ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Comparison of KRAS Mutation, Microsatellite Instability and Histomorphologic Features in Metastatic Colorectal Carcinomas: Single Centre Experience

Metastatik Kolorektal Karsinomlarda KRAS Mutasyonu, Mikrosatellit İnstabilite ve Histomorfolojik Özelliklerin Karşılaştırılması: Tek Merkez Deneyimi

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ABSTRACT

Aim: *Microsatellite instability (MSI) and KRAS mutations change colorectal carcinoma (CRC) treatment protocols. Advanced examinations such as immunohistochemistry and polymerase chain reaction (PCR) are required to determine MSI and KRAS mutations. On the other hand, Crohn-like lymphoid reaction (CLR), tumor-infiltrating lymphocytes (TIL), tumor budding (TB), and desmoplastic response (DR) are histomorphologic features that can be determined only with routine hematoxylin-eosin (H&E) sections. Our study aimed to evaluate relationships between MSI, KRAS mutations, and histomorphologic features. It was thought that the relationships to be determined may be useful in predicting KRAS mutations and MSI by evaluating only H&E sections.*

Material and Method: *One hundred nine metastatic CRC cases were reviewed retrospectively. Polymerase chain reaction results were obtained from the molecular pathology report archive and performed on all cases for KRAS mutation detection upon clinical request during routine pathologic examinations. MLH1, MSH2, MSH6, and PMS2 immunohistochemistry, performed on 70 cases for MSI interpretation upon clinical request during routine pathological examinations, was re-evaluated for standardization. Routine H&E sections with tumors were examined for CLR, TIL, TB, and DR according to study-specific criteria.*

Results: *KRAS mutations were found in 35.77% (39/109), MSI in 24.28% (17/70), CLR in 32.11% (35/109), TIL in 44.95% (49/109), TB in 73.39% (80/109), DR in 84.40% (92/109) of the cases. CLR, TIL, DR, and KRAS mutations were higher in microsatellite stable (MSS) cases, and TB was higher in MSI cases. Crohn-like lymphoid reaction, TIL, DR, and MSI were higher in KRAS wild cases, and TB in KRAS mutant cases. Only the MSS-DR correlation was statistically significant.*

Conclusion: *The MSS-DR correlation was statistically significant in our study. However, desmoplasia was determined in 92.45% of MSS cases, and was also determined in 58.82% of MSI cases. Because DR is an expected feature in tumor stroma, its guidance in terms of MSI was limited. Also, no significant relationship was found between MSI and DR in Turkish or English literature. In our study, histomorphologic features were insufficient to predict MSI and KRAS mutations. It is vital to immediately refer patients with metastases evaluated in centers without immunohistochemistry and PCR facilities to an advanced center for MSI and KRAS mutation determination diagnosing CRC, especially for treatment selection.*

Key words: *colorectal cancer; KRAS protein; microsatellite instability*

ÖZET

Amaç: *Kolorektal karsinomlarda (KRK) mikrosatellit instabilite (MSİ) ve KRAS mutasyonu tedavi protokollerini değiştirir. Mikrosatellit instabilite ve KRAS mutasyonunu belirlemek için immünohistokimya ve PCR gibi ileri incelemeler gerekir. Diğer taraftan Crohn benzeri lenfoid reaksiyon (CBLR), tümörü infiltre eden lenfositler (TİEL), tümör tomurcuklanması (TT) ve desmoplastik yanıt (DY) yalnızca rutin hematoksilen-eozin (H&E) kesitlerle belirlenebilen histomorfolojik özelliklerdir. Çalışmamızda MSİ, KRAS mutasyonu ve histomorfolojik özellikler arasındaki ilişkilerin değerlendirilmesi amaçlanmıştır. Saptanacak ilişkilerin sadece H&E kesitler değerlendirilerek MSİ ve KRAS mutasyonunu öngörmede faydalı olabileceği düşünülmüştür.*

Materyal ve Metot: *Çalışmada 109 metastatik KRK olgusu retrospektif olarak incelenmiştir. Rutin patolojik inceleme yapılırken klinik istek üzerine KRAS mutasyonu tespiti için tüm olgulara uygulanmış olan PCR sonuçlarına moleküler patoloji rapor arşivinden ulaşılmıştır. Rutin patolojik inceleme yapılırken klinik istek üzerine MSİ yorumlaması için 70 olguya uygulanmış olan MLH1, MSH2, MSH6, PMS2 immünohistokimyaları standarizasyon amacıyla tekrar değerlendirilmiştir. Rutin tümörlü H&E kesitleri CBLR, TİEL, TT, DY açısından çalışma için oluşturulan kriterlere göre incelenmiştir.*

Bulgular: *Olguların %35,77'sinde (39/109) KRAS mutasyonu, %24,28'inde (17/70) MSİ, %32,11'inde (35/109) CBLR, %44,95'inde (49/109) TİEL, %73,39'unda (80/109) TT, %84,40'ında (92/109) DY saptanmıştır. Mikrosatellit stabil (MSS) olgularda CBLR, TİEL, DY, KRAS mutasyonu, MSİ olgularda ise TT daha sıktır. KRAS wild olgularda CBLR, TİEL, DY, MSİ, KRAS mutant olgularında TT daha sıktır. Sadece MSS-DY korelasyonu istatistiksel olarak anlamlı bulunmuştur.*

Sonuç: *Çalışmamıza göre MSS-DY korelasyonu istatistiksel olarak anlamlıdır. Ancak MSS olguların %92,45'inde saptanan desmoplazi, MSİ olguların da %58,82'sinde gözlenmiştir. Desmoplastik yanıtın tümör stromasında beklenen bir bulgu olması sebebiyle MSİ açısından yönlendiriciliğinin sınırlıdır. Ayrıca Türkçe ve İngilizce literatürler tarandığında MSİ ile DY arasında istatistiksel olarak anlamlı ilişki bulunamamıştır. Çalışmamızda histomorfolojik özellikler MSİ ve KRAS mutasyonunu öngörmede yetersiz kalmıştır. İmmünohistokimya ve PCR olanakları bulunmayan merkezlerde değerlendirilen hastaların KRK tanısı konulduktan sonra MSİ ve KRAS mutasyonu tayini için ivedilikle ileri bir merkeze sevk edilmesi tedavi seçimi açısından hayati önem taşımaktadır.*

Anahtar kelimeler: *kolorektal kanser; KRAS proteini; mikrosatellit instabilite*

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Introduction

Colorectal carcinomas (CRC) are third after female breast and lung cancers in terms of incidence and second after lung cancers in cancer-related deaths[1]. Surgical resection is the main treatment modality in CRC[2,3]. Chemotherapy, radiotherapy, targeted therapy, or immunotherapy are other treatment modalities that can be preferred when surgery cannot be performed or is insufficient. At this stage, the presence of microsatellite instability (MSI) or KRAS mutations in the patient will be decisive in terms of treatment selection because 5-fluorouracil (one of the classic chemotherapeutics) and anti-EGFR/VEGFR drugs are not preferred in the presence of MSI[3–5] and KRAS mutations,[3,6,7] respectively, due to low treatment response or profit/loss rates. In addition, anti-PD-1/PD-L1 drugs are a treatment option for patients with metastases in the presence of MSI[3,6,8].

Microsatellite instability is found in 15% of patients with CRC, which is a good prognostic factor. Early-onset disease, proximal colon localization, high histologic grade, mucinous or medullary differentiation, signet ring cell changes, Crohn-like lymphoid reaction (CLR), and tumor-infiltrating lymphocytes (TIL) are expected clinical and microscopic features in the presence of MSI[4–6,9,10]. Microsatellite instability is usually interpreted by evaluating MLH1, MSH2, MSH6, and PMS2 expressions in tumoral tissue using immunohistochemistry because it is more accessible. Loss of expression in any of them indicates MSI-high. However, it should be kept in mind that MSI can be detected using polymerase chain reaction (PCR) in 5% of patients who are MSI-low in immunohistochemistry[4,5]. KRAS mutations are evaluated using PCR and found in 30–45% of CRC cases[6,9,10]. KRAS mutations have been associated with poor prognosis in most studies[6].

Crohn-like lymphoid reaction, TIL, tumor budding (TB), and desmoplastic response (DR) are some of the histomorphologic features evaluated in CRC cases. Crohn-like lymphoid reaction and TIL have been associated with good prognosis, [11–15] whereas TB has been associated with poor prognosis[2]. The prognostic role of DR is unclear[16–20]. Among these features, only the TB has been clearly defined and standard criteria have been established for its evaluation[2].

We aimed to evaluate the relationships between MSI and KRAS mutations, which require immunohistochemistry or PCR for evaluation and histomorphologic features (CLR, TIL, TB, DR), which can be determined using just hematoxylin-eosin (H&E) sections. Knowledge of these relationships may be useful for predicting MSI or KRAS mutations in centers that do not have immunohistochemistry or PCR facilities, thus providing faster and cheaper access to treatment without advanced tests.

Materials and Methods

In our study, we reviewed 109 patients with metastases who were diagnosed as having carcinoma in colorectal resections at the pathology department of Karadeniz Technical University Medical Faculty between 2016 and 2019 retrospectively. Results of PCR, which had been performed on all cases for KRAS mutation detection upon clinical request during routine pathologic examinations, were obtained from the molecular pathology report archive. KRAS mutation analysis through PCR was performed on formalin-fixed, paraffin-embedded tumor tissue (Device: Qiagen Rotor-Gene Q real-time PCR device (Version 1.7.87), Kit: Easy® KRAS, Company: Diatech Pharmacogenetics, Detectable mutations: Codon 12–13–59–61–117–146). MLH1, MSH2, MSH6, and PMS2 immunohistochemistry, performed on 70 cases for MSI interpretation upon clinical request during routine pathological examinations, was re-evaluated by one pathologist for standardization. A Nikon Eclipse E200 microscope was used for evaluation. Immunohistochemistry had been performed on formalin-fixed, paraffin-embedded tumor tissue (Device: Ventana BenchMark ULTRA automated staining system, Clone (Respectively): M1, G219–1129, SP93, A16–4, Company: Ventana Medical Systems). Normal colonic mucosa contiguous to the tumors were available as an internal control in all cases. Nuclear staining was considered significant (Fig. 1). Despite positive internal control, complete negativity in tumor cells was considered a loss of expression. Loss of expression of at least one marker was considered MSI.

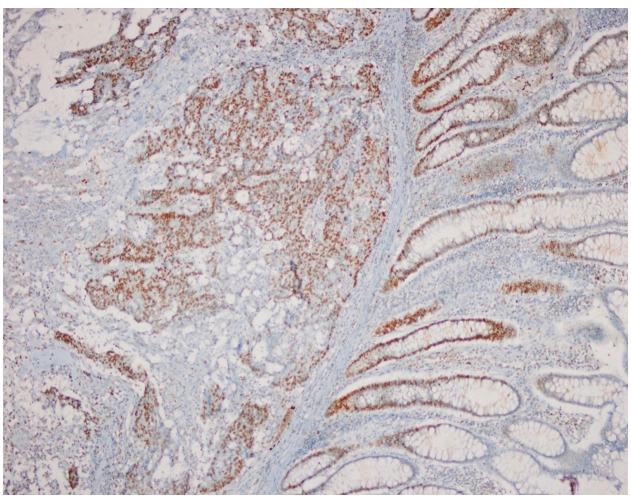

Figure 1. *Nuclear expression in tumor tissue (left) and normal colon mucosa as an internal control (right) (MLH1; ×100)*

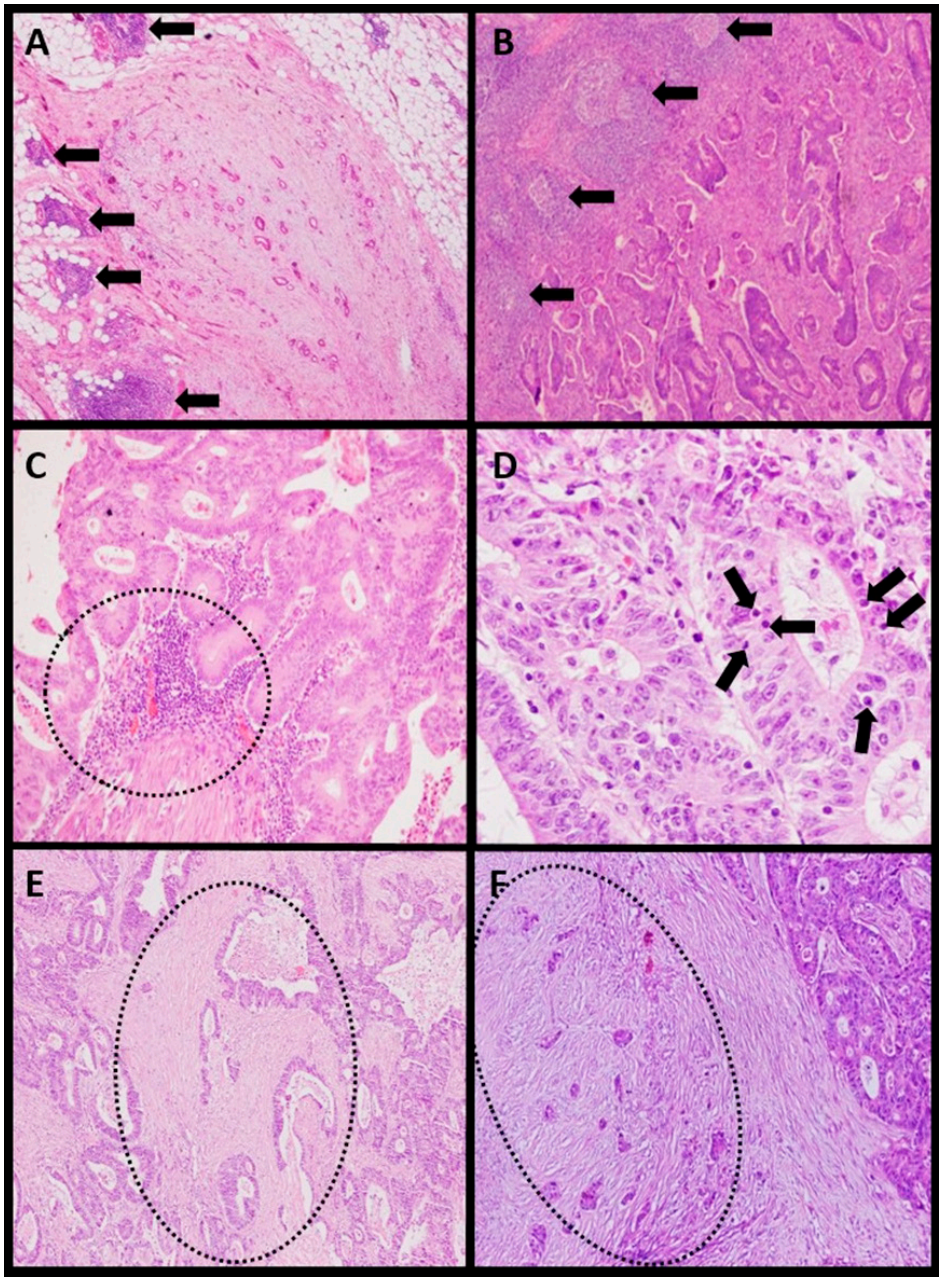

Figure 2. a–e. *Crohn-like lymphoid reaction (arrows, LA without germinal center) (H&E; ×40)*(a), *CLR (arrows, LA with the germinal center) (H&E; ×100)*(b), *Stromal lymphocytosis (ring, Cluster of lymphocytes spacing the glands) (H&E; ×200)* (c), *Intraepithelial lymphocytosis (arrows, Lymphocytes infiltrating gland epithelium) (H&E; ×400)*(d), *Desmoplastic response (ring, Dense fibrous connective tissue increase in the stroma) (H&E; X100)*(e), *TT (ring, Tumor buds progressing at the invasion margin) (H&E; ×100)*(e).

All H&E sections with tumors of all cases were evaluated for CLR, TIL, TB, and DR by one pathologist. There were standardized criteria for only TB in the English literature[2]. However, we established criteria for our study to evaluate features including TB. Lymphoid aggregates (LA) without a germinal center but with a diameter over 1 mm (Fig. 2a) or LA with a germinal center at the invasion margin of the tumor (Fig. 2b) were included in the calculation for CLR. The presence of CLR was accepted in cases with a mean LA count of ≥1 per section. When evaluating TIL, the density of stromal lymphocytes was given as the percentage of the one high-power field in the hotspot area, and a density of ≥10% was accepted as stromal lymphocytosis (Fig. 2c). The presence of only prominent lymphocytes infiltrating neoplastic glands was considered intraepithelial lymphocytosis (Fig. 2d). The presence of only stromal lymphocytosis, only intraepithelial lymphocytosis or both were accepted as TIL. Only prominent and diffuse fibrous connective tissue increase in the tumor stroma was accepted as DR (Fig. 2e). In our study, single tumor cells or less than five tumor cell groups at the invasion margin of the tumor were accepted as TBs as in the standard evaluation criteria. However, instead of being classified as low/medium/high according to the hotspot 0.785 mm^2 area, TB was accepted in cases with ≥1 bud in one high-power field of the hotspot area (Fig. 2f).

Table 1. *Clinical and microscopic features*

Clinical and microscopic features			Number (%)(N: 109)
Age	Maximum	85	
	Minimum	27	
	Average	60	
	≤60		47 (43.12%)
	>60		62 (56.88%)
Gender	Female		39 (35.78%)
	Male		70 (64.22%)
Tumor localization	Right colon		18 (16.51%)
	Left colon		57 (52.29%)
	Rectum		34 (31.20%)
Diagnosis	Adenocarcinoma		96 (88.07%)
	Mucinous carcinoma		13 (11.93%)
Histological grade	1		76 (69.72%)
	2		25 (22.93%)
	3		8 (7.35%)
Tumor diameter	Maximum	15 cm	
	Minimum	0.1 cm	
	Average	4 cm	
	≤4 cm		65 (59.63%)
	>4 cm		44 (40.37%)
Depth of invasion	Muscularis propria		10 (9.17%)
	Subserosa/adventisia		77 (70.64%)
	Serosa		22 (20.19%)
Lymphovascular invasion			43 (39.44%)
Perineural invasion			21 (19.26%)
Metastasis	Lymph node		67 (61.46%)
	Liver		100 (91.74%)
	Lung		15 (13.76%)
	Adrenal gland		6 (5.50%)
	Bone		2 (1.83%)
	Brain		1 (0.91%)
	Peritoneal carcinomatosis		11 (10.09%)

Table 2. *Histomorphologic features, MSI and KRAS mutation*

Parameters			Number (%)
Histomorphologic features (N: 109)	CLR		35 (32.11%)
	TIL		49 (44.95%)
	DR		92 (84.40%)
	TB		80 (73.39%)
MSI (N: 70)	MSI		17 (24.28%)
	Loss of expression	MLH1	10
		MSH2	1
		MSH6	8
		PMS2	14
	Combinations of expression losses	MLH1+PMS2	5
		MLH1+MSH6+PMS2	4
		Only PMS2	3
		Only MSH6	3
		MSH6+PMS2	1
		MLH1+MSH2+PMS2	1
KRAS mutation (N: 109)	KRAS mutation		39
	Codon-based mutations	Codon 12	30
		Codon 13	2
		Codon 61	2
		Codon 117	2
		Codon 146	3

CLR: Crohn-like lymphoid reaction, TIL: tumor-infiltrating lymphocytes, TB: tumor budding, DR: desmoplastic response, MSI: microsatellite instability, MSS: microsatellite stability

The hospital records of patients were reviewed. In all cases, there was at least one diagnosis of metastasis in magnetic resonance imaging (MRI), computed tomography (CT) or positron emission tomography (PET)/CT reports, and some were histopathologically correlated. None of the patients had a history of different malignancies, inflammatory bowel disease, colorectal cancer syndromes, or neoadjuvant chemotherapy.

Ethical approval for the study was obtained from the Ethics Committee of Karadeniz Technical University Medical Faculty (Number: 24237859-568, Date: 19.07.2019).

The IBM Statistical Package for Social Sciences (SPSS) program version 23.0 for Windows, was used for all statistical calculations. The Chi-square test was used to compare categorical data. P <0.05 was considered statistically significant.

Results

Clinical and microscopic features are given in Table 1, and histomorphologic features, MSI, and KRAS mutations are given in Table 2. The relationship between MSI, KRAS mutations, and histomorphologic features is given in Table 3 and Table 4.

Discussion

Microsatellite Instability and Histomorphologic Features

Crohn-like lymphoid reaction and TIL are histomorphologic features that are accepted to be related to MSI[4–6,9,10]. Different evaluation criteria for CLR have been established over time. In the semiquantitative Graham-Appelman criteria, cases with no LA are graded as '0' , cases with few LA with no germinal center are graded as '1' , and cases with many LA with germinal centers are graded as '2'[11]. In the Ueno criteria based on LA size, cases with LA with <1 mm maximum diameter are classified as 'inactive LA' , and cases with >1 mm diameter LA are classified as 'active LA'[12]. In the Vayrynen-Makinen criteria based on LA density, cases with <0.38 LA per mm^2 are graded as 'low CLR' , and those with >0.38 LA per mm^2 are graded as 'high CLR'[13]. Various semiquantitative criteria have

Table 3. *Relationship between MSI, KRAS mutation, and Histomorphologic features*

	MSI (N: 17)	MSS (N: 53)	KRAS mutant (N: 39)	KRAS wild (N: 70)
CLR (+)	5(23.81%) (29.41%)	16(76.19%) (30%)	11(31.42%) (28.20%)	24(68.58%) (34.28%)
CLR (-)	12(24.48%) (70.59%)	37(75.52%) (70%)	28(37.83%) (71.8%)	46(62.17%) (65.72%)
P value	0.951		0.515	
TIL (+)	7(20.58%) (41.17%)	27(79.42%) (50.94%)	14(28.57%) (35.89%)	35(71.43%) (50%)
TIL (-)	10(27.77%) (58.83%)	26(72.23%) (49.06%)	25(41.66%) (64.11%)	35(58.33%) (50%)
P value	0.483		0.156	
DR (+)	10(16.94%) (58.82%)	49(83.06%) (92.45%)	32(34.78%) (82.05%)	60(65.22%) (85.71%)
DR (-)	7(63.63%) (41.18%)	4(36.37%) (7.55%)	7(41.17%) (17.95%)	10(59.83%) (14.29%)
P value	0.030		0.613	
TB (+)	16(29.62%) (94.11%)	38(70.38%) (71.69%)	32(40%) (82.05%)	48(60%) (68.57%)
TB (-)	1(6.25%) (5.89%)	15(93.75%) (28.31%)	7(24.13%) (17.95%)	22(75.87%) (31.43%)
P value	0.940		0.778	

CLR: Crohn-like lymphoid reaction, TIL: tumor-infiltrating lymphocytes, TB: tumor budding, DR: desmoplastic response, MSI: microsatellite instability, MSS: microsatellite stability

Table 4. *Association between MSI and KRAS mutation*

	MSI	MSS	Total
KRAS mutant	4(16%) (23.52%)	21(84%) (39.62%)	25(100%)
KRAS wild	13(28.88%) (76.48%)	32(71.12%) (60.38%)	45(100%)
Total	17 (100%)	53 (100%)	P: 0.228

MSI: microsatellite instability, MSS: microsatellite stability

been used for TIL interpretation in different studies. The classifications were prepared according to the density and localization of lymphocytes (intraepithelial and stromal in the tumor, at the tumor invasion margin)[14,15]. However, standard criteria have not been established for CLR and TIL.

In the study of Ueno et al.,[12] active LA was present in 35.3% of the cases, and loss of expression of at least one of MLH1 and MSH2 was observed in immunohistochemistry. Thirteen percent of the cases that had preserved expression of both had active LA. Rozek et al.[21] evaluated MSI using PCR. While evaluating CLR, they accepted the presence of 3 LA per section as the cut-off value. Crohn-like lymphoid reaction was determined in 58.7% of instable cases and 45.3% of stable cases. Contrary to these studies, we found CLR slightly more frequently in MSS cases.

Rozek et al.[21] observed TIL in 56.3% of MSI cases and 22.6% of MSS cases. The correlation of MSI-TIL was statistically significant. In our study, TIL was present in 50.94% of MSS cases, and 41.17% of MSI cases. Contrary to expectations, TIL was found more frequently in MSS cases. Hu et al. evaluated TIL using a computerized system by immunohistochemistry. Tumor-infiltrating lymphocytes was grouped according to ITGAE and CD8 immunoreactivity as 'low' and 'high'. Tumor-infiltrating lymphocytes were found 'high' in 65.9% of MSS cases and 34.1% of MSI cases[22]. However, studies associating TIL with MSS, including our study, were not statistically significant.

Lymphocytic reactions (CLR and TIL), which have been proven to be correlated with MSI, were found more frequently in MSS cases in our study. All patients in our population had metastases. This may lead to the hypothesis of increased metastasis capacity in patients with MSI when the expected lymphocytic response does not accompany it. However, this hypothesis should be supported by new studies.

Fujiyoshi et al.[23] evaluated MSI using PCR. They found moderate/high TB using standard criteria in 42.79% of stable cases and 33.76% of instable cases. Graham et al.[24] evaluated MSI using PCR, and they classified TB as absent/low/high indicating that they had >10 TBs in a 0.95 mm^2 hotspot area. In these studies, TB was statistically significantly more frequent in MSS cases. By contrast, the frequency of TB was higher in MSI cases in our study.

We only found the DR-MSS correlation as statistically significant. However, desmoplasia, which was determined in 92.45% of MSS cases, was observed in 58.82% of MSI cases. Because DR is an expected finding in tumor stroma, its guidance in terms of MSI is limited. Also, no significant relationship was found between MSI and DR in the Turkish or English literature.

KRAS Mutation and Histomorphologic Features

In 212 patients with MSI, Kim et al.[11] found CLR more frequent in KRAS mutant cases using the Vayrynen-Makinen and Graham-Appleman criteria and in KRAS wild cases using the Ueno criteria. Lee et al.[25] evaluated cases for CLR according to possessing ≥1 mm peritumoral LA. They observed prominent CLR more frequently in KRAS wild cases, as in our study. Due to the different results and high p-values, CLR was not considered a predictive feature for KRAS mutation.

Lee et al.[25] classified TIL in terms of the density of peritumoral lymphocytes according to the 50%

cut-off value. High TIL was observed more frequently in KRAS wild cases, as in our study. Although the data were not statistically significant, they supported the KRAS-TIL inverse correlation.

Shin et al.[20] evaluated the maturation of tumor stroma and desmoplasia according to the structure of collagen fibers and cytomorphology of fibroblasts, and Akimoto et al.[26] examined the structure of collagen fibers and the presence of myxoid changes for desmoplasia interpretation; no significant relationship was found between DR-KRAS mutation in any study, including ours.

The results of Fujiyoshi et al.[23] and Lee et al.[25] demonstrated TB more frequently in KRAS wild cases. In contrast, Bonetti et al.[27] and Graham et al.[24] observed TB in KRAS mutant cases with higher rates, as in our study. Due to the different results and high p-values, TB was not considered a predictive feature for KRAS mutation.

Microsatellite instability and KRAS Mutation

Niu et al.[28] used immunohistochemistry for MSI interpretation. KRAS mutations were detected in 60% of MSI cases and 47.6% of MSS cases. The results statistically significantly supported the MSI-KRAS mutation correlation. However, in our study, MSI was observed in KRAS mutant cases at a rate of 16% and in KRAS wild cases at 28.88%. In addition, KRAS mutations were not determined in most MSI cases (76.48%). Although not statistically significant, there was an inverse correlation between MSI and KRAS mutations. In the study of 205 cases by Huang et al.[29], 20.3% of the cases in which KRAS or BRAF mutations were determined using PCR were MSI, and 79.7% were MSS. Microsatellite instability was observed statistically significantly less in mutant cases. KRAS mutation was determined in 14.2% of MSI cases and 38.3% of MSS cases in the N0147 study and 16.8% of MSI cases and 34.4% of MSS cases in the PETACC8 study. KRAS mutations were statistically significantly lower in MSI cases in these two studies with large populations, similar to our results[30]. In conclusion, studies in the English literature associated KRAS mutation with MSS or conversely with MSI with statistically significant results, as in our study.

In patients with metastases, histomorphologic features were insufficient to predict MSI and KRAS mutations. Therefore, it is vital to immediately refer patients who are evaluated in centers without immunohistochemistry and PCR facilities to an advanced center for MSI and KRAS mutation determination, with a paraffin block representing tumor tissue including normal mucosa preferably, after the diagnosis of CRC.

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Authors' Contribution

The authors share the responsibility for the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare no potential conflicts of interest regarding this article.

Disclaimer

The content is solely the responsibility of the authors.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209–49.
2. Burgart LJ, Chopp WV, Jain D, with guidance from CAP Cancer and CAP Pathology Electronic Reporting Committees. Protocol for the examination of specimens from patients with primary carcinoma of the colon and rectum. Version:4.2.0.2. Protocol posting date: June 2022.
3. American society of clinical oncology. Colorectal cancer: Types of treatment. https://www.cancer. net/cancer-types/colorectal-cancer/types-treatment;05/2022 [accessed 01.07.2023].
4. Losso GM, Moraes RS, Gentili AC, Messias-Reason IT. Microsatellite instability-MSI markers (BAT26, BAT25, D2S123, D5S346, D17S250) in rectal cancer. Arq Bras Cir Dig. 2012;25:240–4.

5. Murphy KM, Zhang S, Geiger T, Hafez MJ, Bacher J, Berg KD, et al. Comparison of the microsatellite instability analysis system and the Bethesda panel for the determination of microsatellite instability in colorectal cancers. J Mol Diagn. 2006;8:305–11.
6. Nguyen HT, Duong H-Q. The molecular characteristics of colorectal cancer: Implications for diagnosis and therapy. Oncol Lett. 2018;16:9–18.
7. Keskin SE, Güzdolu E, Sertdemir N, Demir G, Sünnetçi D, Çabuk D, et al. The mutation profiles of K-RAS/N-RAS genes in metastatic colorectal cancer patients. KOU Sag Bil Derg. 2022;8(3):172–8.
8. Çokmert S, Altun Z, Öztop I, Aktaş S, Olgun N. Kolorektal Kanser ve İmmünoterapi. DEÜ Tıp Fak Derg. 2016;30(3):131–7.
9. Bartlett JMS, Shaaban A, Schmitt F. Molecular pathology: A practical guide for surgical pathologist and cytopathologist. United Kingdom: Cambridge University Press, 2015:200–21.
10. Tariq K, Ghias K. Colorectal cancer carcinogenesis: A review of mechanisms. Cancer Biol Med. 2016;13:120–35.
11. Kim JH, Kim KJ, Bae JM, Rhee YE, Cho NY, Lee HS, et al. Comparative validation of assessment criteria for Crohn-like lymphoid reaction in colorectal carcinoma. J Clin Pathol. 2015;68:22–8.
12. Ueno H, Hashiguchi Y, Shimazaki H, Shinto E, Kajiwara Y, Nakanishi K, et al. Objective criteria for Crohn-like lymphoid reaction in colorectal cancer. Am J Clin Pathol. 2013;139:434–41.
13. Vayrynen JP, Sajanti SA, Klintrup K, Makela J, Herzig KH, Karttunen TJ, et al. Characteristics and significance of colorectal cancer associated lymphoid reaction. Int J Cancer. 2014;134:2126–35.
14. Klintrup K, Makinen JM, Kauppila S, Vare PO, Melkko J, Touminen H, et al. Inflammation and prognosis in colorectal cancer. Eur J Cancer. 2005;41:2645–54.
15. Jakubowska K, Kisielewski W, Kanczuga-Koda L, Koda M, Famulski W. Stromal and intraepithelial tumor-infiltrating lymphocytes in colorectal carcinoma. Oncol Lett. 2017;14:6421–32.
16. Caporale A, Vestri AR, Benvenuto E, Giluliani A, Mingazzini P, Angelico F. Is desmoplasia a protective factor for survival in patients with colorectal carcinoma? Clin Gastroenterol Hepatol. 2005;3:370–5.
17. Ueno H, Jones AM, Wilkinson KH, Jass JR, Talbot IC. Histological categorization of fibrotic cancer stroma in advanced rectal cancer. Gut. 2004;53:581–6.
18. Conti J, Thomas G. The role of tumor stroma in colorectal cancer invasion and metastasis. Cancers (Basel) . 2011;3:2160–8.
19. Tsujino T, Seshimo I, Yamamoto H, Ngan CY, Ezumi K, Takemasa I, et al. Stromal myofibroblasts predict disease recurrence for colorectal cancer. Clin Cancer Res. 2007;13:2082–90.
20. Shin N, Son GM, Shin DH, Kwon MS, Park BS, Kim HS, et al. Cancer-associated fibroblasts and desmoplastic reactions related to cancer invasiveness in patients with colorectal cancer. Ann Coloproctol. 2019;35:36–46.
21. Rozek LS, Schmit SL, Greenson JK, Tomsho LP, Rennert HS, Rennert G, et al. Tumor-infiltrating lymphocytes, Crohn's-like lymphoid reaction, and survival from colorectal cancer. J Natl Cancer Inst. 2016;108: djw027.
22. Hu X, Li YQ, Li QG, Ma YL, Peng JJ, Cai SJ. ITGAE defines CD8+ tumor-infiltrating lymphocytes predicting a better prognostic survival in colorectal cancer. Ebiomedicine. 2018;35:178–88.
23. Fujiyoshi K, Vayrnen JP, Borowsky J, Papke DJ, Arima K, Haruki K, et al. Tumor budding, poorly differentiated clusters, and T-cell response in colorectal cancer. Ebiomedicine. 2020;57:102860.
24. Graham RP, Viekart RA, Tillmans LS, Wang AH, Laird PW, Weisenberg DJ, et al. Tumor budding in colorectal carcinoma: Confirmation of prognostic significance and histologic cut off in a population-based cohort. Am J Surg Pathol. 2015;39:1340–6.
25. Lee HS, Hwang DY, Han HS. Histology and its prognostic effect on KRAS-mutated colorectal carcinomas in Korea. Oncol Lett. 2020;20:655–66.
26. Akimoto N, Vayrynen JP, Zhao M, Ugari T, Fujiyoshi K, Borowsky J, et al. Desmoplastic reaction, immune cell response, and prognosis in colorectal cancer. Front Immunol. 2022;13:840198.
27. Bonetti LR, Baressi V, Maiorana A, Manfredini S, Caprera C, Bettelli S. Clinical impact and prognostic role of KRAS/BRAF/PIK3CA mutations in stage I colorectal cancer. Dis Markers. 2018;2018:2959801.
28. Niu W, Wang G, Feng J, Li Z, Li C, Shan B. Correlation between microsatellite instability and RAS gene mutation and stage III colorectal cancer. Oncol Lett. 2018;17:332–8.
29. Huang CJ, Huang SH, Chien CC, Lee HHC, Yang SH, Chang CC, et al. Impact of microsatellite status on chemotherapy for colorectal cancer patients with KRAS or BRAF mutation. Oncol Lett. 2016;12:4427–34.
30. Taieb J, Malicot K Le, Shi Q, Penault-Llorca F, Bouche O, Tabernero J, et al. Prognostic value of BRAF and KRAS Mutations in MSI and MSS stage III colon cancer. Natl Cancer Inst. 2017;109: djw272.