The Relationship of Microsatellite Instability with BRAF and p53 Mutations and Histopathological Parameters in Colorectal Adenocarcinoma

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Aim: This study aims to elucidate the associations between microsatellite instability (MSI) status, *BRAF* mutation, and *p53* reactions with pathological parameters and survival outcomes in colorectal carcinoma.
Material and Method: MutL homologous 1 (*MLH1*), Postmeiotic segregation increased 2 (*PMS2*), MutS homologous 2 (*MSH2*), MutS homologous 6 (*MSH6*), *BRAF*, and *p53* antibodies were performed on 130 adenocarcinoma samples, including 65 from the right colon and 65 from the left colon. The relationships of MSI status with *BRAF* mutation, *p53* reaction, clinical and pathological parameters, and survival times were statistically analyzed.
Results: A statistically significant relationship was found between MSI and right colon localization, tumor size, histological grade, intraepithelial tumor-infiltrating lymphocytes, Crohn-like lymphocytic reaction, expansive growth pattern, and *BRAF* mutation ($p < 0.05$). No significant correlation was found between MSI status and the disease-free or overall survival times ($p > 0.05$).
Conclusion: In colorectal adenocarcinoma, MSI and *BRAF* mutation are associated with parameters, indicating the host immune response and prognostic histopathological parameters, including tumor size and histological grade. The evaluation of MSI status and *BRAF* mutation can be particularly informative for predicting the prognosis and guiding the treatment management in poorly differentiated colorectal adenocarcinoma. Understanding the mechanisms of molecular carcinogenesis in colorectal carcinoma and organizing treatment algorithms based on molecular foundations will increase the success of the treatment.

Keywords: colorectal adenocarcinoma; MSI; *BRAF*; *p53*; histopathology; survival

Introduction

Approximately 90% of colorectal carcinomas are sporadic, while genetic syndromes play a role in about 10% of cases [1]. Colorectal carcinoma is a heterogeneous tumor group with various genetic pathways. The main pathways include chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylation phenotype (CIMP). These three developmental mechanisms in colorectal carcinomas are not entirely separate; several pathways can coexist in the same tumor [2]. About 85% of sporadic colorectal carcinomas use the CIN pathway, at the end of which the *p53* gene, the most commonly mutated tumor suppressor gene in cancer cells, is found. *P53* mutation is an important parameter for the biological behavior of colorectal carcinomas and is associated with prognostic indicators such as invasion depth and metastasis [3, 4, 5]. MSI pathway is encountered in 15% of sporadic colorectal carcinomas and develops due to genetic defects in DNA mismatch repair (*MMR*) genes [6].

Microsatellites are short DNA strands with 1-6 base repeats in the form of $[A]_n$ or $[CA]_n$. This system consists of MutL homologous 1 (*MLH1*), MutS homologous 2 (*MSH2*), MutS homologous 6 (*MSH6*), and Postmeiotic segregation increased 2 (PMS2) proteins [7]. The presence of MSI is considered to have clinical prognostic significance, with early-stage MSI tumors having better prognosis compared to microsatellite stable (MSS) and CIN pathway-using tumors. The CIMP pathway was defined as a separate pathway related to epigenetic instability and is generally seen in sporadic MSI colorectal carcinomas. This pathway is associated with silencing the *MLH1* gene by methylation and includes sporadic cases other than Lynch syndrome. In the CIMP pathway, *BRAF* mutation is one of the main genetic alterations [1]. *BRAF* is an oncogene involved in cell differentiation and proliferation. The *BRAF* V600E mutation is found in about 8% of all colorectal carcinomas but in 39% of those with MSI [8, 9]. It is suggested that MSS/*BRAF* V600E mutated colon carcinoma has a more mortal course and requires more aggressive adjuvant ther-

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apy, whereas *MSI/BRAF* V600E mutant tumors have a better prognosis [10, 11]. From this perspective, assessing the presence of *BRAF* mutation is essential for more effective clinical management.

Immunohistochemical studies that represent these three genetic pathways in colorectal carcinomas are available. In this study, immunohistochemical applications of *MLH1*, *MSH2*, *MSH6*, *PMS2*, *BRAF*, and *p53* antibodies were conducted on sections containing adenocarcinoma and normal mucosa from 65 right colon and 65 left colon-located total 130 cases diagnosed with colorectal adenocarcinoma. The aim is to determine the MSI status, *BRAF* mutation, and *p53* reaction and to investigate the relationship of MSI status with *BRAF* mutation, *p53* reaction, histopathological parameters, and survival.

Material and Method

Study Group Identification

The present study involves resection specimens from 65 right colon and 65 left colon cases diagnosed with adenocarcinoma between 2011 and 2018 in our department. The cases receiving neoadjuvant treatment were excluded. Patients' clinical data such as age, gender, disease-free and total survival times, and presence of metastasis were obtained from the patient files. In contrast, parameters such as tumor localization, tumor size, and growth pattern were obtained from the pathology reports. The study was approved by the Clinical Research Ethical Committee of Taksim Training and Research Hospital, Provincial Directorate of Health, on 07.02.2018 with the number of 10.

Histomorphological Assessment

Hematoxylin-eosin (H&E)-stained histopathological slides of the colon resection materials were retrieved from the archives and re-examined in terms of histopathological diagnosis, histological grade, growth pattern, mucinous differentiation, signet-ring cell differentiation, invasion depth, lymphovascular invasion, perineural invasion, stromal tumor-infiltrating lymphocytes (TIL), intraepithelial TIL, Crohn-like lymphocytic reaction, tumor necrosis, tumor heterogeneity, tumor deposit, development from adenoma, presence of adenoma, and lymph node metastasis based on the recent World Health Organization 2019 classification system [12]. Histological grade and tumor stage were evaluated according to the 2017 AJCC Cancer Staging Guideline's 8th Edition and the 2020 CAP Guideline [13, 14]. Histological grade 1 corresponds to well-differentiated, grade 2 to moderately differentiated, grade 3 to poorly differentiated, and grade 4 to undifferentiated. Cases with grades 1 and 2 were classified as Group 1, and grades 3 and 4 as Group 2 [13, 14].

Assessing the Intraepithelial TIL Response

The area of deepest tumor invasion was selected under $\times 40$ magnification to examine the intraepithelial lymphocytic reaction. The lymphocytes were counted in the five most intense sequential $\times 400$ magnification areas, and the mean value was calculated. Accordingly, the absence of intraepithelial TIL was classified as 0, and the presence of intraepithelial TIL 1 as 1 [15]. Stromal TIL response: the area of deepest tumor invasion was detected using a microscope at $\times 4$ magnification to examine the stromal lymphocytic reaction. The lymphocytes were counted in the five most intense sequential $\times 400$ magnification areas, and the mean value was calculated. They were classified as weak (0–10%): 1, moderate (20–40%): 2, and strong (50–90%): 3. Stromal TIL responses were divided into 2 groups as group 1: weak; group 2: moderate and strong [16]. When assessing the Crohn-like lymphocytic reaction, the lymphoid follicles at the sections' deepest point, where the tumor invaded the intestinal wall, were counted, and the mean values were calculated by dividing them by the total number of samples. The mean values ≥ 3 were accepted to be positive for the presence of lymphoid follicles. The lymphoid follicles located at the mucosa, those with lymph-node appearance, and those with irregular-shaped aggregate forms were excluded. All tumoral sections were examined, and $<10\%$ coagulation necrosis in tumor tissue was scored as 0, and $\geq 10\%$ was scored as 1. Detection of 2 or more histological types in the tumor was considered as tumor heterogeneity. For mucinous tumors, the absence of histological grade and structural differences in non-mucinous regions was not considered to be tumor heterogeneity [17]. The tumor nodules in areas other than lymph nodes or vascular structure and in pericolic/perirectal adipose tissue or mesentery, which were separated from the tumor mass, were considered as tumor deposits [13].

Immunohistochemical Method

A tissue block containing both invasive tumor and adjacent normal colonic mucosa was selected for each case. Using Ventana Benchmark XT model fully-automated immunohistochemical staining device, the cross-sections were subjected to *MLH1* (clone: G168-15, ready-to-use, Biocare), *MSH2* (clone: FE11, ready-to-use, Biocare), *PMS2* (clone: A16-4, ready-to-use, Biocare), *MSH6* (clone: 44, ready-to-use, Biocare), *p53* (clone: BP53-12, ready-to-use, Biocare), and *BRAF* V600E (clone: VE1, ready-to-use, Ventana) antibodies by using Optiview 3,3′-diaminobenzidine tetrahydrochloride (DAB) (catalog number: 05269806001, Ventana Medical Systems, Inc. 1910 E., Tucson, AZ, USA).

Immunohistochemical Assessment

The absence of nuclear reaction with at least one of *MLH1*, *MSH2*, *MSH6*, and *PMS2* antibodies in tumor cells was considered to be MSI. Focal or common nuclear reaction with all antibodies was considered to be MSS. Normal colon mucosa, inflammatory, and stromal cells were considered positive controls [18]. 50% or more nuclear positivity for *p53* antibody in tumor cells was considered posi-

tive, whereas <50% nuclear reaction was considered negative [19]. High-grade serous carcinoma of the ovary was used as positive control while assessing the *p53* antibody. No reaction with *BRAF* V600E antibody in tumor cells was scored as 0, whereas low-level cytoplasmic reaction was 1, moderate-level cytoplasmic reaction was 2, and strong cytoplasmic reaction was 3. Scores 0 and 1 were considered negative, whereas scores 2 and 3 were considered positive [20]. Papillary thyroid carcinomas showing *BRAF* V600E mutation with a molecular method were used as a positive control.

Statistical Analysis

The variables showing normal distribution between two groups were analyzed using independent samples *t*-test, whereas those not distributed normally were analyzed using Mann Whitney U test. Nominal variables were analyzed using the Chi-Square test, Yates-corrected Chi-Square, and Fisher's exact probability test. The coherence between *p53* and *BRAF* was tested using the McNemar test. The level of statistical significance was set at $p < 0.05$ and taken bilaterally. The survival analysis was performed using Kaplan-Meier analysis, and the survival times were compared using the Logrank test. Analyses were performed using NCSS 10 (NCSS, Kaysville, UT, USA) software.

Results

Clinical and Histopathological Results

The mean age was 63.45 ± 12.49 (26–87) years. 68 (52.3%) cases were male and 62 (47.7%) were female. Tumors were located in the right colon in 65 (50%) and the left colon in 65 (50%). The localizations were cecum in 32 (24.6%), ascending colon in 19 (14.6%), hepatic flexure in 8 (6.2%), transverse colon in 7 (5.4%), splenic flexure in 1 (0.8%), descending colon in 7 (5.4%), sigmoid colon in 27 (20.8%), rectum in 24 (18.5%), and rectosigmoid in 5 (3.8%). Mean tumor size was 5.74 ± 2.60 (2–15) cm. 115 (88.5%) cases were of histological grade 1–2, whereas 15 (11.5%) of the cases were classified as grade 3. Muscularis propria invasion (stage 2) was found in 22 (16.9%), pericolic fatty tissue (subserosa, stage 3) invasion in 78 (60%), and serosal invasion (stage 4) in 30 (23.1%). 87 (66.9%) showed an ulcerovegetative growth pattern, 23 (17.7%) showed a polypoid growth pattern, and 20 (15.4%) showed an ulceroinfiltrative growth pattern. An expansive growth pattern was observed in 26 (20%). Mucinous differentiation was found in 52 (40%), signet-ring cell differentiation in 10 (7.7%), and tumor heterogeneity in 7 (5.4%), whereas adenoma-originated tumor development was found in 12 (9.2%). Lymphovascular invasion was detected in 94 (72.3%) and perineural invasion in 41 (31.5%). Tumor deposit was found in 25 (19.2%) and tumor necrosis in 43 (33.1%). Among 84 stromal TIL group 1 cases, 53 (40.8%) were weak, 31 (23.8%) were negative; 46 (35.4%) cases were in stromal TIL group 2, and 3 (2.3%) of them were severe, and 43 (33.1%) were moderate. Intraepithelial TIL was found in 49 (37.7%) and Crohn-like lymphocytic reaction in 34 (26.2%). Moreover, adenoma was found in 25 cases (19.2%) at different localizations. Lymph node metastasis was found in 59 (45.4%), whereas 42 (32.3%) of them had capsular invasion and 26 (20%) had non-capsule invasion. According to AJCC 2017 Guideline, 18 (13.8%) were stage 1, 40 (30.8%) were stage IIA, 9 (6.9%) were stage IIB, 4 (3.1%) were stage IIIA, 30 (23.1%) were stage IIIB, 18 (13.8%) were stage IIIC, 10 (7.7%) were stage IVA, and 1 (0.8%) was stage IVC. Disease-free survival times could be calculated for 103 out of 130 cases, and the mean disease-free survival time was 80 ± 4 months. The mean total survival time ± SD was 72 ± 4 months. Metastasis was found in 9 cases (7%) at the time of diagnosis, 2 were MSI, and 7 were MSS.

Immunohistochemical Results

Among 130 cases, there were 25 (19.2%) cases with MSI and 105 (80.8%) with MSS. *BRAF* mutation was found in 14 (10.8%), with 10 (7.7%) being moderate-strength reactions, and 4 (3.1%) being severe reactions. In 116 cases with no *BRAF* mutation, 13 (10%) cases were found to have a weak response, whereas no reaction was observed in 103 (79.2%) cases. *P53* reaction was positive in 60 (46.2%) cases and negative in 70 (53.8%) cases.

Microsatellite Instability Status' Relationship with Clinical, Histopathological Parameters and BRAF Mutation, p53 Reaction

The median age was 67 (25–75 percentile) in the MSI group (54–77) and 64 (25–75 percentile) in the MSS group (57–71), and there was no statistically significant difference between the two groups ($p = 0.743$). There were 13 (52%) women and 12 (48%) men in the MSI group and 49 (46.7%) women and 56 (53.3%) men in the MSS group. No significant relationship was found between gender and MSI ($p = 0.797$). The median value in the MSI group was found to be 7 median (25–75 percentile) (5–9.5) cm, and the median in the MSS group was found to be 5 medians (25–75 percentile) (4–6) cm. The tumor size was significantly larger in the MSI group ($p = 0.003$). In the MSI group, there were 20 cases (80%) with right colon localization and 5 cases (20%) with left colon localization, and the presence of MSI was significantly higher in right-colon localization ($p = 0.002$). MSI was found to have a statistically significant relationship with histological grade, intraepithelial TIL, Crohn-like lymphocytic reaction, and expansive growth pattern ($p < 0.05$) (Fig. 1). The relationship between MSI and histopathological parameters is summarized in Table 1. A statistically significant relationship was found between MSI and *BRAF* mutation ($p < 0.001$), whereas no statistically significant relationship was found with *p53* reaction ($p = 0.175$) (Table 2).

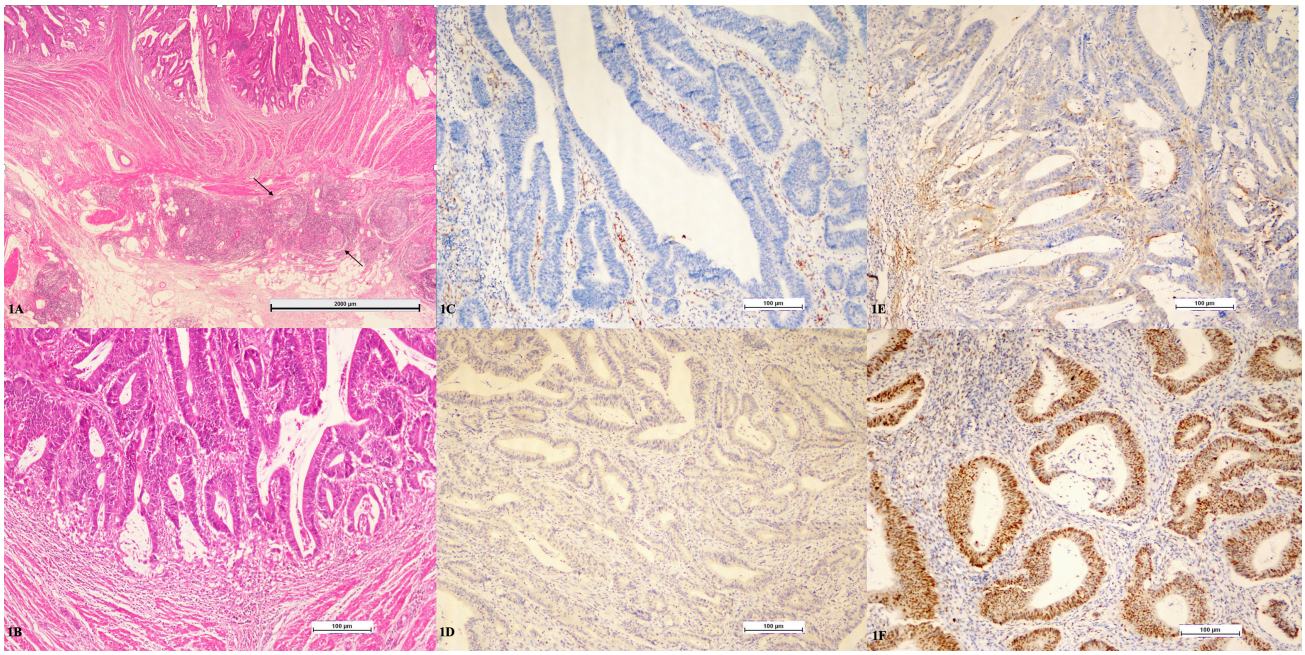

Fig. 1. Hematoxylin and Eosin and Immunohistochemical Images of the Cases. (A) Crohn-like lymphocytic reaction (→) area in colon adenocarcinoma showing expansive growth pattern (H&E × 20). (B) The area showing increased intraepithelial tumor-infiltrating lymphocytes (H&E × 100). (C) In the area of colon adenocarcinoma, there is a loss of reaction with *MLH1*, with stromal lymphocytes seen as a positive control (IHC × 100). (D) Weak cytoplasmic reaction with *BRAF* (IHC × 100). (E) Loss of reaction with *PMS2*, with lymphocytes seen as a positive control (IHC × 100). (F) Positive reaction with *p53*. H&E, Hematoxylin-eosin; *MLH1*, MutL homologous 1; *PMS2*, Postmeiotic segregation increased 2; IHC, Immunohistochemistry.

In the MSI group, there were 25 (19.2%) cases composed of 18 (13.8%) *MLH1* (-)/*PMS2* (-) cases, 2 (1.5%) *MSH2* (-)/*MSH6* (-) cases, and 5 (3.8%) *PMS2* (-) cases. However, there was not only an *MSH6*-negative case. Considering the *BRAF* mutation, there was a significant relationship between the *MLH1* (-)/*PMS2* (-) group and *MSH2* (-)/*MSH6* (-) and *PMS2* (-) groups ($p = 0.02$). No significant difference was found between these groups in terms of *p53* mutation, tumor localization, gender, and histopathological parameters ($p > 0.05$) (Table 3).

The Relationship of Tumor Localization with Clinical and Histopathological Parameters

Statistically significant differences were found between right and left-colon localization regarding histological grade, mucinous differentiation, signet-ring cell differentiation, presence of tumor deposit, and expansive growth pattern ($p < 0.05$). No statistically significant difference was found in gender and other histopathological parameters ($p > 0.05$). A statistically significant relationship was found between right-colon localization and left-colon localization in terms of *BRAF* mutation ($p < 0.05$), but there was no relationship in terms of *p53* immune reaction ($p > 0.05$).

P53 Reaction's Relationship with Clinical and Histopathological Parameters

P53 reaction was found in 30 cases (50%) with right-colon localization and 30 cases (50%) with left-colon localization. *p53* reaction was found to have a statistically significant relationship with clinical and histopathological parameters ($p > 0.05$).

BRAF Mutation's Relationship with Clinical and Histopathological Parameters

BRAF mutation was found in 14 (100%) cases with right-colon localization but none of the cases with left-colon localization. *BRAF* mutation was found to have a statistically significant relationship with histological grade ($p = 0.009$) and right-left colon localization ($p = 0.003$), whereas no relationship was found with gender and other histopathological parameters ($p > 0.05$).

BRAF Mutation's Relationship with p53 Reaction

BRAF mutation and *p53* reaction were found in 6 (4.6%) cases. Besides that, 8 cases (6.2%) were found to have *BRAF* mutation but no *p53* reaction, whereas 62 cases (47.7%) were found to be negative for *BRAF* mutation and *p53* reaction. Fifty-four cases (41.5%) were positive for p52 reaction but negative for *BRAF* mutation. A statistically significant inverse relationship was found between *BRAF* mutation and *p53* reaction ($p < 0.001$).

Survival Analysis

No statistically significant difference was found between MSI and MSS cases in terms of disease-free survival and total survival ($p > 0.05$) (Figs. 2,3). No statistically signif-

Table 1. Relationship between microsatellite instability status and histopathological parameter.

Parameters		Microsatellite unstable		Microsatellite stable		*p*
		Number	%	Number	%	
Localization	Right colon	20	80	45	43	0.002
	Left colon	5	20	60	57	
Tumor size (cm) (median)		7 (5–9.5)		5 (4–6)		0.003
Histological grade	1–2	19	76	96	91.4	0.041
	3	6	24	9	8.6	
Intraepithelial TIL	Yes	16	64	33	31.4	0.005
	No	9	36	72	68.6	
Crohn-like reaction	Yes	13	52	21	20	0.003
	No	12	48	84	80	
Expansive growth pattern	Yes	10	40	16	15.2	0.01
	No	15	60	89	84.8	
Mucinous differentiation	Yes	10	40	42	40	1
	No	15	60	63	60	
Signet-ring cell differentiation	Yes	3	12	7	6.7	0.404
	No	22	88	98	93.3	
Tumor heterogeneity	Yes	2	8	5	4.8	0.619
	No	23	92	100	95.2	
Adenoma basis	Yes	3	12	9	8.6	0.70
	No	22	88	96	91.4	
Tumor deposit	Yes	6	24	19	18.1	0.573
	No	19	76	86	81.9	
Lymphovascular invasion	Yes	16	64	78	74.3	0.433
	No	9	36	27	25.7	
Perineural invasion	Yes	7	28	34	32.4	0.854
	No	18	72	71	67.6	
Tumor necrosis	Yes	7	28	36	34.3	0.716
	No	18	72	69	65.7	
Stromal TIL	Yes	11	44	35	33.3	0.441
	No	14	56	70	66.7	
Adenoma	Yes	3	12	22	21	0.404
	No	22	88	83	79	
Lymph node metastasis	Yes	13	52	46	43.8	0.606
	No	12	48	59	56.2	

TIL, tumor-infiltrating lymphocytes.

icant relationship was found between disease-free survival, total survival, and metastasis at the moment of diagnosis in terms of *BRAF* mutation, *p53* immune reaction, right-left colon localization, intraepithelial TIL, stromal TIL and Crohn-like reaction ($p > 0.05$) (Tables 4,5) (Immunohistochemistry (IHC) $\times$ 100).

Discussion

The MSI pathway is related to germline mutations in Lynch syndrome and somatic mutations and *MLH1* epigenetic mutations in sporadic cases, and it is generally accompanied by aberrations in the methylation pathway [1]. In literature, the rate of MSI in colorectal carcinomas was reported to vary between 9 and 28%. This difference has been observed related to patients' ages and tumor localizations [21]. In the present study, this rate was found to be 19.2%. Similar to the present study, Benatti *et al*. [22] reported the MSI rate to be 20%. MSI is detected more frequently in the right colon (82.1% in the right colon and 17.9% in the left colon) [23]. Similarly, it was determined in the present study that 80% of MSI cases have right colon localization. In the literature, the mean age of cases with detected MSI was reported to be 67 years (20–90), and, in parallel with the literature, the mean age in the present study was found to be 67 years (54–77) [24].

Table 2. Microsatellite instability status' relationship with *BRAF* mutation and *p53* reaction.

		Microsatellite unstable		Microsatellite stable		*p*
		Number	%	Number	%	
BRAF mutation	Yes	10	40	4	3.8	<0.001
	No	15	60	101	96.2	
p53 reaction	Yes	8	32	52	49.5	0.175
	No	17	68	53	50.3	

Table 3. Distribution of BRAF mutation and *p53* reaction in *MLH1* (-)/*PMS2* (-), *MSH2* (-)/*MSH6* (-) and *PMS2* (-) groups.

		MLH1 (-)/*PMS2* (-)		*MSH2* (-)/*MSH6* (-), *PMS2* (-)		*p*
		Number	%	Number	%	
BRAF mutation	Yes	10	55.6	0	0	0.02
	No	8	44.4	7	100	
p53 reaction	Yes	6	33.3	2	28.6	1
	No	12	66.7	5	71.4	

MSH2, MutS homologous 2; *MSH6*, MutS homologous 6.

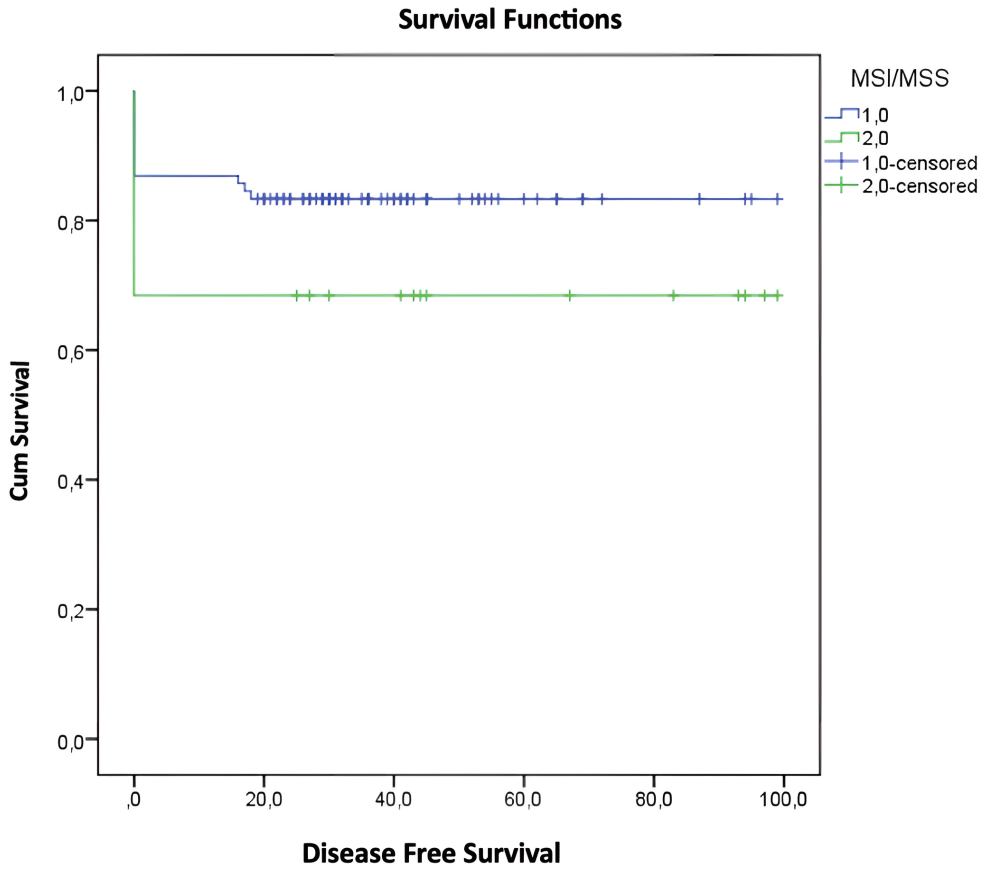

Fig. 2. The relationship between disease-free survival and microsatellite instability (MSI)/microsatellite stable (MSS) status. No statistically significant relationship was detected between disease-free survival and MSI/MSS status ($p > 0.05$).

Benatti *et al*. [22] reported a relationship between MSI and right colon localization, poor differentiation, mucinous differentiation, expansive growth pattern, and early-stage. In different studies, besides these parameters, MSI was found to be also related to female gender, tumor size, signet-ring cell differentiation, tumor heterogeneity, intraepithelial TIL, Crohn-like lymphocytic reaction, and tumor necrosis, and it was reported that MSI had a positive contribution to the survival of colorectal carcinomas [16, 23]. In the present study, MSI was found to have a statistically significant relationship with right colon localization, histological grade, tumor size, intraepithelial TIL, Crohn-like

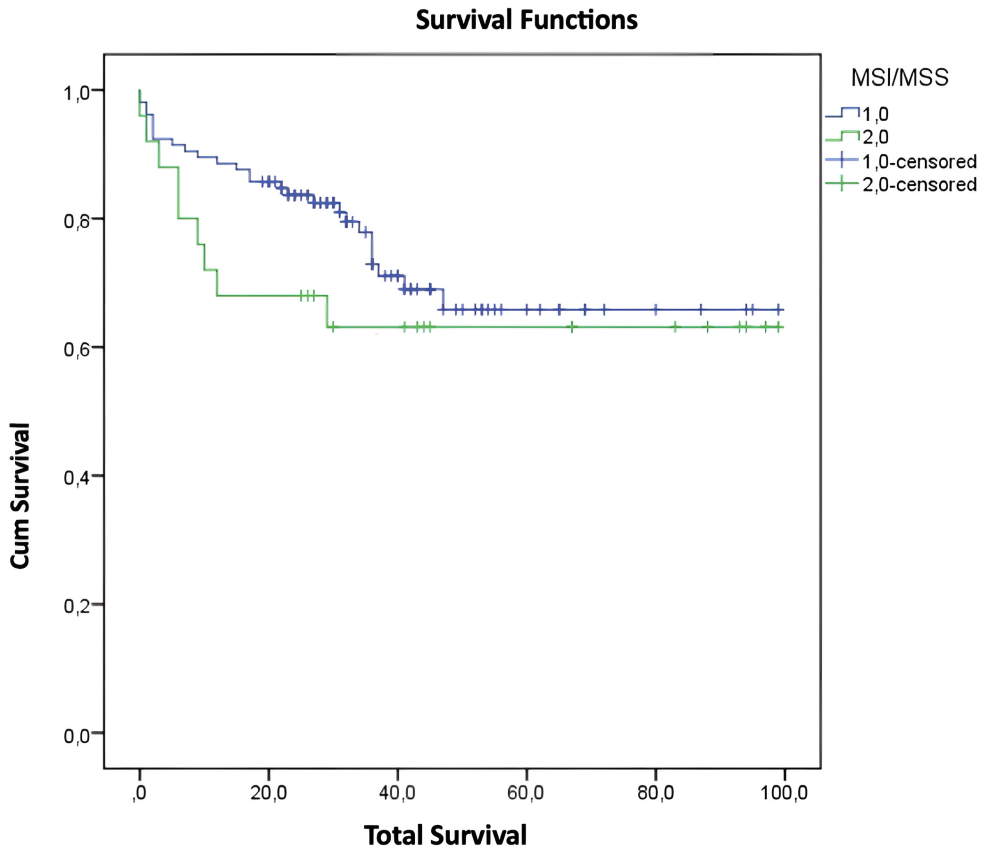

Fig. 3. The relationship between total survival and MSI/MSS status. No statistically significant relationship was detected between total survival and MSI/MSS status ($p > 0.05$).

lymphocytic reaction, and expansile growth pattern. Local anti-tumoral immune response development and organization constitute the tumor microenvironment and indicate the immune response quality. The presence of stromal and intraepithelial TIL at the deepest invasion is the first defense mechanism against metastasis. It was reported that lymphovascular invasion, perineural invasion, and metastasis are more frequently seen in the absence of TIL, and the disease-free survival is shorter. For this reason, it is thought that the presence of TIL is important in the progression of colorectal carcinoma. In the present study, stromal TIL, intraepithelial TIL, and Crohn-like reaction were found at a higher rate in the MSI group, and a statistically significant relationship was found between intraepithelial TIL and Crohn-like reaction and MSI. In contrast, no significant relationship was observed with stromal TIL. Klintrup *et al*. [25] determined that the rate of intraepithelial TIL was higher in cases with MSI, and the presence of intraepithelial TIL had positive effects on 5-year survival. Accordingly, due to the higher rate of TIL, MSI colorectal carcinoma is related to an earlier stage and better prognosis [15]. The present study examined the relationship between intraepithelial TIL, stromal TIL, and Crohn-like reaction and survival, but no statistically significant relationship was found. It might be because of the relative sparsity of cases with MSI.

Buckowitz *et al*. [26] determined that Crohn-like reaction is more frequently seen in the presence of MSI, and even at an advanced pathological stage, these tumors rarely had distant metastasis. This situation was associated with the mutations developed in metastasis promotor genes and the host immune response related to lymphocytic infiltration. It is estimated that the immune response increases and metastatic potential decreases in MSI tumors because of the frameshift mutation in most gene regions, where there are tumor-specific neo-peptides. For this reason, it was asserted that a vaccine developed against these neo-peptides might be used to treat MSI tumors [26]. Neumann *et al*. [18] related the lower prevalence of distant metastasis in the presence of MSI to stem cell-related genes. In parallel with the present study, the results suggest that the development of anti-tumoral response is related to MSI and should be considered an indicator of a better prognosis.

Rosty *et al*. [27] determined that the MSI rate was higher in poorly differentiated colorectal carcinomas and that MSI poorly differentiated tumors had a better prognosis than MSS ones, and they even acted as well-differentiated tumors. In the present study, MSI was at higher rates in poorly differentiated tumors than in moderate and well-differentiated ones. This finding can be explained by the better prognostic effect of the presence of MSI, which is

Table 4. Relationship between disease-free survival and MSI, *BRAF*, *p53*, and histopathological parameters.

Parameters		Number	Mean ± SD (month)	*p*
MSI/MSS	MSS	84	83.11 ± 3.89	0.132
	MSI	19	67.73 ± 10.56	
BRAF mutation	No	93	79.32 ± 4.04	0.435
	Yes	10	83.70 ± 8.82	
p53 reaction	No	55	83.12 ± 4.84	0.403
	Yes	48	77.00 ± 5.84	
Localization	Right colon	46	79.97 ± 5.69	0.965
	Left colon	57	56.29 ± 3.46	
Stromal TIL	1	24	63.37 ± 8.59	0.135
	2	43	80.59 ± 5.87	
	3	33	87.45 ± 5.39	
Intraepithelial TIL	0	63	80.66 ± 4.77	0.908
	1	40	79.65 ± 6.13	
Crohn like reaction	0	79	81.87 ± 4.16	0.436
	1	24	75.00 ± 8.51	

TIL, tumor-infiltrating lymphocytes.

more effective than the poor prognostic effect of high histologic grade. Thus, the evaluation of the presence of MSI gains importance in predicting survival [27].

Tie *et al*. [28] determined the rate of *BRAF* mutation in colorectal carcinoma to be 10%, and *BRAF* mutation was found to be at a higher rate in right-colon localization and in poorly differentiated tumors among the women. Salem *et al*. [29] also found a statistically significant relationship between MSI and *BRAF* mutation. Like Salem *et al*. [29], we also depicted a significant relationship between *BRAF* mutation and the presence of MSI. Jang *et al*. [30] reported that MSI tumors are seen at earlier ages, in the proximal colon, and are related to Crohn-like lymphocytic reactions and the presence of TIL. The authors also emphasized that *BRAF* mutation is related to advanced stage, poor differentiation, proximal colon, and tumor size [30]. In the present study, MSI was detected at higher rates in right-colon adenocarcinomas, and *BRAF* mutation was found only in right-colon localization. Moreover, *BRAF* mutation was found to be at a higher rate in the MSI group than in the MSS group.

There was no difference between the MSI tumors with sporadic and germline mutations regarding histopathological results. Both were observed more frequently in the right colon, sporadic cases were observed more frequently among women aged 70 years or older, and germline cases were observed more frequently among young men [28]. In the present study, a statistically significant difference was found between *BRAF* mutations in *MLH1* (-)/*PMS2* (-) cases, which generally represent the sporadic cases, and in *MSH2* (-)/*MSH6* (-) and *PMS2* (-) cases, which typically represent the cases with germline mutation. As stated in different studies, this finding can be explained by the relationship between *MLH1* gene defect and *BRAF* mutation. In parallel with the literature, this result corroborates that *BRAF* mutation could be used to distinguish sporadic cases. Hu W. *et al*. [31] reported the relationship between *BRAF* mutation and *MLH1* gene defect in their study. Moreover, it was also reported that TIL has an effective role in treatment response, that not every case with MSI responds to immune checkpoint inhibitors, and that there might be cases with poor prognosis and different molecular findings [31].

Comparing the survival times in the literature, it can be seen that the cases with MSI had longer total survival and disease-free survival times than those with MSS. The present study found no statistically significant difference between total survival and disease-free survival times of MSI and MSS cases ($p > 0.05$). Although the presence of *BRAF* mutation was related to the poor prognosis, there are also studies reporting that the MSI/*BRAF* mutation cases had longer disease-free survival time. The positive prognostic effect of MSI can explain this finding. Comparing those with MSS/*BRAF* mutation and MSS/*BRAF* wild-type, it was determined that those with MSS/*BRAF* mutation had a poorer prognosis. This finding can also be explained by the poorer prognostic effect of *BRAF* mutation [32]. In the present study, no statistically significant difference was found in total survival and disease-free survival times between those with MSI/*BRAF* mutation and those with MSI/*BRAF* wild-type, and between those with MSS/*BRAF* mutation and those with MSS/*BRAF* wild-type. It can be explained by the lower number of cases in the groups in the present study.

Since distant and local metastases are observed less frequently in MSI tumors independently from the histopathological findings, the number of Stage III and IV is lower. Still, a better prognosis is observed in MSI cases, even for tumors at the same stage [22]. Depending on the status of MSI and *BRAF*, the option of adjuvant therapy is an impor-

Table 5. Total survival relationship with MSI, *BRAF*, *p53*, and histopathological parameters.

		Number	Mean ± SD (month)	*p*
MSI/MSS	MSS	105	73.13 ± 4.26	0.329
	MSI	25	65.80 ± 8.86	
BRAF mutation	No	116	72.76 ± 4.04	0.719
	Yes	14	64.92 ± 10.05	
p53 reaction	No	70	73.89 ± 5.12	0.770
	Yes	60	71.03 ± 5.41	
Localization	Right colon	65	69.53 ± 5.10	0.417
	Left colon	65	54.43 ± 3.47	
Stromal TIL	1	31	59.15 ± 7.51	0.302
	2	53	72.28 ± 5.84	
	3	43	77.89 ± 6.19	
Intraepithelial TIL	0	81	70.83 ± 4.80	0.691
	1	49	74.69 ± 6.06	
Crohn like reaction	0	96	73.27 ± 4.43	0.552
	1	34	69.20 ± 7.32	

tant point. Seppälä *et al*. [10] reported in their study that adjuvant therapy is not necessary for Stage I–III MSI/*BRAF* mutant cases, but due to the increased mortality risk, aggressive adjuvant therapy is needed for MSS/*BRAF* mutant cases (even for Stage I–II). The present study determined that *BRAF* mutation increased the mortality, but the presence of MSI decreased the mortality. Besides its prognostic importance, examining the *BRAF* mutation and MSI might be important for directing the treatment options by categorizing the cases.

Samowitz *et al*. [33] found that the *p53* reaction in the MSS group was significantly higher than in the MSI group, and no statistically significant difference was found between the right and left colons regarding *p53* reactions. This finding might be related to different tumoral growth pathways in MSI and *p53* [33]. In the present study, the rate of *p53* was found to be higher in the MSI group than in the MSS group, but the difference was not statistically significant.

Oncological treatments are progressing to immune checkpoint inhibitors and immunotherapy. It was asserted that 5-FU treatment was ineffective in Stage II and III MSI tumors and might even be harmful because there might be additional adverse effects [22]. In Stage III colorectal carcinomas, using oxaliplatin instead of leucovorin and 5-FU treatment extends the disease-free survival in the presence of MSI. Oxaliplatin was superior to 5-FU in the presence of *p53* mutation [7]. In 2015, the FDA declared that the anti-PD-1 medication pembrolizumab could be used in metastatic and resistant MSI colorectal carcinomas [32]. When used as monotherapy, PD-1/PD-L1 inhibitors decrease the tumor size, providing long-term effects and less toxicity. Mutation-related neo-antigens increase in the presence of MSI, stimulate the immune system against tumors, and increase the anti-PD-1 response [34]. TIL prevalence in MSI tumors is higher than in MSS tumors. Despite the immune environment around the tumor, MSI tumors cannot be totally eliminated due to cancer-specific checkpoint inhibitors such as PD-1. This active microenvironment is balanced by the immune inhibitory signals. For this reason, MSI colorectal carcinomas are a candidate for immunotherapy. It was found that, among MSI and MSS colorectal carcinoma cases under pembrolizumab treatment, MSI cases had a longer immune response and disease-free survival times. Moreover, in MSI sporadic cases, the response to pembrolizumab is better than that of hereditary cases. This finding is explained by fewer frame-shift mutations among the germline mutation cases [32]. In colorectal adenocarcinomas, PD-L1 expression is related to poor differentiation, the presence of MSI, *BRAF* mutation, and the presence of TIL [35]. The presence of TIL accompanied by PD-1 expression is related to a good prognosis in MSI colorectal tumors. In contrast, the expression of PD-L1 in tumors is related to short survival time and poor prognosis [36].

Conclusion

In the present study, MSI was detected at a rate of 19.2%. MSI was associated with *BRAF* mutation, tumor localization, and significant prognostic histopathological parameters (tumor size, lower histological grade, intraepithelial TIL, Crohn-like lymphocytic reaction, and expansive growth pattern). It was observed that MSI cases were more frequently located in the right colon. MSI plays a critical role in colorectal carcinomas for investigating Lynch syndrome, determining the clinical course as a favorable prognostic parameter, and assessing the treatment options as a predictive factor. When examined with the presence of MSI in managing colorectal carcinomas, *BRAF* mutation is important in excluding Lynch syndrome. Evaluating the *BRAF* mutation in conjunction with MSI status might be useful in effective treatment management, assessing the

clinical course, and predicting the response to chemotherapy agents in colorectal carcinoma. Understanding the mechanisms of molecular carcinogenesis and establishing treatment algorithms based on this molecular foundation will enhance the success rate of therapies. Hence, assessing the MSI status and *BRAF* mutation in colorectal adenocarcinomas can provide valuable treatment management and prognosis insights, particularly in poorly differentiated tumors.

Availability of Data and Materials

All data in this study are confidential, kept by the corresponding author, and can be made available by the corresponding author only in some cases of necessity. In this article, the data cannot be shared due to ethical concerns.

Author Contributions

ÖG: defining the subject, writing, planning; SB: defining the subject, making corrections, writing; EY: sharing clinical information, generating ideas, evaluation; DG: sharing clinical information, generating ideas, evaluation. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The ethical committee approval was granted by the Taksim Training and Research Hospital Clinical Research Ethics Committee on 07.02.2018, under the approval letter number 10. The consent of the patients was taken prior to the writing of the manuscript. The study is in accordance with the Declaration of Helsinki.

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Conflict of Interest

The authors declare no conflict of interest.

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