**p**
Female	39(37.5)	6(40.0)	8(36.3)	0.714
Male	65(62.5)	9(60.0)	19(70.4)	
Age				
0-49	$18(17.3)_a$	$4(26.7)_{a,b}$	$11(40.7)_b$	**0.032**
≥50	$86(82.7)_a$	$11(73.3)_{a,b}$	$16\ (59.3)_b$	
Tumor site				
right side	$31(29.8)_a$	$7(46.7)_{a,b}$	$17(63.0)_b$	**0.005**
left side	$73(70.2)_a$	$8(53.3)_{a,b}$	$10(37.0)_b$	
Histologic subtype				
Adenocarcinoma	$74(71.2)_a$	$9(60.0)_{a,b}$	$13(48.1)_b$	**0.037**
Mucinous adenocarcinoma	$30(28.8)_a$	$6(40.0)_{a,b}$	$14(51.9)_b$	
Grade				
well-moderate	90(86.5)	11(73.3)	21(77.8)	0.295
Poor	14(13.5)	4(26.7)	6 (22.2)	
Lymphovascular invasion				
no	23(22.1)	2(13.3)	8(29.6)	0.469
yes	81(77.9)	13(86.7)	19(70.4)	
Perineural invasion				
no	42(40.4)	7(46.7)	15(55.6)	0.357
yes	62(59.6)	8 (53.3)	12 (44.4)	
Budding				
Low	$41(39.4)_a$	$1(6.7)_b$	$11(40.7)_a$	
Intermediate	$30(28.8)_a$	$9(60.0)_b$	$12(44.4)_{a,b}$	**0.029**
High	$33(31.7)_a$	$5(33.3)_a$	$4\ (14.8)_a$	
Stage				
I-II	$54(51.9)_a$	$3(20.0)_b$	$21(77.8)_c$	**0.001**
III-IV	$50(48.1)_a$	$12(80.0)_b$	$6(22.2)_c$	
Distant Metastasis				
M0	88(84.6)	12(80.0)	26(96.3)	0.214
M1	16(15.4)	3(20.0)	1(3.7)	
Survival				
Alive	$75(72.1)_a$	$9(60.0)_a$	$25(92.6)_b$	**0.036**
Dead	$29(27.9)_a$	$6(40.0)_a$	$2(7.4)_b$	
TILs				
no	$56(53.8)_a$	$2(13.3)_b$	$0(0.0)_b$	**<0.001**
yes	$48(46.2)_a$	$13(86.7)_b$	$27(100)_b$	
Lymph node metastasis				
no	$51(49.0)_a$	$10(66.7)_{a,b}$	$22(81.5)_b$	
pN1	$30(28.8)_a$	$4(26.7)_a$	$3(11.1)_a$	**0.029**
pN2	$23(22.1)_a$	$1(6.7)_a$	$2(7.4)_a$	

TILs:tumor infiltrating lymphocytes

*The symbols a and b indicate the statically difference between cells. There is a statistically significant difference between cells with different symbols.

RESULTS

The mean age of 146 patients enrolled in the study was 57.77±12.66 (min:16-max:80). Of these patients, 53 (36.3%) were female and 93 (63.6%) were male. While there was no statistically significant difference in terms of gender-specific microsatellite characteristic, it was found that the rate of MSS CRCs was higher in patients over 50 years of age. It was found that MSS CRCs were more localized to the left colon, while MSI-H CRCs were more localized to the right one. Of the colon cancer cases in our series, 96 (65.7%) were made up of adenocarcinoma, of the 50 (34.2%) mucinous carcinoma subtypes, 28 (19.2%) were adenocarcinomas with mucinous component and 22 (15%) were pure adenocarcinomas.

In MSI-H tumors, TILs response, histological subtype of mucinous adenocarcinomas was found to be statistically significantly higher. When compared in terms of the presence of lymph node metastasis, it was found that those without lymph node involvement were statistically significantly higher in the MSI-H group. In terms of microsatellite characteristics, no significant difference was found in terms of gender, histological grade, lymphovascular invasion, perineural invasion, and distant metastasis (Table 1).

Table 2. Distributions of loss of expression in microsatellite proteins

	n	%
MLH-1	12	28.6
MSH-2	2	4.8
MLH-1, PMS-2	11	26.2
MSH-2, MSH-6	9	21.4
MSH-2, MSH-6, MLH-1	3	7.1
PMS-2	1	2.4
MLH-1, MLH-2	1	2.4
MLH-1, MLH-6	3	7.1
Total	42	100.0

In terms of survival, the mortality rate can be seen to be higher in MSS and MSI-L CRCs than in MSI-H tumors. MSI-H CRCs were evaluated as earlier stage (T1-T2) than other MSS and MSI-L tumors. There were 104 (71.2%) MSS cases and, 42 (28.8%) MSI cases. 15 (10.3%) MSI tumors were found to be MSI-L, and 27 (18.5%) to be MSI-H. The most common loss of expression in microsatellite proteins is shown in MLH-1, MLH-1/PMS-2, MSH2/MSH-6 markers, respectively (Figure 3). The distributions of loss of expression in microsatellite proteins are given in Table 2. It was found that there was no significant difference in tumors with right or left involvement in terms of age, gender, histological subtype, grade, lymphovascular invasion, perineural invasion, stage, distant metastasis, survival, TILs, TBS and lymph node involvement (Table 3).

The mean follow-up period is 898.80 days (min=4-max=5249). The Cox-regression model with produced using significant variables was found to be significant in survival (p=0.008). The variables included in the model are TILs (reference category: no TILs), TBS (reference category: low budding) and MSI (reference category: stable). It was found that TILs and TBS variables contributed significantly to the model and that the presence of TILs exhibited a protective effect (Hazard ratio (HR)=0.446), which decreased the mortality risk by 2.24 times for the follow-up period, while the presence of high TBS increased the risk of mortality by HR=3.22 (95%CI HR=1.07-9.68) (Table 4) (Chart 1, 2).

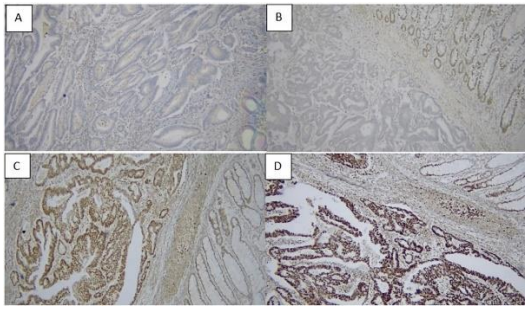

Figure 3. In this MSI CRC, there was loss of expression in (a) MLH1 and (b) PMS2 stained by immunohistochemical method, while nuclear expression was present in (c) MSH2 and (d) MSH6 proteins. (High-power image ×100)

Based on the Kaplan-Meier survival analysis performed according to the presence of TILs, a significant difference was found between the groups in terms of survival. It was found that the patients with TILs had a significantly longer life expectancy than those without (Table 5).

Microsatellite status was evaluated as stable in case of a nuclear staining over 1% in all MSH1, MSH2, MSH6 and PMS2 antibodies. However, we investigated whether there was a statistically significant difference in prognostic parameters in MSS tumors according to the percentage of staining in our study. When the percentages of staining were

compared according to survival and metastasis, TBS, TILs status and lymph node metastasis characteristics in MSS CRCs, no statistically significant difference was determined.

Table 3. Comparison of histopathological parameters by tumor localization

	Tumor Side		
Sex	Right colon	Left colon	p
Woman	21(38.2)	32(35.2)	0.850
Male	34(61.8)	59(64.8)	
Age			
0-49	14(25.5)	19(20.9)	0.663
≥50	41(74.5)	72(79.1)	
Histologic subtype			
Adenocarcinoma	31(56.4)	65(71.4)	0.093
Mucinous adenocarcinoma	24(43.6)	26(28.6)	
Grade			
well-moderate	45(81.8)	77(84.6)	0.833
Poor	10(18.2)	14(15.4)	
Lymphovascular invasion			
no	14(25.5)	19(20.9)	0.663
yes	41(74.5)	72(79.1)	
Perineural invasion			
no	29(52.7)	35(38.5)	0.131
yes	26(47.3)	56 (61.5)	
Budding			
Low	22(40.0)	31(34.1)	
Intermediate	17(30.9)	34(37.4)	0.688
High	16(29.1)	26(28.6)	
Stage			
I-II	33(60.0)	45(49.5)	0.286
III-IV	22(40.0)	46(50.5)	
Distant Metastasis			
M0	48(87.3)	78 (85.7)	0.986
M1	7(12.7)	13(14.3)	
Survival			
Alive	43(78.2)	66(72.5)	0.572
Dead	12(21.8)	25(27.5)	
TILs			
no	19(34.5)	39(42.9)	0.412
yes	36(65.5)	52(57.1)	
Lymph node metastasis			
no	36(65.5)	47(51.6)	0.239
pN1	12(21.8)	25(27.5)	
pN2	7(12.7)	19(20.9)	

TILs:tumor infiltrating lymphocytes

Table 4. The distributions of loss of expression in microsatellite proteins (Cox regression analysis)

	B	p	H.R.	95.0% CI for HR	
				Bottom	Top
TILs	-0.808	0.038	0.446	0.208	0.956
Low budding		0.112			
İntermediate budding	0.878	0.123	2.405	0.789	7.331
High budding	1.170	0.037	3.221	1.071	9.685
MSI	-0.277	0.550	0.758	0.305	1.883

TILs: tumor infiltrating lymphocytes; MSI: microsatellite instability

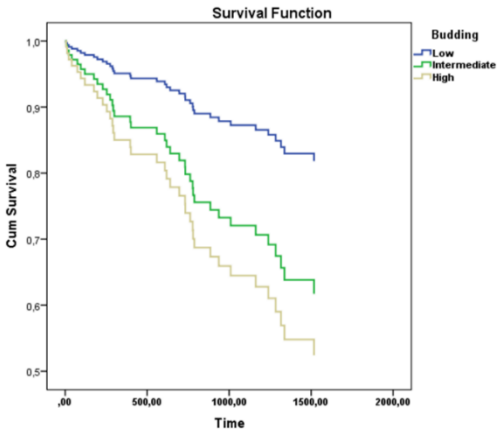

Figure 1. TBS-survival relationship

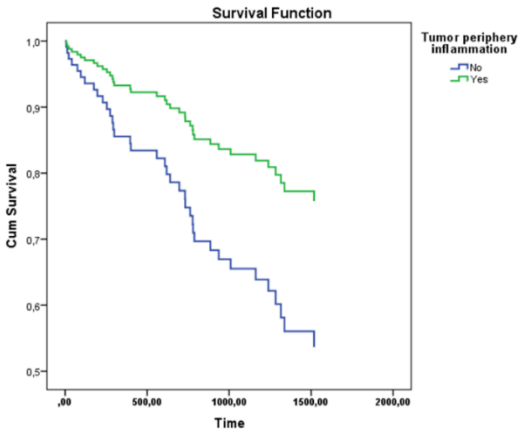

Figure 2. TIL-survival relationship

Table 5. Survival times according to the presence of tumor-infiltrating lymphocytes

Peritumoral inflamation	**Mean**				
	Calculated	Std. Error	95% Confidence Interval		
			Lower Limit	Upper Limit	p
No	1294.523	117.736	1063.760	1525.286	0.003
Yes	3976.171	316.772	3355.298	4597.044	
Total	3414.188	254.509	2915.351	3913.025	

Std: standard deviation

DISCUSSION

CRCs are a heterogeneous group of diseases exhibiting different molecular and genetic changes. MSI is one of the main causes leading to the development of CRCs. MSI, which has been reported in approximately 10% to 15% of colon cancers in the literature, was found to be 28.8% in our study group[3, 12, 13].

MSI tumors exhibit different clinicopathological features and variations in survival rate. In our study group, CRCs were more common in patients over the age of 50 in parallel with the literature[13]. El Agy et al. found in their study that MSI tumors were more common in patients under the age of 57[9].

In our patient group, male predominance was present in both MSI and MSS CRCs, and no significant difference was observed in terms of microsatellite characteristic. While El Agy et al. reported a male predominance in MSI CRCs in their study[9], Whitehall et al. reported female predominance in their study[13].

There are some studies showing that tumors with DNA dMMR have such specific clinicopathological features as being poorly differentiated and located in the right colon, presence of multiple TILs in addition to mucinous histology[13]. In terms of poorly differentiated carcinoma morphology, no significant difference could be shown between MSS and MSI CRCs in our study. Chapusot et al. reported that approximately one-third of MSI CRCs are localized in the right colon and the right colon localization presents poorly differentiated adenocarcinoma morphology[14]. In our study, MSI-H CRSs were found to be more localized to the right colon, consistent with the literature, El Agy et al. reported in their study that MSI CRCs were frequently located in the right colon[9], which also determines the response to immunotherapy in right colon tumors.

In our study, lymph node involvement was less frequently seen in MSI CRCs compared to MSS CRCs, which is consistent with the literature. In a study by El Agy et al, the number of metastatic lymph

nodes was also found to be lower in MSI CRCs (0.62 ±1.49) compared to MSS CRCs (1.33 ±3.10)[9].

El Agy et al. did not report any distant metastases in MSI CRCs in their study[9]. However, in our patient group, 1 (3.7%) of MSI-H CRCs had distant metastases. In MSI tumors related to the pathological disease stage, the early stage (I-II) is more common[9]. In our study, MSI-H tumors were mostly in the early stage (I-II) category.

Greenson et al. suggested in their study that not only pure mucinous adenocarcinomas, but also tumors with less than 50% mucinous component are associated with MSI[15]. Also, in our study, when mucinous adenocarcinoma and adenocarcinomas containing mucinous component were evaluated together, MSI-H CRCs presented more mucinous morphology, which is in line with the literature.

Elias et al. reported no statistically significant difference between MSI and MSS CRCs in terms of survival rate similar to our study[16]. However, in our study, the mortality rate was higher in MSS and MSI-L CRCs than in MSI-H tumors. In many studies, it has been reported that MSI CRCs have a better prognosis[16].

When we examined the histopathological features (Table 3) observed in tumors located in the right and left colon in our study, we could not detect a statistically significant difference. However, in the literature, Karahan et al. reported that tumors located in the right colon were larger in size, poorly differentiated and exhibited frequent mucinous features, lymph node metastasis, with a female predominance[17]. El Agy et al. reported in their study that CRCs located in the right colon appeared at a younger age than CRCs located in the left colon, exhibiting male predominance and higher survival rates. In addition, it has also been shown that the mucinous carcinoma subtype is more common in CRCs located in the right colon, and the presence of distant metastases is less frequent. In their study, 30.2% of patients with right-localized CRCs were present with MSI compared to 5.9% of patients with left-localized CRCs[9]. On the other hand, in our study, 19.7% of patients with left-localized CRCs and 43.6% of patients with right-localized CRCs exhibited MSI. Some studies have documented worse overall survival in right CRCs compared to left CRCs[18]. Philips et al. reported no significant difference between the two groups in terms of survival similar to our study[19]. However, the small number of cases may have led to this result.

El Agy et al. found that 293 of 330 CRC patients were MSS (88.8%) while 37 (11.2%) were MSI and the most common loss of expression of MMR proteins was present in MLH1/PMS2 pair[9]. In our study, 104 (71.2%) of CRC cases were MSS and 42 (28.8%) were MSI. The most common loss of expression was in the MLH-1 protein, followed by the MLH1/PMS2 pair. As in our study, MLH1 is the main protein most frequently affected in sporadic MSI CRCs[6].

In most studies conducted in recent years, it has been found that the presence of TILs is more prominent in MSI CRCs than in MSS CRCs. In our study, the presence of TILs in MSI CRCs was 100%, particularly in MSI-H tumors, which is consistent with the literature[14, 20].

MSI-H CRCs have a better prognosis than MSS CRCs[21]. There are some studies associating this condition with TILs. TILs play a protective role against tumor development by suppressing tumor growth and mediating the maturation and activation of immune cells. Therefore, as mentioned in a meta-analysis, it was emphasized that TILs density could be a strong prognostic marker for survival in patients with CRC[22]. In our study, we scored these as "yes" or "no" rather than TILs intensity. Consistent with the literature, we found that the presence of TILs exhibits a protective feature (Hazard ratio (HR)=0.446) and decreases the risk of mortality 2.24 times during the follow-up period. In a retrospective study by Rozek et al.[23] with 2,369 patients, and Sinicrope et al.[24] with 2,293, it was demonstrated that TILs provide a prognostic advantage with a maximum probability of overall survival (HR = 0.65).

Tumor budding has been identified as an unfavorable prognostic factor by the International Union against Cancer (UICC) based on data obtained from several studies. The UICC has classified tumor budding, together with histological grade, perineural invasion, and surgical margin into the category of "additional prognostic markers"[25]. It is known that the rates of lymph node metastasis, vascular invasion and distant metastasis are significantly increased in patients with high TBS. Tumor budding is less frequently encountered in MSI-H CRCs than in MSS CRCs[26]. In our study, we could not find a significant difference between MSS and MSI-H ones in terms of TBS. Graham et al. demonstrated in the multivariate analysis of a cohort study with 553 cases that patients

with high TBS were 2.5 times more likely to die from CRCs on average[27]. In our study, we found that the presence of high TBS increases the risk of mortality by HR=3.22 times. The presence of TILs is inversely proportional to higher TBS[26]. Several studies have proven that the adaptive immune response represented by cytotoxic T cells plays a key role in suppressing tumor invasion and metastasis. There are also studies showing that combined budding-immune cell score may be a better predictor of survival than using both parameters alone[28].

MSI-H tumors have an antigenic microenvironment with high density of TILs and express multiple immune checkpoint molecules, including PD-1 and PD-L1 due to mutation-related neo-antigens[29]. Today, a detailed understanding of the biology of dMMR is required not only to determine hereditary susceptibility to cancer, but to guide oncologists in coming up with the treatment options associated with both conventional chemotherapy and novel immunotherapies as well.

In our study, when MSS CRCs were compared in terms of staining percentages and some prognostic parameters (TILs status, TBS, lymph node metastasis or presence of distant metastases), we did not find any significant difference between these groups. In fact, it should be kept in mind that MMR markers in the tumor may display heterogeneous staining in IHC due to such reasons as brand of fixation, process or ambient temperature. However, there are studies reporting that staining loss may also occur in MMR markers following preoperative chemoradiation therapy[30].

The limitations in this study is that we could not test the microsatellite state with molecular approaches as we demonstrated with immunohistochemical studies.

In conclusion, it is emphasized in our study that patients with MSI CRC may show unique clinicopathological characteristics and should be evaluated together with some guiding parameters in survival. Pathologists should seek histopathological clues for MSI in all patients with CRCs, and search for Microsatellite proteins by IHC method, and assume a guiding role in chemotherapy protocols and in the follow-up of other family members before they get cancer.

Yazar Katkıları: Çalışma konsepti/Tasarımı: TT, AA, MA; Veri toplama: MA, TT; Veri analizi ve yorumlama: TT, ŞE, SP; Yazı taslağı: TT, AA, BM; İçeriğin eleştirel incelenmesi: ŞE, SP, EKB; Son onay ve sorumluluk: TT, KEE. EKB, AA, MA, SP, BM, ŞE; Teknik ve malzeme desteği: -; Süpervizyon: TT, KEE; Fon sağlama (mevcut ise): yok.

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