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Clinicopathological and molecular features of sporadic colorectal cancers with DNA mismatch repair deficiency: A single center experience

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Abstract

DNA mismatch repair (MMR) proteins may play an important role in colorectal carcinogenesis. In our study, the clinicopathological features of defective MMR in sporadic colorectal adenocarcinomas (CRCs) cases were examined. This is a retrospective study, 457 consecutive cases of colorectal carcinoma with immunohistochemical (IHC) studies for DNA MMR were included.The immunohistochemically (IHC) MMR results of 457 cases were; nuclear expression was intact (proficient, pMMR) in 401 (87.7%) cases and loss of nuclear expression (deficient, dMMR) was found in 56 cases (12.3%). High probability of Lynch syndrome ratio was 2.4% (11/457) in all cases. The loss of PMS2 was predominantly detected in dMMRcases (78.6%). Seventy eight percent of dMMR tumors were located in the proximal colon. In dMMR tumors, prominent peritumoral lymphoid aggregates (LAs) (85.7%) and tumor-infiltrating lymphocytes (TILs) (78.6%) were observed. Among 56 colorectal cancers, we observed expanding /pushing growth pattern in 41 tumors (73.2%), and infiltrative growth pattern in 15 cancers (16.8%). Medullary, mucinous and signet ring cell carcinomas were observed in approximately half of the cases, but there was no statistically significant relationship. Eighty nine percent of dMMR cases had advanced pathologic tumor stage (pT3 or pT4), and this rate was 82.5% in pMMR cases. The average number of positive lymph nodes in cases with dMMR was higher than in pMMR. KRAS mutations were detected in 7.2% (4/13) patients and 14.3% (8/13) patients with MLH1 promoter methylation was observed. Seventy percent of patients with dMMR were alive (n=44) and the mean age of the patients who died was higher. A statistically significant relationship was found between the patients who died and the mean age of surviving patients (p = 0.036). We conclude that the dMMR patients constitutes have a number of distinctive clinicopathological features subtype of sporadic CRC. The overall frequency of defective MMR in colorectal carcinoma cases was found to be Turkish population similar to western studies. dMMR in CRCs were more likely to be of advanced pathologic tumor stage to have a mucinous tumor component and positive LN to show PMS2 loss and to harbour higher numbers of both peritumoral LAs and TILs. They were also more likely to be proximal colon and to occur in male.

Keywords: Colorectal carcinoma, DNA mismatch-repair protein, microsatellite instability, histopathology, immunohistochemistry

Introduction

Colorectal adenocarcinomas (CRCs) are the most often malignancies of the digestive system [1]. It is the fourth most common cancer in the world (after lung, prostate, stomach) in men, and the third most common (after breast and uterine cervix) in women [2]. According to 2012 world cancer statistics, 9.4% of all cancers accounted for more than 1.4 million new cases and 693,000 deaths were reported each year [3].There are two known pathways of carcinogenesis for CRCs. The most common pathway is multiple tumor suppressor loci, such as 5q, 17p, 18q associated with loss of heterozygosity with chromosomal instability. The less common pathway is the loss of DNA mismatch repair (MMR) protein which is mostly caused by germline mutation or hypermethylation of the promoterregion of the MLH1 gene [4]. MMR deficiency (dMMR) is one of the well-known prognostic factors for CRCs. Compared with microsatellite stable (MSS) patients there is a 15% higher survival rate. Microsatellite instability (MSI) is an important factor while deciding treatment, especially in stage II CRCs. The American National Comprehensive Cancer Network (NCCN) protocols do not proposed chemotherapy for MSI-high (MSI-H) patients with stage II colorectal carcinoma with improved prognosis. On the other hand, the reason of their good prognosis is still remains to be explained [5]. The major aim of this study was to clarify the clinicopathological features and frequencies of DNA MMR proteins biomarkers in Turkish patients with CRCs.

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Material and Methods

Case Selection

The study population was based on a series of 1039 cases undergoing curative surgery for colorectal cancer at Marmara University-Pendik Training and Research Hospital between 31 December 2014 and 31 December 2018.Five hundred eighty two cases were excluded because no immunohistochemical study was performed for MMR proteins. Patients who met the Amsterdam

II criteria were excluded. Thus, 457 patients with MMR proteins immunohistochemistry results were in the study. All hematoxylin-eosin and immunohistochemical slides of deficient DNA mismatch repair (dMMR) patients (n=56 patients) were re-evaluated. The definition of ‘’high probability of Lynch syndrome’’ is defined as co-loss of MSH2 and MSH6 or loss of MSH6 or PMS2 only. Loss of MLH1 and PMS2 together indicates suggests the possibility of Lynch syndrome. MLH1/PMS2, MSH2/MSH6 and similar losses were classified as the group with ‘’combined expression loss’’. American Joint Committee on Cancer tumor-node-metastasis grading system (AJCC, TNM) manual 8th edition 2017 and The College of American Pathologists (CAP) colon and rectum 2017 were used for evaluating histologic grade, pathologic tumor stage and histologic tumor type. Briefly, for MLH1 (Pacheco CA, Biocare, G168-15 clone, 1:100 dilution), PMS2 (Santa Clara CA, Dako, EP51 clone, 1:1200 dilution), MSH6 (United Kingdom, Novocastro, PU29 clone, 1:100 dilution), MSH2 (Rocklin California, Cell Marque, G219-1129 clone, 1:100 dilution) antibodies were performed on formalin-fixed and paraffin-embedded (FFPE) tissue sections.

Statistical Analysis

Descriptive statistics were used for mean, standard deviation and categorical variables, and constantly changing parametric variables. Descriptive statistics for categorical variables were conducted by Pearson's chi-squared test ($\chi 2$ test), t test, Fisher’s exact test are reported. Overall Survival and disease-free survival were determined according to the method of Kaplan and Meier and their comparisons were made by the log rank test. Statistical Package for the Social Sciences Windows version 25.0 (SPSS, Armonk, NY: IBM Corp). Significance for all statistics were recorded if $p<0.05$.

Results

Clinical Details, Age, Sex, Histologic Type And Survival Analysis

In the study, 270 (59.1%) of the cases were male, 187 were female (40.9%), with the female / male ratio of 1/1.44, the mean age was 63.8 (range: 18 to 102) for both sexes (see Table 1). Detailed histopathological features of dMMR cases; histological types of tumors were adenocarcinoma NOS 24 (42.9%), mucinous adenocarcinoma 13 (23.2%), medullary carcinoma 4 (7.1%), signet ring cell carcinoma 2 (3.6%), serrated adenocarcinoma 2 (%3.6), adenocarcinoma + mucinous adenocarcinoma 2 (3.6%), mucinous adenocarcinoma + signet ring cell carcinoma 2 (3.6%), serrated adenocarcinoma + adenocarcinoma NOS 2 (3.6%), mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN) 1 (% 1.8), mixed tumors (different percentages, medullary, mucinous, signet ring cell, poorly differentiated, serrated, NOS) 4 (7.2%) (Figure 1,2,3). Histological grade (except for mucinous and medullary carcinomas) respectively; was grade 1 for 3 (5.4%) cases, was grade 2 for 23 (41.1%) cases, was grade 3 for 13 (23,2%) cases.While 78.6% (n=44 patients) of patients were alive(26 June 2019), 21.4% (12 patients) were died (the cause of death could not be reached in the current death notification system). The mean overall survival was 16.6 months (± 10.3) in overall population, whereas 6.85 months in patients with exitus, and 18.86 months (± 10.1) in surviving patients.A statistically significant relationship was found between the patients who died and the mean age of surviving patients ($p = 0.036$). According to this, the mean age of the patients who died was 69.33 years, while it was alive 57.39 years. There was no statistically significant relationship between dMMR and overall survival($p = 0.092$).

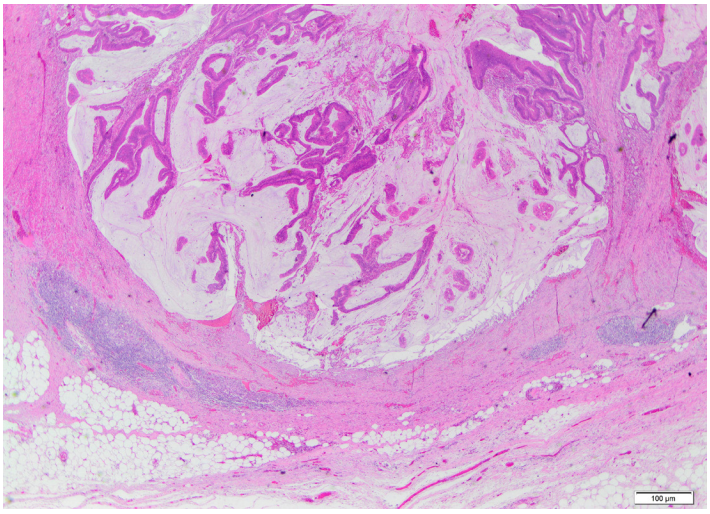

Figure 1. Histopathological features of microsatellite instability colorectal carcinoma. Mucinous carcinoma with Crohn’s like lymphoid reaction

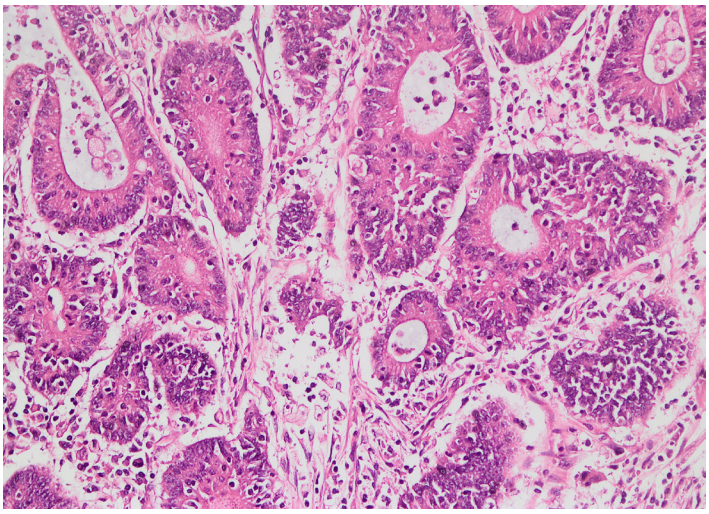

Figure 2. Adenocarcinoma, NOS, dMMR tumors. A tumor-infiltrating lymphocytes is present.

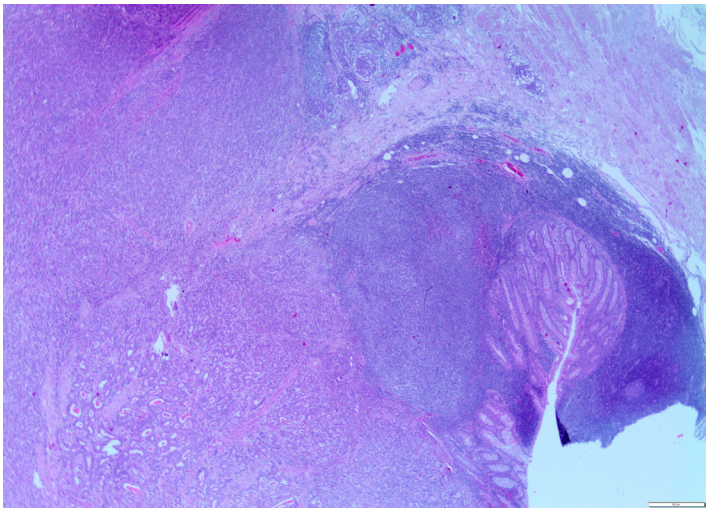

Figure 3: A, Medullary carcinoma and adenocarcinoma showing an expansive growth pattern with lymphoid infiltration in the peritumoral area

Table 1. Characteristics of cases with sporadic colorectal cancer according to MMR status

	Total	dMMR	pMMR
Patient Number	457	56 (%12.3)	401 (%87.7)
Gender			
Men	270 (59.1%)	32	238
Female	187 (40.9%)	24	163
Age mean (y+ SD)	63.8 (±12.97)	59.95 (±2.48)	64.34 (±0.61)
Anatomiclocalization			
Rectum	121 (26.5%)	2	117
Sigmoid colon	103 (22.5%)	2	101
Recto-sigmoid colon	65 (14.2%)	1	64
Right (ascending) colon	48 (10.5%)	12	36
Cecum	48 (10.5%)	19	29
Left (descending) colon	20 (4.4%)	1	19
Splenic flexura	16 (3.5%)	1	15
Transverse colon	15 (3.3%)	5	10
Ileocecal valve	11 (2.4%)	2	9
Hepatic flexura	10 (2.2%)	2	8
Histologic Type			
Adenocarcinoma	394 (86.2%)	24	370
Mucinous adenocarcinoma	42 (9.2%)	13	29
Signet-ring cell carcinoma	4 (0.9%)	2	2
Medullary carcinoma	4 (0.9%)	4	0
Other	13 (2.8%)	13	
Pathologic Stage Classification			
Primary Tumor (pT)			
pTis	6 (1.3%)	0	6
pT1	30 (6.6%)		27
pT2	40 (8.8%)	3	37
pT3	252 (55.1%)	29	223
pT4a	121 (26.5%)	19	102
pT4b	8 (1.8%)	2	6
Regional Lymph Nodes (pN)			
pN0	232 (50.8%)	29	203
pN1a	71 (15.5%)	9	62
pN1b	61 (13.3%)	9	52
pN1c	20 (4.4%)	1	19
pN2a	42 (9.2%)	3	39
pN2b	31 (6.8%)	5	26
Number of lymph nodes recovered and mean±SD	9058 and 19.82±11.26	1617 and 28.87±18. 38	7441 and 18.5±9.2
Number of positive lymph nodes and mean±SD	852 and 1.86±4	115 and 2.05±3.7	737 and 1.83±4.07
Resected specimen length. (cm)	21.4 ±11.4 cm	28.3±15.9 cm	20.5±10.2 cm
Greatest dimension of invasive carcinoma (cm)	4.7 ±2.4 cm	6.6±3 cm	4.4±2.2 cm
Mutational Analysis	87 (19%)	23	64

dMMR: Deficient mismatch repair, pMMR: Proficient mismatch repair

Immunohistochemistry (MMR Expression Status) and Histopathologic Features

Of the 457 cases in which MMR protein analysis was performed possible, 401 (87.7%) were no loss of nuclear expression of MMR protein (nuclear expression intact, proficient MMR, pMMR), 56 (12.3%) were loss of nuclear expression of MMR (dMMR, absent expression of at least one MMR protein). The loss of expression the partner MMR protein for MLH1/PMS2 were observed in 67.8% (38/56) and loss of expression MSH2/MSH6 were seen in 8.9% (5/56) of the patients. Isolated loss of PMS2 or MSH6 immunohistochemical expression was showed in 4 and 2 of the patients (Figure 4,5). The frequency of abnormal IHC in our study for PMS2 (78.6%) and MLH1, MSH6, MSH2 was 76.8, 17.9, and 16.1%, respectively. Eighty-three percent (47/56) of the patients with dMMR had a larger tumor size of 4 cm or more. Ninety-three percentof the patients with tumor size over 4 cm had advanced pathologic tumor stage (pT3 or pT4). There was statistically significant relationship between tumor size (>5 cm) and dMMR (p = 0.039). There was no correlation between overall survival and both tumor size (p=0.470) and location of the tumor (proximal or distal) (p=0.197). There was a statistically significant relationship between MLH-1 expression and mean specimen length (p=0.022). While ‘’high probability of Lynch syndrome’’ratio was 2.4% (11/457) in all cases.While combined loss of expression for MMR ratio was 10.3% (47/457) in all cases, 84% of the dMMR cases were combined. The majority of positive lymph nodes (LN) (107/115) of dMMR cases were found in the combined loss group. In dMMR protein loss tumors, prominent peritumoral lymphoid aggregates (LAs, Crohn-like lymphoid reaction (CLR)) and tumor-infiltrating lymphocytes (TILs) were observed 85.7% and 78.6%, respectively (see Table 2). There was a significant relationship between MLH1 and PMS2 loss and the density of TIL (p=0.002 and p=0.002, respectively). Medullary, mucinous and signet ring cell carcinomas were predominantly seen in cases where loss of at least 2 MMR protein is observed, but there was no statistically significant relationship. Our study found, 89.3% of dMMR cases had advanced pathologic tumor stage (pT3 or pT4), whereas this rate was 82.5% in pMMR cases. No correlation was found between dMMR and pathologic tumor stage (p=0.599). There was no correlation between overall survival and pathologic tumor stage (p = 0.103) and lymph node (LN) positivity, lymph-vascular invasion (LVI), TIL, and peritumoral lymphoid aggregates (LAs) (p = 0.184, p = 0.071, p=0.325, p=0.938). The mean number of positive lymph nodes found was 2,05, with a mean of 28,8 LNs recovered per specimen. Although there was no relationship between age and lymphoid aggregates (LAs) (p = 0.369). There was no statistically significant differences between LAs and proximal/distal colon anatomical location (p = 0.236). However, 83.3% of the cases with LA 300 ≥mm or more were located in the proximal colon. A statistically significant correlation was found between LVI in patients with tumor budding (TB) intermediate and high scores (p = 0.021). We found 65% LVI in these cases. However, there was no statistically significant association between poorly differentiated groups (PDC) grade 2 and grade 3 cases and LVI (p=0,245). Additionally, there was a statistically significant relationship between PDC and perineural invasion (PNI) (p=0.020). The majority of cases with PNI showed grade 2 PDC. All cases with PNI were located in proximal colon (especially cecum, ascending colon and ileocecal valve). All but one of these cases were extramural. In 70% of cases with PNI, the distance from the muscularis propria was greater than 5 mm. LVI was observed in more than half (51.8%) of dMMR cases, 39.3% of them were small vessels and 12.5% were extramural venous invasion (EMVI). There was a statistically significant relationship between LVI and tumor location (p=0.037). Accordingly, 89.7% of the LVI cases were located in the proximal colon and 72.4% of them were located in the cecum and ascending colon. There was a statistically significant relationship between LVI and pathological tumor stage (pT) (p = 0.037). LVI was observed in 54% of advanced stage cases. Of the 56 cases, 49 (87.5%) had ≥12 lymph nodes dissected. It was observed that all cases with immature desmoplastic stromas had advanced stage pathologic tumor stage (pT3, pT4) in patients with dMMR, but there was no statistically significant difference. There was a statistically significant relationship between the number of dMMR and PNI with the desmoplastic reaction (reactive fibrous zone) which developed at the tumor invasive front (p = 0.012 and p = 0.039). Mature fibrotic stroma was observed in the majority of patients dMMR and without PNI, whereas patients with PNI had intermediate/keloid-like or immature /myxoid stroma. No significant correlation was found between tumor necrosis and pathologic tumor stage, LVI (p = 0.205 to p = 0.181) however, there was a statistically significant relationship between TN and TB (p = 0.004). Intermediate or high score TB was observed in 33.8% of the patients with TN. On the other hand, there was a statistically significant relationship between TN and tumor size (P = 0.014). While the mean tumor size was 5.1 cm in patients without TN and mean the tumor size was 7.91 cm in patients with TN above 30%. There was no statistically significant relationship between dMMR and tumor growth pattern. However, cases with PNI were found to have equal distrubution among tumor growth pattern, expanding / pushing or infiltrative. Tumor deposit and LVI co-existence were observed in 12.5% of patients with dMMR. LVI was detected in all tumor deposit (TD) positive cases and PNI was detected in more than half of them. All but one of the TD positive patients were pT3 or PT4. No tumor deposit was observed in 87.5% of the cases. On the other hand, LVI was observed in 81.5% of all LN positive patients and this was statistically significant (p=0.000001).

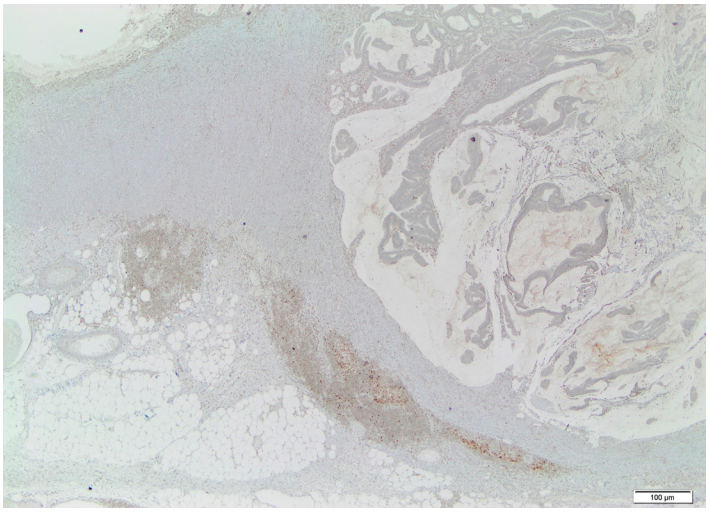

Figure 4. Representative immunohistochemical images of MLH1 loss

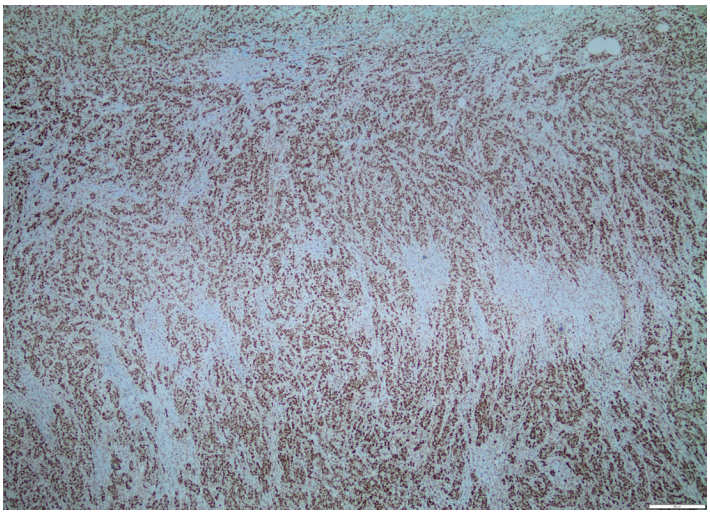

Figure 5. Immunohistochemical staining demonstrating intact expression of MSH2 in a colorectal carcinoma

Table 2. Histopathological features of patients with MMR deficiency

Peritumoral lymphoid aggregates (LAs) (Crohn-like response)	**Number (%)**
None	8 (14.3%)
Lymphoid aggregates (LAs) present 300µm-<1 mm	34 (60.7%)
Lymphoid aggregates (LAs) present ≥1 mm	14 (25.0%)
Intratumoral Lymphocytic Response (tumor-infiltrating lymphocytes. TIL)	
None	12 (21.4%)
Mild to moderate (0-2 per high-power [x400] objective)	17 (30.4%)
Marked (3 or more per high-power field)	27 (48.2%)
Reaktive fibrouse zone (Desmoplastic Reaction, DR)	Number (%)
Mature	29 (51.8%)
Intermediate (keloid-like)	14 (25%)
Immature (myxoid with)	13 (23.2%)
Tumor necrosis (dirty, TN)	Number (%)
None	9 (16.1%)
<%10	20 (35.7%)
%10-30	11 (19.6%)
>%30	16 (28.6%)
The tumor growth pattern	
Ekspansile (pushing)	41 (73.2%)
Infiltrative	15 (26.8%)
Lymph-Vascular Invasion (LVI)	
Small vessel lymphovascular invasion	22 (39.3%)
Large vessel (venous) invasion)	
Intramural (IMVI)	0
Extramural (EMVI)	7 (12.5%)
Perineural invasion (PNI)	
Intramural PNI	1 (1.8%)
Extramural PNI	9 (16.1%)
Extramural dimension <5 mm	2 (3.6%)
Extramural dimension ≥5 mm	7 (12.5%)

Mutational Analysis

Molecular mutation studies were performed in 23 (41.1%) of dMMR cases. KRAS mutations was detected in 4 (7.2%) out of 13 cases. NRAS was studied in 9 cases but no mutation was observed. The NRAS mutation study is not studied in patients with KRAS mutation. BRAF (V600E (c.1799T> A) mutation was detected in 2 of 19 patients (3.6%). MLH1 promoter methylation analysis was performed in 13 cases and hypermethylation was observed in 8 cases (14.3%) (see Table 3).

Table 3. Distribution of mutational analysis according to MMR status

	Total	**dMMR**	**pMMR**
Mutational Analysis	87 (19%)	23	64
KRAS Mutational Analysis			
No mutation detected	42	9	33
Mutation identified			
Codon 12(G12V)	16	1	15
Codon 12(G12A)	2	1	1
Codon 13 (G13D)	6	1	5
Codon 12(G12D)	7	1	6
Codon 12(G12S)	1	0	1
Codon 61 (Q61L, Q61H, Q61)	1	0	1
Codon 12(G12V, G12D)	1	0	1
Codon 12(G12A, G12V)	1	0	1
NRAS Mutational Analysis			
No mutation detected	41	9	32
Codon 61	1	0	1
BRAF Mutational Analysis			
No mutation detected (BRAF V600E (c.1799T>A)	70	17	53
BRAF V600E (c.1799T>A) mutation	4	2	2
MLH1 Mutational Analysis			
MLH1 Promoter Methylation: No mutation detected	5	5	0
MLH1 Promoter Methylation: Detected	14	8	1

Discussion

The molecular pathways of CRCs contain several different tumor contain subcategories showing high level of MSI anddMMR. MMR mutations are caused by addition or deletion of the bases within nucleotide repeats, which are defined as microsatellite regions. Microsatellite instability (MSI) is a well-known feature of hereditary nonpolyposis colorectal cancer syndrome (HNPCC), previously referred to as Lynch syndrome.This form of dMMR is caused by hereditary germline mutations in the main DNA MMR genes. Microsatellite loci contain repeating units, each of these showing 1 to 6 nucleotides in length, and often (CA) n or poly A/T sequences [6]. When no loss is observed in any of these genes, the tumor is defined as MSS and if there are losses in two or more loci, the case is grouped as showing a high frequency of microsatellite instability (MSI-H). If any loss is observed at any location, it is suggested to examine five additional loci. If the abnormal areas are counted below 40% of the test markers, the case is grouped as low level of microsatellite instability (MSI-L) [6,7]. MSI is observed

in approximately 15% of all sporadic CRCs and loss of hMLH1 immunoreactivity related with gene inactivation or hMLH1 promoter methylation. Immunohistochemically (IHC), the most common 1 gene loss is usually observed in hMSH2 or hMLH1 [6]. Our study found, the rate of dMMR among all patients was 12.3% and it was concordant with published data. The most frequent loss of binding partners was MLH1 and PMS2.

Studies based on the MSI-H ratio and annual frequency in CRCs have been found to occur between 20.000 and 26.000 MSI-H CRCs in the United States almost every year. In one study, 22% (67 of 306) of the cases were diagnosed as MSI-H based on alteration of at least two markers. According to the same study, histopathological features of MSI-H cases were signet ring cells, medullary or mucinous carcinoma (or with component), cribriform and poorly differentiation, lymphocytosis or peritumoral CLR [8]. While 55.3% of our cases had mucinous adenocarcinoma, signet ring cell carcinoma, medullary carcinoma or mixed forms, 85.7% had lymphoid aggregate and 78.6% had TILs. These finding were consistent with previous studies (see Table 4).

Table 4. Literature Reports on Immunohistochemical Staining for MMR Proteins in Colorectal Carcinomas

References	MMR status	M/F	Age, years (median)	pT3/pT4	Proximal/Distal colon (%)	Mucinous histology	CLR	TIL
Gologan A[6]	14/43	N/A	40	N/A	18/46	36%	9.7%	6.9%
Alexender J[8]	22% (67/306)	51/41	63	N/A	N/A	15%	49%	21%
Natsume S[9]	34/541	332/243	66.1	375/116	26.3/73.7 %	N/A	N/A	N/A
Young J[11]	13%		74.5	N/A	N/A	18/42	22/45	24/45
Ward R[13]	17.4 %(54/310)	172/128	68.4	97/49	115/195	50/257	44/257	63/245
Benatti P[14]	206/720	483/443	N/A	N/A	753/173	167/759	N/A	N/A
Kim CG[15]	9.7% (261/2679)	1735/1205	63	1156/1231	745/2195	12 (4.6%)	N/A	N/A
Robinson JM[16]	62/40	44/57	72.6	N/A	49/53	23/79	N/A	37/65
Johncilla M[21]	118/1014	42/74	70	35/4	92/24	20% (23/116)		%48 (56)
Parc Y[24]	24/118	77/65	74/69	123	77/65	N/A	N/A	N/A
Cohen R[33]	71/129	75/54	57	46/60	86/35	N/A	N/A	N/A
Vogelaar FJ[34]	43/143	99/87	N/A	185/1	101/85	N/A	N/A	N/A
Lim SB[35]	23/225	147/101	56.6/60.4	63.8%	59/184	16	N/A	N/A
Shin US[36]	8.2% (20/225)	142/103	59.1/62.5	130/23	61/184	7.1%	36	N/A
Klingbiel D[37]	15.1% (190/1064)	N/A	61/54	958/222	501/753	1013/232	N/A	N/A
Hyde A[38]	11%(78/632)	435/275	60.7	N/A	293/339	16/75	67/367	46/91
Yamaura T[39]	33.6% (37/73)	65/45	69	36/16	38/71	5	N/A	N/A
Malesci A[40]	10% (89/804)	519/374	65	597/102	298/595	52	N/A	N/A
Greenson JK[41]	9.85%(52/528)	N/A	N/A	178/14	183/258	121	238/269	156

MMR: Mismatch repair, **M:** Male, **F:** Female, **pT:** Pathologic Tumor Stage, **Proximal Colon:** Cecum, Ascending colon, Hepatic Flexure, and Transverse Colon, **Distal** Colon: Splenic Flexure, Descending, Sigmoid Colon, Rectum, Poorly **dif:** Poorly differentiated carcinoma , **CLR:** Crohn-like lymphoid reaction, **TIL:** Tumor-infiltrating lymphocytes

In a study in which 17 (23%) of 75 colorectal carcinomas were reported as MSI-H, it was reported that 13 cases were under 50 years of age, and 59% of MSI-H tumors showed MLH1 loss and 35% showed MSH2 loss. MSS/MSI-L tumors had more positive LN than MSI-H tumors [6]. We found, that the loss of MLH1 was seen in 89.4% of the among cases with at least two marker loss with IHC whereas the frequency of MSH2 loss was 14.9%. These results are concordant with the literature. The number of cases which were under 50 years was close to the literature (23.3%).

According to one recent study, it was found that 5.9% of 575 cases had MSI therefore MSI, KRAS, BRAF mutations were more common in the right colon. Right colon cancers had found to have a worse prognosis than the left colon cancer. Among the cases with left colon tumors, MSS ones had a worse prognosis than MSI; however, MSI status did not reveal any prognostic difference among right colon cancers. MSS cases which were stage II and located in the right colon [9]. In our study, 78.6% of dMMR tumors were located in the proximal colon.They were less likely located in the rectum and rectosigmoid colon. Our study revealed a statistically significant association and consistent with the literature. In our study, all dMMR cases which had KRAS mutation, BRAF mutation or MLH1 promoter hypermethylation were group with at least two protein loss and most of them were located in right colon.

Statistically significant relationship found between MSI and proximal colon site, female patients, younger and elder ages at

diagnosis, high histopathological differentiation and lower tumor stage. MSI cases were significantly related with better prognosis. Frequency of deaths was 60% lesser in MSI cases than other CRCs [10]. According to our findings, the mean overall survivaltime of dMMR patients was 16 months, the exitus time was in the first 6 months, and the mean age of the exitus patients was older.

Young et al. reported that sporadic MSI-H tumors were seen at a older age than HNPCC cases (74 vs 46), and they were predominantly seen in women[11]. In another study, female predominance was detected in MSI-H tumors but a slight male predominance was also reported in the literature [12]. In our study, pMMR cases were older age (mean age 63) than the patients with dMMR CRCs (mean age 59.9). Although different findings on this subject were reported in the literature; slight predominance was observed in men in our study (M/F:1.13/1).

A slow progression pattern from local stage II disease to metastatic stage III disease is a feature of MSI cancers. In a study, among the MSI subgroups, it was found that MSI-H cancer patients had a longer survival time had less recurrence; although it was not statistically significant one can say that MSI-H status have a favorable effect on overall survival [13]. In a recent study, it was shown that the type of MSI may have a better effect in the prognosis of CRC compared with MSI-L cases especially in stages II and III, in the some study; it is reported that fluorouracil-containing therapies might not be useful for survival in MSI-H cases [14].

The prevalence of general recurrence (14.0% - 7.7%) and systemic recurrence (13.1% - 6.5%) was more common in cases with MSI-L/MSS CRC than cases with MSI-H CRC. Recurrences were mostly seen in the liver, followed by lung and peritoneum. In cases with MSI-H CRC, local recurrence and peritoneal metastasis are isolated peritoneal or intraabdominal LN metastases, whereas MSS/MSI-L CRCs more frequently had lung and liver metastases [15]. In our study, 89.3% of dMMR cases were pT3 or pT4. We did not find any statistically significant relationship between MMR status and stage and overall life time. Recurrence was observed in only four of our dMMR cases, thus the number was not sufficient for further interpretation.

Colon cancers with MSI are characteristic with an inflammatory response in the form of TILs, and TILs are an significant finding for prognosis. TIL is an important descriptive histopathologic finding for MSI-H cancers, but its association with TIL and apoptosis has not been completely documented. While high apoptotic ratesand higher amount of TILs have a good prognosis for MSI-H cancers, both TIL and apoptosis may have free properties for MSI-H tumors [16]. Graham and Appelman first described the CLR in CRCs in 1990. The degree of CLR has been reported to be related with survival in CRCs. The presence of CLR has been shown to be more frequent in the right colon CRCs which exceeding muscularis propria than in tumors which were limited to the colon wall in particular. Intensive CLR at the invasive front lower rates of LN metastasis, and a positive effect on 10-year survival were determined. However, whether CLR alone is an independent prognostic factor is left to future research [17]. The maximum size of LAs appears to be more important than the number for survival. It was shown that patients with 1 mm or greater LAs had less recurrence and longer survival than patients with LAs which are less than 1 mm [18]. In our study, more prominent peritumoral LAs and TILs were observed in dMMR tumors. Similarly, no relationship was found between disease death, disease-free survival and recurrence. These findings seen to be different from the literature. There was a significant association between MLH1 and PMS2 loss and higher frequency of TILs. It was concordant with the literature.

Poorly differentiated groups (PDC) of five or more cancer cells (that do not make gland within the invasive front of cancer have an important role of predicting survival and planning surgery. Tumor budding was defined as a single cancer cell or a cluster of less than five cancer cells which are also observed at the invasive front. Ueno et al. found that, the five-year survival was 95% for the grade 1 PDC group and 59% for the grade 3 PDC group, for stage II and III cases [19]. TB and PDCs (invasive front features) are associated with advanced stage and worse clinicohistological sign. They are very closely related to TILs, CLR and MSI. When PDCs and TB grades were compared with disease-free 3-year periods, survival rate was 94% for grade 1 PDCs and TB, and 67-68% for grade 3 PDCs and TB. Based on these findings, it was suggested that PDC and TB grades can be used to stratify high and low risk in stage II cancers and may be useful while deciding adjuvant chemotherapy [20]. Johncilla et al identified that tumor budding was absent dMMR tumors and there was no correlation between TB and stage at the time of cancer diagnosis [21]. Several studies have demonstrated that TB was positively correlated with high grade pathologic tumor stage 4 and LVI; while it was reversely correlated with decreased overall survival time [21,22]. In our study, a statistically significant correlation was found between LVI and intermediate/high scores of TB it was concordant with previously published data. Tumor budding was absent in most (64.3%) of the dMMR CRCs, and there was no relationship between tumor budding and MMR status. This result is consistent with the literature.

There are several limitations to our study. First, it is not clear that how medullary carcinoma, signet ring cells carcinoma and mucinous adenocarcinoma cases would be evaluated for TB or PDCs. Second, mutation analysis was not performed in all cases.

PNI which are exceeding the muscularis propria and into muscularis propria are observed in 15 to 20% and 10 to 15% of CRCs, respectively.PNI has been accepted as an important histopathological finding in NCCN protocols for stage II CRC cases, especially while deciding adjuvant chemotherapy. Extramural PNI depth of 5 mm or 10 mm did not affect survival, but multiple extramural PNI foci were associated with poor prognosis. Intramural PNI is usually seen in the left colon and the recurrence rate is lower, in those cases while extramural PNI is mostly observed in the rectum and they have a higher incidence of recurrence in the liver, lung, and LN [23]. Park et al. reported that there was no difference among MMR status between age, PNI, LVI in colorectal carcinomas [24]. In our study, PNI was found the most commonly at extramural location. Unlike the literature, almost all of the dMMR patients with PNI were right colon tumors.

LVI positive cases (25.2%) were significantly older than LVI negative ones. In addition, LVI positive tumors were more likely to be poorly differentiated tumors with elevated serum CEA

levels,advanced pathologic stage (T and N) and diffuse metastases. LVI positive CRC patients had higher recurrence rates than LVI negative ones [25]. In our study, LN positive was detected in 75.9% of LVI (small and large) positive cases, while 93.1% of LVIpositive patients had advanced pathologic tumor stages. These results were consistent with the literature.Venous invasion (VI) is believed to be an important prognostic factor in CRCs.Several studies have been reported in the literature at different VI rates (11-89.5%). Sato et al. found that VI rate was 64% (146/229). Venous invasion were also associated with high LN metastases and patients with VI had a shorter 5-year survival (73.4% vs. 92.2%). Whether intramural (IMVI) or extramural (EMVI) venous invasion appears to have an impact on survival. Patients with IMVI had longer survival (78.7 vs 70.3%) [26]. We found, six cases were classified into the extramural invasion and 1 cases (62%) were classified into the intramural invasion.The incidence of VI in our cases is lower than the reports in the literature.In another study, it was reported that MSI-H tumors were more frequent in patients with a resection of 12 or more LNs and showed less LVI [15]. In our study, In our dMMR cases, the minimum number of lymph nodes recommended to be dissected was sufficient.

Desmoplastic reaction (DR) is a host response to tumors and has not been adequately studied compared to other prognostic factors of CRCs.In the study by Ueno and colleagues reported the DR classified as mature, intermediate and immature; 39.6%, 34.3% and 26.1% with the frequency of respectively. Immature DR was frequently observed in the rectal localization, but had also higher T and N categories than other DR types. Immature DR was associated with more frequent TB than other DR types, whereas mature DR was associated with lower risk factors. pT4 (43%) and pN2 (28.5%) cases mostly had immature desmoplastic reaction. The rate of recurrence especially lung metastasis was highest in immature DR [27]. All of the immature desmoplastic stromas had advanced pathologic tumor stage among our cases with dMMR. It was statistically significant and consistent with the literature. There was no relationship between DR type and other prognostic histopathological factors and this result is is discordant with the literature.

A small foci of tumor necrosis (TN) was observed in 365/381 (96%) patients, widespread necrosis was observed in 17%. The prevalence of TN was significantly associated with higher pathologic tumor stage (pT and pN), LVI, maximum tumor size and tumor differentiation. Tumor necrosis was reported to be an important prognostic factor [28]. In the same study, no correlation was found between MMR proteins and tumor necrosis. Five-year disease-free survival rates were ranged from 93% to 38% depending on the prevalence of tumor necrosis, while tumor necrosis and death were evaluated from 7% to 52% [28]. In our study, no significant difference was found between TN and pathologic tumor stage or LVI and it was discordant with from the literature. There was a statistically significant relationship between TN and TB scores and it was concordant with the literature. In our study, TN was found to be over 30% in the majority of cases with high TB scores.

Morikawa et al. found a pushing growth pattern in 33% of the 1139 CRCs and an infiltrative growth pattern in 14%. The survival time of CRCs with infiltrative growth pattern was found to be shorter, although the predictive role of infiltrative growth pattern on survival was limited to stage I-III cases. While an infiltrative growth pattern was reversely related to MSI-H status, it was found to be favorable associated with BRAF mutation [29]. We found a statistically significant association between dMMR and tumor growth pattern (p=0.049). Accordingly, all patients with infiltrative growth pattern were group with at least two protein loss patients.

The associated and significance of CRCs with pericolic/perirectal tumor deposits (TD) has been shown in previous studies.TDs are closely associated with previously known bad prognostic factors [30]. When TD is present the pathologic LN stage is classified as N1c, independently from pT stage and N1c stage is important in adjuvant treatment decision [31]. In a study conducted by Jin et al., it was reported that N1c patients had worse survival than N0 and N1 patients while showed better survival than the N2 cases. The prognosis of TD was similar to LN positive patients [32]. In our study, no statistically significantdifferences was found between dMMR protein markers and tumor deposit. Approximately all TD positive patients were pT3 or PT4 and this was consistent with prior literature.

Conclusion

CRCs with dMMR were frequently located in the proximal colon and had more peritumoral LAs and TILs in our study. Medullary carcinomas, mucinous adenocarcinomas, signet ring cell carcinomas and the mucinous tumor component was observed close to half of the cases. The immature desmoplastic stroma was associated with advanced pathologic tumor stage in the cases with dMMR. The prevalence of defective MMR in colorectal carcinoma in our study is similar to that seen in previous studies. The diagnosis of CRCcases with dMMR tumors is important because it helps in selecting cases for family history, prognosis and potential for effective immunotherapeutic. Especially TILs and CLR may benefit from immunotherapy in CRCs with dMMR detected. A longer follow-up is needed to make further comments on prognosis.

Conflict of interests
The authors declare that they have no competing interests.

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Ethical approval
This study was approved by the ADYAK Committee for the Marmara University, Pendik Training and Research Hospital in Research. (Kararlar;10.04.2019/3).

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