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SURGICAL SCIENCES / CERRAHİ TIP BİLİMLERİ

The Effect of Microsatellite Instability on Clinicopathological Data and Survival in Colorectal Cancer

Kolorektal Kanserde Mikrosatellit İnstabilitesinin Klinikopatolojik Veriler ve Sağkalım Üzerine Etkisi

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Abstract

Objectives: Colorectal cancer is known as the third most common type of cancer worldwide. The microsatellite instability (MSI) pathway is effective in the development of 15-20% of colorectal cancers. MSI is mainly caused by mutational inactivation of one of the four main *MMR* genes (MSH2, MLH1, MSH6 or PMS2). This study was planned to investigate clinicopathological features of MSI in colorectal cancer and its effect on prognosis. For this purpose, demographic and clinicopathological data of patient groups with MSI and microsatellite stability (MSS) were compared.

Materials and Methods: In this study, the pathology reports of 109 patients who were diagnosed with colorectal cancer and were operated on between 01.01.2015 and 01.01.2019 in the Surgery Oncology Clinic of Cumhuriyet University Medical Faculty Hospital were analyzed retrospectively. MLH-1, MSH-2, MSH-6, and PMS-2 antibodies were evaluated together with demographic and histopathological features and survival time of the patients.

Results: The histological grade distribution difference between the MSS and MSI groups was not statistically significant (p=0.838). Mostly observed T-stage was T3 in both groups, and the differences between the groups were not statistically significant (p=0.405). Regarding the N stage, N0 was more common in MSS and N2 in MSI; however, no significant difference was observed between the two groups (p=0.844). Lymphovascular invasion (LVI) was not observed in most cases in both groups, and the differences between the groups were not statistically significant (p=0.493). Perineural invasion (PNI) was present in most cases in both groups, and the differences between the groups were not statistically significant (p=0.987). Survival rates according to the groups were evaluated using the Kaplan Meier test, and no statistically significant difference was found in the 2-year survival rates (p>0.05).

Conclusion: In this study, the relationship of MLH1, MSH2, MSH6, and PMS2's immunohistochemical expression with clinicopathological parameters and survival in patients with colorectal cancer was investigated. According to study results, the losses of expression in the cases were 13.7% for MLH1, 9.1% for MSH2, 12.8% for MSH6, and 14.6% for PMS2. Although it was not statistically significant in the study, we think that the survival rate is higher in cases with MSI. However, there was no statistical difference in MSI in terms of gender, age, grade, localization, LVI, and PNI. More parameters should be studied to detect MSI.

Key Words: Colorectal Cancer, Microsatellite Instability, Microsatellite Stability

Öz

Amaç: Kolorektal kanserler tüm dünyada en yaygın görülen üçüncü kanser türü olarak bilinmektedir. Mikrosatellit instabilite (MSİ) yolağı kolorektal kanserlerin %15-20'sinin gelişiminde etkilidir. MSİ, esas olarak dört ana *MMR* genlerinden birinin (MSH2, MLH1, MSH6 veya PMS2) mutasyonla inaktivasyonundan kaynaklanır. Bu çalışma kolorektal kanserde MSİ'nin klinikopatolojik özellikler ve prognoz üzerine etkisini araştırmak için planlandı. Bu amaçla; MSİ ve mikrosatellit stabilite (MSS) hasta gruplarına ait demografik ve klinikopatolojik veriler karşılaştırıldı.

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Gereç ve Yöntem: Bu çalışmada, çalışma grubu olarak Cumhuriyet Üniversitesi Tıp Fakültesi Hastanesi Cerrahi Onkoloji Kliniği'nde 01.01.2015-01.01.2019 tarihleri arasında kolorektal kanser tanısı almış ve opere edilmiş 109 hastaya ait patoloji raporları retrospektif olarak incelendi. MLH-1, MSH-2, MSH-6, PMS-2 antikorları, hastaların demografik ve histopatolojik özellikleriyle ve sağkalım süreleriyle birlikte değerlendirildi.

Bulgular: MSS ve MSI grupları arasındaki histolojik derece dağılım farkı istatistiksel olarak anlamsızdı (p=0,838). Her iki grupta da T-evresi çoğunlukla T3 olup, gruplar arasındaki farklılıklar istatistiksel olarak anlamlı değildi (p=0,405). N evresi de MSS'de N0, MSİ'de ise N2 çoğunluktaydı ve her iki grup arasında anlamlı bir fark görülmedi (p=0,844). Lenfovasküler invazyon (LVI) her iki grupta da olguların çoğunluğunda olmayıp, gruplar arasındaki farklılıklar da istatistiksel olarak anlamlı değildi (p=0,493). Perinöral invazyon (PNI) her iki grupta da olguların çoğunluğunda var iken, gruplar arasındaki farklılıklar istatistiksel olarak anlamlı değildi (p=0,987). Gruplara göre sağkalım oranları Kaplan-Meier testi ile değerlendirildiğinde 2 yıllık sağkalım oranları arasında istatistiksel olarak anlamlı farklılık saptanmamıştır (p>0,05).

Sonuç: Olgularımız arasında ekspresyon kaybı MLH1 için %13,7, MSH2 için %9,1, MSH6 için %12,8 ve PMS2 için %14,6 olarak bulunmuştur. Çalışmamızda MSİ gösteren olgularda, istatistiksel olarak anlamlı olmamakla birlikte sağ kalım oranının yüksek olduğunu düşünüyoruz. Ancak MSI için cinsiyet, yaş, grade, lokalizasyon, LVI, PNI açısından istatistiksel olarak anlamlı bir farklılık yoktu. MSI tespit etmek için daha çok parametrenin çalışılması gerektiği kanaatindeyiz.

Anahtar Kelimeler: Kolorektal Kanser, Mikrosatellit İnstabilitesi, Mikrosatellit Stabilitesi

Introduction

Colorectal cancers are the third most common type of cancer worldwide; regarding cancer-related deaths, it ranks second among the deadliest cancer types in both sexes (1). The mortality due to malignant tumors developing in the gastrointestinal tract is estimated to be higher than the mortality of cardiovascular diseases in the coming years (2).

Colorectal cancer occurs through several mechanisms that lead to the transformation of normal mucosa into adenoma and then carcinoma. The defined molecular pathways are microsatellite instability (MSI) pathway, chromosomal instability pathway, and CpG island methylator phenotype pathway (3). The chromosomal instability pathway mainly depends on the mutations in the *APC* gene of the fifth chromosome (5q21). This mutation is thought to be the earliest event in colorectal cancer initiation and progression. The MSI pathway is effective in the development of 15-20% of colorectal cancers (4). Microsatellites are repetitive DNA sequences of one to four base pairs distributed throughout the human genome (5). The CpG island methylator phenotype is associated with the transcriptional inactivation of tumor suppressor genes in neoplasia. Short repeating nucleotide sequences distributed throughout the genome are called microsatellites. Errors that occur during repetitions are recognized and repaired by the DNA mismatch repair system (MMR). MSI reflects the failure of the MMR system in recognizing and repairing errors (6). Somatic mutations and hypermethylation in the MMR system are responsible for 15% of sporadic colorectal cancers. The human MMR system consists of MLH1, MLH3, MSH2, MSH3, MSH6, PMSI, and PMS2 proteins. MSI is mainly caused by the mutational inactivation of one of the four main *DNA MMR* genes (MSH2, MLH1, MSH6, or PMS2) (5,7). Colorectal cancers are divided into three groups in terms of MSI. Tumors showing MSI in >30-40% of the investigated loci are called MSI-High (MSI-H). Tumors with MSI in <30-40% of the investigated loci are called Low-Level MSI (MSI-L). Tumors in which no MSI is detected in any locus are called microsatellite stable (MSS) (8). MSI are important prognostic markers and have been defined as a favorable prognostic factor in colorectal cancers (9,10). MSI-H tumors have been suggested to have a better prognosis because the likelihood of metastasis is less (11,12). MSI-H tumors are mostly localized in the right colon and have mucinous features histopathologically (13).

This study was planned to investigate clinicopathological features of MSI in colorectal cancer and its effect of on prognosis. For this purpose, demographic and clinicopathological data of patient groups with MSI and MSS were compared.

Materials and Methods

Ethical approval was obtained from the ethics commission of the Sivas Cumhuriyet University where the study was conducted (2022-04/24 number and 27.04.2022 date).

In this study, the pathology reports of 109 patients diagnosed with colorectal cancer and operated between 01.01.2015 and 01.01.2019 in the Surgery Oncology Clinic of Sivas Cumhuriyet University Medical Faculty Hospital were retrospectively analyzed.

Patients' age at diagnosis, gender, tumor localization, lymph node status, histological type, degree of differentiation, T-stage, lymphovascular invasion, and perineural invasion were evaluated. Immunohistochemically detected MLH-1, MSH-2, MSH-6, and PMS-2 antibodies than are used to identify MSI, the demographic and histopathological characteristics of the patients, and their survival period were evaluated together.

Statistical Analysis

All analyzes were performed in SPSS 17.0 for Windows and the confidence interval was taken as 95%. Nominal and ordinal parameters were defined by frequency analysis, and age, which is the only numerical measurement value of the study, was defined

by mean and standard deviation (SD). Chi-square similarity ratio and chi-square test were employed for the differentiation of categorical data. The Kolmogorov-Smirnov test was used to confirm that age showed had a normal distribution.

Results

The ages of 109 patients were ranged between 49 and 81, with a mean age ± SD of 70.51±6.41 years. Fourty (36.6%) of the cases were female and 69 (63.3%) were male.

Regarding the nuclear expression of MMR proteins, MSS was detected in 83 cases (76.1%), whereas MSI was detected in 26 cases (23.8%). The expression patterns and distributions of MLH1, MSH2, MSH6, PMS2 proteins of the cases are given in Table 1. The examples of MMR proteins' immunoreaction patterns are given in Figure 1.

Regarding the tumor grade, 36 cases (33.0%) were grade 1 (G1), and 73 (66.9%) were grade 2 (G2). T-stage of the tumor was distributed as T3 in 72 cases (66.0%), followed by T4 in 24 (22.0%) and T2 in 13 (11.9%) cases. Regarding the N stage showing lymph node involvement of the cases, 52 cases (47.7%) were in N0, 31 (28.4%) in N1, and 26 (23.8%) in N2. Lymphovascular invasion was detected in 36 (33.0%) and perineural invasion in 59 (54.1%) patients.

Regarding the relationships of MSS and MSI with clinicopathological parameters; The mean age was 70.0 in the MSS group and 70.5 in the MSI group, and the difference between the groups was not significant (p>0.05). Males were in the majority in both MSS and MSI groups, and the differences according to gender were not significant (p=0.091). The most common histological grade was G2 in both MSS and MSI groups. Besides, the histological grade distribution difference between the MSS and MSI groups was not statistically significant (p=0838). Mostly observed T-stage was T3 in both groups, and the differences between the groups were not statistically significant (p=0.405). Regarding the N stage, N0 was more common in MSS and N2 in MSI; however, no significant difference was observed between the two groups (p=0.844). Lymphovascular invasion (LVI) was not observed in most cases in both groups, and the differences between the groups were not statistically significant (p=0.493). Perineural invasion (PNI) was present in most cases in both groups, and the differences between the groups were not statistically significant (p=0.987) (Table 2). The mean tumor size was 5.2 cm in the MSS group and 4.6 cm in the MSI group, and the difference between the groups was not statistically significant (p=0.452).

Out of 83 cases with MSS, 16 deaths occurred in 2 years, and 67 cases (80.7%) survived, the mean survival time was found to be 21.1±0.69 months. Whereas out of 26 cases with MSI, 6 deaths occurred, and 20 cases (76.9%) survived, the mean survival time was found to be 21.7±1.05 months (Table 3). Survival rates according to the groups were evaluated using the Kaplan-Meier test, and no statistically significant difference was found between the 2-year survival rates (p>0.05) (Figure 2).

It was found that there was no progression in 65 (78.3%) of the 83 cases showing MSS; progression was observed in 18 cases; the average survival period was 23.5±0.25 months. It was found that there was no progression in 22 of the 26 cases showing MSI (84.6%); progression was observed in 4 cases; the average survival time was 23.1±0.63 months (Table 4). When the survival rates were evaluated according to the groups, there was no statistically significant difference between the 2-year survival rates (p>0.05) (Figure 3).

Discussion

Colorectal cancer is the second most common cancer in women after breast cancer and the third most common in men after lung and prostate cancers (14). The mean age of incidence is 62, and the risk group is 60-79-year-olds (15). MSI are sporadic or inherited defects in DNA repair genes (16).

Table 1: Immunohistochemical evaluation of MMR proteins' nuclear expressions

MLH 1	MSH 2	MSH 6	PMS 2	MSS or MSI	Number of cases	Percentage
+	+	+	+	MSS	83	76.1
-	+	+	-	MSI	5	4.5
+	+	+	-	MSI	2	1.8
+	-	-	+	MSI	1	0.9
+	+	-	+	MSI	5	4.5
-	-	+	+	MSI	4	3.6
-	-	+	-	MSI	1	0.9
-	-	-	-	MSI	4	3.6
+	+	-	-	MSI	3	2.7
-	+	-	-	MSI	1	0.9

MSS: Microsatellite stability, MSI: Microsatellite instability, MMR: Mismatch repair system

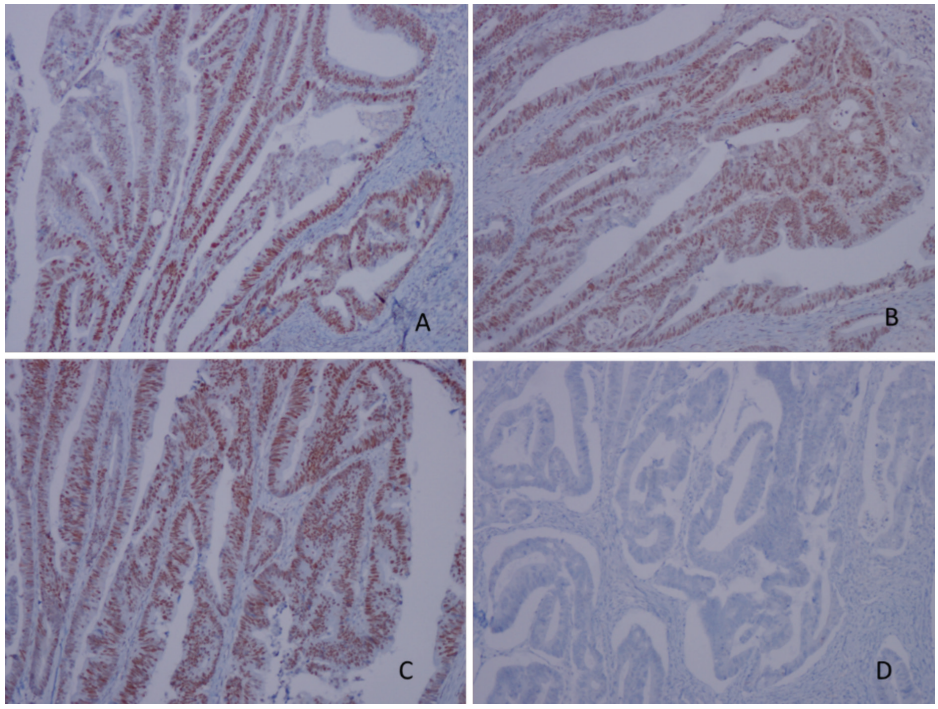

Figure 1: Immunoreactions patterns of MMR proteins magnified by 100, (A: MSH2 expression, B: PMS2 expression, C: MLH1 expression, D: MSH6 expression loss)

MMR: Mismatch repair system

Table 2: The relationships of microsatellite stability and instability with clinicopathological parameters

		MSS		MSI		p-value
		Count	Row N %	Count	Row N %	
Gender	Male	56	81.2%	13	18.8%	0.091
	Female	27	67.5%	13	32.5%	
Grade	1	28	77.7%	8	22.2%	0.838
	2	55	75.3%	18	24.7%	
Localization	Right column	21	77.8%	6	22.2%	0.516
	Left column	25	69.4%	11	30.6%	
	Rectosigmoid	15	71.4%	6	28.5%	
	Rectum	15	88.2%	2	11.8%	
	Transverse colon	7	87.5%	1	12.5%	
T	T 2	11	84.6%	2	15.3%	
	T 3	56	77.7%	16	22.2%	0.405
	T 4	16	66.6%	8	33.3%	
N	N 0	41	78.8%	11	21.1%	0.844
	N 1	25	80.6%	6	19.3%	
	N 2	17	65.3%	9	34.6%	
Lymphovascular invasion	Yes	29	80.5%	7	19.4%	0.493
	No	54	74.0%	19	26.0%	
Perineural invasion	Yes	45	76.2%	14	23.7%	0.987
	No	38	76.0%	12	24.0%	
Two-year survival	Alive	67	77.0%	20	22.9%	0.674
	Ex	16	72.7%	6	27.2%	

MSS: Microsatellite stability, MSI: Microsatellite instability

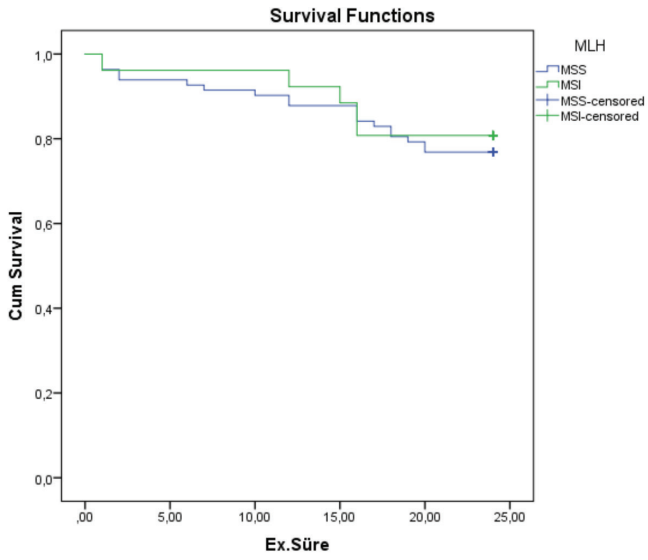

Figure 2: Comparison of 2-year survival rates of the groups with MSS and MSI

MSS: Microsatellite stable, MSI: Microsatellite instability

Colon cancers with MSI show different clinical and pathological features. MSI is detected in more than 90% of patients with hereditary non-polyposis colorectal cancer and approximately 15% of sporadic colorectal cancers (17,18). Sporadic colorectal cancers are much more common than hereditary forms, so most tumors with MSI are sporadic tumors (19).

Knowing the presence of MSI in patients with colorectal cancer is important for prognosis, treatment, and family guidance (16). Studies have shown that tumors with MSI cause fewer lymph nodes and distant organ metastases than those without MSI, even if they have a better prognosis and advanced stages (20,21).

The immunohistochemical technique is 94% sensitive in detecting germline mutations in DNA MMR proteins for MLH1, MSH2, MSH6, PMS2 (22,23). Negativity in at least one of the antibodies is considered as MSI (24).

In the literature, immunohistochemical staining loss is mostly observed in MLH1 and PMS2 among the four proteins

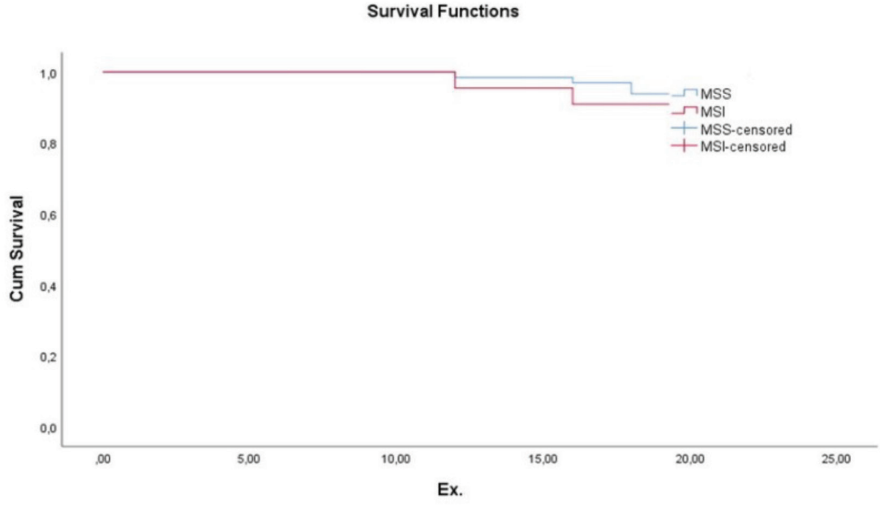

Figure 3: Progression-free survival graph based on MS status

Table 3: Survival analysis according to microsatellite status

MS status	N	Ex	Living	Survival rate	Average survival time	95% Confidence interval lower-upper
MSS	83	16	67	80.7%	21.1±0.69	19.72-22.44
MSI	26	6	20	76.9%	21.7±1.05	19.64-23.75

MSS: Microsatellite stability, MSI: Microsatellite instability

Table 4: Progression-free survival analysis according to MS status

MS status	N	Progression	Survival rate	Average survival time		95% Confidence interval lower-upper
	Yes	None				
MSS	83	18	65	78.3%	23.5±0.25	22.95-24.12
MSI	26	4	22	84.6%	23.1±0.63	22.84-24.01

MSS: Microsatellite stability, MSI: Microsatellite instability

(22,25). The negativity of all four antibodies was reported as 1.4% (22). In this study, the loss was mostly observed in PMS2 (14.6%), followed by MLH1 (13.7%) and MSH6 (12.8%). The least negativity was observed in MSH2 (9.1%). The negativity of all four antibodies was observed in 4 cases in this study, which is 3.6%, slightly higher than the rate stated in the literature.

In the study of Karahan et al. (26), consisting of 186 cases, the loss of MLH1, MSH2, MSH6, PMS2 was found to be correlated with poor differentiation and mucinous histology. Mucinous components, lymphovascular invasion, and intense intratumoral lymphocyte infiltration were more common in cases with loss of MLH1 and PMS2. There was no correlation between the localization and MLH1 and MSH2 negativity. All four antibodies were found to be negative in 2 cases (26).

DNA-MMR enzymes work in pairs. When the MLH1 function is impaired, the immunoreactivity of PMS2 is impaired, and when the function of MSH2 is impaired, MSH6's immunoreactivity is impaired (14). In this study, the loss of PMS2 expression was observed in 11 of 15 cases evaluated as MLH1 focal and negative. MSH6 expression loss was observed in 5 of 10 cases with MSH2 loss.

In this study, MSI was detected in 23.9% of the cases. Regarding the results of studies in the literature, MSI has been detected in a wide range of 9-28% due to various variables and limitations. In this context, the rate of the cases with MSI seems compatible with these data (27,28).

Some studies supporting MSI as a positive predictor in patients with colorectal cancer have been reported (29,30). Although MSI tumors are mucinous adenocarcinoma type and tend to be poorly differentiated, stage-specific survival rates are higher (31). In a study, MSI status was investigated in 91 patients with rectal cancer, and it was shown that disease-free survival and overall survival rates were higher in patients with MSI. On the other hand, another study including 181 patients reported that the MSI status of the patients with sporadic colorectal cancer did not make a statistically significant difference for prognosis (32-34). A cohort study consisting of 738 patients suggested that MSI has a positive contribution to metastasis-free survival (35). The current study showed that MSI status did not affect 2-year survival of the patients with colorectal cancer.

In a study conducted by Goldstein et al. (36) with 55 MSI cases, the mean age of the cases was 67. On the other hand, the mean age of patients with MSI was 70.5 in this study, slightly above the average reported in the literature.

Poor differentiation, in other words, high histological grade, is another histopathological parameter associated with MSI in the literature (37,38). Xiao et al. (28) found that poor differentiation was more common in MSI tumors compared to MSS tumors. In their study investigating the effects of histological grade and MSI status on survival on colorectal carcinoma, Rosty et al. (25) reported that MSI was more common in high-grade tumors than low-grade tumors.

MSI colorectal cancers tend to be poorly differentiated (27). In this study, grade 2 patients were in the majority in both MSI and MSI groups, but no statistical difference was found between the two groups according to grade.

In the literature, the presence of MSI is associated with clinical parameters such as female sex and right colon location (39,40). The study conducted by Batur et al. (41) on 145 cases showed that the tumor of the cases with MSI was more frequently located in the right colon, and there was a significant relationship between MSI and the female sex. In our study, no significant relationship was found between MSI and the tumor localization in the right colon; therefore, it differs from the literature in terms of tumor localization. Regarding gender, half of the MSI cases were male, and the other half were female. There was no significant relationship between MSI and gender.

In our study, LVI was detected in 32.1% of the cases. A significant correlation was observed between the advanced T-stage and the advanced N stage. In this respect, it is in line with the literature. In a study conducted by Parc et al. (40), MSI was detected in 17% of the cases, and no significant difference was observed between the MSI and MSS groups in terms of age, LVI, and PNI. Consistent with these results, there was no significant difference in LVI and PNI between patients with MSI and CNS in this study.

Study Limitations

Our study has some limitations. First, it was a retrospective data collection study. Due to its retrospective nature, we used short-term survival analyses due to the lack of data. Second, our study was single-center. Third the low number of patients in our study.

Conclusion

In this study, the relationship of MLH1, MSH2, MSH6, and PMS2's immunohistochemical expression with clinicopathological parameters and survival in patients with colorectal cancer was investigated. According to study results, the losses of expression in the cases were 13.7% for MLH1, 9.1% for MSH2, 12.8% for MSH6, and 14.6% for PMS2. Although it was not statistically significant in the study, we think that the survival rate is higher in cases with MSI. However, there was no statistical difference in MSI according to gender, age, grade, localization, LVI, PNI. More parameters should be studied to detect MSI.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the ethics commission of the Sivas Cumhuriyet University where the study was conducted, (2022-04/24 number and 27.04.2022 date).

Informed Consent: Retrospective study.

Peer-reviewed: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.E.B., M.C.M., K.K., Concept: M.E.B., M.C.M., H.Ö., K.K., Design: M.E.B., H.Ö., Data Collection or Processing: M.E.B., M.C.M., H.Ö., K.K., Analysis or Interpretation: M.E.B., M.C.M., H.Ö., K.K., Literature Search: M.E.B., M.C.M., K.K., Writing: M.E.B., H.Ö., K.K.

Conflict of Interest: The authors declare no conflicts of interest.

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