RESEARCH ARTICLE

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The Effect of Gene Mutations on Metastasis and Overall Survival in Metastatic and Nonmetastatic Colon Cancers

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Abstract

Objective: It is known that many genes are associated with colon cancer. We aimed to investigate the effect of gene mutations on metastasis and overall survival in metastatic and non metastatic colon cancers. **Methods:** A total of 50 patients with metastatic (n=25) and non metastatic (n=25) diagnosed with colon cancer between 2010 and 2018 were included in the study. *APC, MUTYH, RAD50, MEN1, ATM, PALB2, NSH2, BRCA1, BRCA2, MLH1, BRIP1, TP53, PTEN, BARD1, MSH6, PMS2, NBN,* and *FAM175A* gene mutations were evaluated using the next generation sequencing method. The effect of gene mutations on metastasis and overall survival were evaluated. **Results:** The mean age of patients with colon cancer without distant metastasis was 48.64±14.72 years and for patients with distance metases was 56.68±11.65. The mean survival time of colon cancer patients with distant organ metastasis after the metastasis date was 104.36±58.59 weeks. The presence of *APC, MUTYH*, and *TP53* genetic mutations was observed with a higher rate in metastatic colon cancer ($p<0.05$). **Conclusion:** We showed that *APC, MUTYH*, and *TP53* mutations are associated with distant organ metastasis.

Keywords: Colon cancer- metastasis- gene mutuation

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Introduction

Colorectal cancer (CRC), with more than 1.8 million new cases diagnosed in 2018, is one of the most common cancers worldwide (Schrembs et al, 2018). CRC is the second most common cancer in women (9.2%) and third cancer in men (10%) and CRC cases (55%) are more common in Western countries (Marmol et al., 2017). The incidence of CRC has increased from 1990 to 2012, with the number of new cases exceeding the previous year's new cases by 200,000 cases (Brody et al., 2015). Improvement in survival in CRC has been observed due to efforts spent on screening and early detection and advances in systemic and local treatment methods (Schrembs et al., 2018). However, CRC is the fourth most common cause of cancer-related deaths, causing an average of 700,000 deaths per year (Marmol et al., 2017).

Identifying individual risk factors for the development of CRC and even risk factors causing local or distant metastasis and recurrence is very important as it can provide prognostic information for clinicians (Xu et al., 2020) CRC was one of the first tumors in which the various genes and pathways involved in the development and progression of the disease were investigated in detail (Fear on et al., 2011; Vogelstein et al., 1988;Vogelstein et al., 2013) From a genomic perspective, it is assumed that colorectal cancer is not a single disease, but a heterogeneous group of malignancies occurring in the colon. Therefore, genomic analysis of CRC will provide important prognostic information. Critical pathways include *APC, TP53, KRAS, NRAS, BRAF, BRCA1/2, BMPR1A, Her2, PIK3CA,* transforming growth factor (TFG)-β, and mismatch repair (MMR) genes *MLH1, MSH2, MSH6,* and *PMS2* (Pearlman et al., 2016).

The major cause of death from CRC is development of metastases. When CRCs are diagnosed, approximately 22% are metastatic, and approximately 70% of patients will eventually develop metastatic relapse (Le Voyer et al., 2003; Markl et al., 2013). Considering that most of the deaths in CRC are caused by metastatic cancer, it is

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of vital importance to diagnose patients with CRC early and to reveal the factors that affect this process (Van der Geest et al., 2015).

To date, risk factors for CRC (increased BMI, red meat intake, cigarette smoking, low physical activity, low vegetable consumption, low fruit consumption etc.) (Johnson et al., 2013) are well defined. However, few studies investigate the effects of these risk factors on distant metastasis and overall survival. In this study, we aimed to detect germline mutations in 18 genes by Next-Generation sequencing in metastatic and nonmetastatic colon cancers and to investigate the effect of these mutations on metastasis and overall survival.

Materials and Methods

The institutional human study review committees of the Erciyes University 2019/794 approved this retrospective case-control study. This study was conducted in accordance with the tenets of the Declaration of Helsinki. In this study, a total of 50 patients with metastatic (n = 25) and nonmetastatic (n = 25) colon cancer who were followed up in Erciyes University Medical Oncology Department between 2010 and 2018 were included in the study. Overall survival was calculated as the time from diagnosis to death or to the last follow-up date.

Next-Generation sequencing

DNA was extracted from the sections taken from 5 micron thick paraffin blocks according to the manufacturer's instructions using QIAamp DNA FFPE Tissue kit. Sequence analysis was performed on the DNA. All variants reported here are detected with >99% confidence, depending on the frequency of the mutation present and the amplicon extent. Mutations were analyzed in Sophia DDM program using a new generation sequencing kit containing 18 genes (*APC, MUTYH, RAD50, MEN1, ATM, PALB2, NSH2, BRCA1, BRCA2, MLH1, BRIP1, TP53, PTEN, BARD1, MSH6, PMS2, NBN, FAM175A*).

Statistical Analysis

The data were evaluated using the IBM SPSS Statistics Standard Concurrent User V 25 (IBM Corp., Armonk, New York, USA) statistical program. For descriptive statistics, unit number (n), percent (%), mean ± standard deviation ($\bar{x} \pm ss$), median (M), smallest value (min), largest value (max) are given as values. The normal distribution of data for post-metastasis survival of patients with age and colon cancer patients with distant organ metastasis was evaluated using Shapiro-Wilk normality test and Q-Q charts. For the age variable, the difference between groups with and without distant organ metastasis was evaluated by independent samples t-test. The relationship between the presence of genetic mutation status and smoking status, gender, grade, cancer stage, lymph node metastasis, family history, tumor location, disease classification, and patient survival categorical variables were examined using the Exact method of the chi-square test in 2x2 and r x c tables. Subgroup analyses were performed using Bonferroni corrected two-rate tests. Post-metastasis survival (weeks) of patients with colon cancer with distant organ metastasis was determined. A regression model was created, and Cox regression analysis was performed to determine the prognostic and predictive values of factors affecting the life span of patients with colon cancer with distant organ metastasis. A $p < 0.05$ value was considered statistically significant.

Results

The mean age of patients with colon cancer without distant organ metastasis (n = 25) included in the study 48.64 ± 14.72 (Min: 15, Max: 75) years, and the mean age of patients with colon cancer with distant organ metastasis (n = 25) was 56.68 ± 11.65 (Min: 30, Max: 73) years. The mean age of patients without distant organ metastasis was lower than those with distant organ metastasis ($p = 0.037$). The average life span of colon cancer patients with distant organ metastasis after the date of metastasis was found to be 104.36±58.59 (Min: 22, Max: 267) weeks.

The frequency distributions, percentages, and the relationship between variables according to the genetic mutation status of all colon cancer patients are given in Table 1. A significant relationship was found between the groups with the presence of APC, MUTYH, and TP53 genetic mutations ($p < 0.05$). The presence of APC and MUHTY genetic mutations is 3.083 times higher in patients with colon cancer with distant organ metastasis compared to patients with colon cancer without distant organ metastasis (OR = 3.083, 95% CI: 1.937-4.909). Similarly, the presence of TP53 genetic mutations is 6,364 times higher in patients with colon cancer with distant organ metastasis than in patients with colon cancer without distant organ metastasis (OR = 2.125, 95% CI: 1.488-3.035). There were no significant differences in the presence of other genetic mutations and between the groups ($p > 0.05$).

The results of the effects of genetic mutations of all colon cancer patients on overall survival are given in Table 2. There was not a significant relationship between the presence of genetic mutation and overall survival ($p > 0.05$).

The effects of sociodemographic and clinical characteristics on overall survival of patients with colon cancer without distant organ metastasis are given in Table 3. No significant relationships were found between smoking, gender, stage, family history, tumor location and pathogenicity with disease status categories ($p > 0.05$). According to Table 4, no significant correlation was found between the presences of genetic mutations on overall survival in patients with colon cancer without distant organ metastasis ($p > 0.05$).

The sociodemographic and clinical characteristics of patients with colon cancer with distant organ metastasis and the effects of genetic mutations on overall survival are given in Table 5 and Table 6. No significant relationships were found between smoking, gender, stage, family history, tumor location, pathogenicity, presence of genetic mutation and overall survival ($p > 0.05$).

A significant relationship was found between the presence of *APC, MUTHY*, and *NSH2* genetic mutations

Table 1. Frequency Distributions, Percentages and Relationships with Metastasis in All Patients with Colon Cancer by Genetic Mutation Status

Variables	Without distant organ metastasis (n/%)	With distant organ metastasis (n/%)	p value
APC			
No	6 (33.3) [a]	- [b]	0.003‡
Yes	12 (66.7) [a]	25 (100) [b]	
MUTYH			
No	6 (33.3) [a]	- b	0.003‡
Yes	12 (66.7) [a]	25 (100) [b]	
RAD50			
No	1 (5.6)	-	0.419‡
Yes	17 (94.4)	25 (100)	
MEN1			
No	1 (5.6)	1 (4)	1.000‡
Yes	17 (94.4)	24 (96)	
ATM			
No	3 (16.7)	4 (16)	1.000‡
Yes	15 (83.3)	21 (84)	
PALB2			
No	1 (5.6)	-	0.419‡
Yes	17 (94.4)	25 (100)	
NSH2			
No	3 (16.7)	1 (4)	0.293‡
Yes	15 (83.3)	24 (96)	
BRCA1			
No	1 (5.6)	-	0.419‡
Yes	17 (94.4)	25 (100)	
BRCA2			
No	2 (11.1)	-	0.169‡
Yes	16 (88.9)	25 (100)	
MLH1			
No	-	1 (4)	1.000‡
Yes	18 (100)	24 (96)	
BRIP1			
No	-	1 (4)	1.000‡
Yes	18 (100)	24 (96)	
TP53			
No	- [a]	9 (36) [b]	0.006‡
Yes	18 (100) [a]	16 (64) [b]	
PTEN			
No	-	2 (8)	0.502‡
Yes	18 (100)	23 (92)	
BARD1			
No	-	1 (4)	1.000‡
Yes	18 (100)	24 (96)	
MSH6			
No	-	3 (12)	0.252‡
Yes	18 (100)	22 (88)	
PMS2			
No	-	1 (4)	1.000‡
Yes	18 (100)	24 (96)	
NBN			
No	-	1 (4)	1.000‡
Yes	18 (100)	24 (96)	
FAM175A			
No	-	1 (4)	1.000‡
Yes	18 (100)	24 (96)	

*, Column percentages are indicated; ‡, Fisher Exact Test; Superscripts *a* and *b* show the difference between groups. The distributions with the same letter are similar.

Table 2. Examination of the Relationship between Overall Survival and Genetic Mutations of All Patients Included in the Study

Variables	Overall Survival		
	Alive (n/%)	Dead (n/%)	p value
APC			
No	5 (22.7)	1 (4.8)	0.185‡
Yes	17 (77.3)	20 (95.2)	
MUTYH			
No	5 (22.7)	1 (4.8)	0.185‡
Yes	17 (77.3)	20 (95.2)	
RAD50			
No	1 (4.5)	-	1.000‡
Yes	21 (95.5)	21 (100)	
MEN1			
No	1 (4.5)	1 (4.8)	1.000‡
Yes	21 (95.5)	20 (95.2)	
ATM			
No	4 (18.2)	3 (14.3)	1.000‡
Yes	18 (81.8)	18 (85.7)	
PALB2			
No	1 (4.5)	-	1.000‡
Yes	21 (95.5)	21 (100)	
NSH2			
No	3 (3.6)	1 (4,8)	0.607‡
Yes	19 (86.4)	20 (95,2)	
BRCA1			
No	1 (4.5)	-	1.000‡
Yes	21 (95.5)	21 (100)	
BRCA2			
No	2 (9.1)	-	0.488‡
Yes	20 (90.9)	21 (100)	
MLH1			
No	1 (4.5)	-	1.000‡
Yes	21 (95.5)	21 (100)	
BRIP1			
No	1 (4.5)	-	1.000‡
Yes	21 (95.5)	21 (100)	
TP53			
No	2 (9.1)	7 (33.3)	0.069‡
Yes	20 (90.9)	14 (66.7)	

Table 2. Continued

Variables	Overall Survival		
	Alive (n/%)	Dead (n/%)	p value
PTEN			
No	-	2 (9.5)	0.233‡
Yes	22 (100)	19 (90.5)	
BARD1			
No	-	1 (4.8)	0.488‡
Yes	22 (100)	20 (95.2)	
MSH6			
No	2 (9.1)	1 (4.8)	1.000‡
Yes	20 (90.9)	20 (95.2)	
PMS2			
No	-	1 (4.8)	0.488‡
Yes	22 (100)	20 (95.2)	
NBN			
No	1 (4.5)	-	1.000‡
Yes	21 (95.5)	21 (100)	
FAM175A			
No	1 (4.5)	-	1.000‡s
Yes	21 (95.5)	21 (100)	

*, Column percentages are indicated; ‡, Fisher Exact Test

and cancer stage (Table 7) (p = 0.014; p = 0.014; p = 0.016). There were no significant relationships between the presence of other genetic mutations categories and cancer stage categories (p> 0.05). The relationship between each

Table 3. Investigation of the Relationship between Sociodemographic and Clinic Pathological Characteristics of Patients with Colon Cancer without Distant Organ Metastasis on Overall Survival

Variables	Overall Survival		
	Alive (n/%)	Dead (n/%)	p value
Smoking status			
No	10 (47,6)	2 (50)	1,000‡
Yes	11 (52,4)	2 (50)	
Gender			
Female	10 (47,6)	2 (50)	1,000‡
Male	11 (52,4)	2 (50)	
Staging			
II	6 (54,5)	-	-
III	4 (36,4)	-	
IV	1 (9,1)	-	
Family story			
No	16 (80)	2 (100)	1,000‡
Yes	4 (20)	-	
Tumor Location			
Right column	3 (21,4)	1 (100)	0,267‡
Left column	11 (78,6)	-	
Classification			
Significance unknown	9 (81,8)	1 (100)	1,000‡
Pathogenic / Possibly pathogenic	2 (18,2)	-	

*, Column percentages are indicated; ‡, Fisher Exact Test

Table 4. The Relationship of Patients with Colon Cancer without Distant Organ Metastases with Overall Survival According to Their Genetic Mutation Status

Variables	Overall Survival		
	Alive (n/%)	Dead (n/%)	p values
APC			
No	5 (31.3)	1 (50)	1.000‡
Yes	11 (68.7)	1 (50)	
MUTYH			
No	5 (31.3)	1 (50)	1.000‡
Yes	11 (68.7)	1 (50)	
RAD50			
No	1 (6.3)	-	1.000‡
Yes	15 (93.7)	2 (100)	
MEN1			
No	1 (6.3)	-	1.000‡
Yes	15 (93.7)	2 (100)	
ATM			
No	2 (12.5)	1 (50)	0.314‡
Yes	14 (87.5)	1 (50)	
PALB2			
No	1 (6.3)	-	1.000‡
Yes	15 (93.7)	2 (100)	
NSH2			
No	3 (18.7)	-	1.000‡
Yes	13 (81.3)	2 (100)	
BRCA1			
No	1 (6.3)	-	1.000‡
Yes	15 (93.7)	2 (100)	
BRCA2			
No	2 (12.5)	-	1.000‡
Yes	14 (87.5)	2 (100)	
MLH1			
No	-	-	-
Yes	16 (100)	2 (100)	
BRIP1			
No	-	-	-
Yes	16 (100)	2 (100)	
TP53			
No	-	-	-
Yes	16 (100)	2 (100)	
PTEN			
No	-	-	-
Yes	16 (100)	2 (100)	
BARD1			
No	-	-	-
Yes	16 (100)	2 (100)	
MSH6			
No	-	-	-
Yes	16 (100)	2 (100)	
PMS2			
No	-	-	-
Yes	16 (100)	2 (100)	

Table 4. Continued

Variables	Overall Survival		
	Alive (n/%)	Dead (n/%)	p values
NBN			
No	-	-	-
Yes	16 (100)	2 (100)	
FAM175A			
No	-	-	-
Yes	16 (100)	2 (100)	

*, Column percentages are indicated; ‡, Fisher Exact Test

genetic mutation status and gender, smoking status was examined, and no significant relationships were found (p> 0.05).

A significant relationship was found between the stage and overall survival status of patients with colon cancer without distant organ metastasis and with distant organ metastasis. The duration of life (weeks) after metastasis of colon cancer patients with distant organ metastasis was taken as the dependent variable. Cox regression analysis was performed to determine the prognostic and predictive values. It was found that age has a significant effect in determining the survival time for the colon cancer patient group with distant organ metastasis (Age = 1.110; 95% CI: 1.011-1.129; p = 0.028).

Table 5. The Relationship between Sociodemographic and Clinic Pathological Characteristics of Patients with Colon Cancer with Distant Organ Metastasis on Overall Survival

Variables	Overall Survival		
	Alive (n/%)	Dead (n/%)	p value
Smoking status			
No	4 (66.7)	9 (47.4)	0.645‡
Yes	2 (33.3)	10 (52.6)	
Gender			
Female	3 (50)	7 (36.8)	0.653‡
Male	3 (50)	12 (63.2)	
Staging			
II	1 (16.7)	-	0.290‡
III	-	2 (10.5)	
IV	5 (83.3)	17 (89.5)	
Family History			
No	4 (66.7)	15 (100)	0.071‡
Yes	2 (33.3)	-	
Tumor Location			
Right column	4 (66.7)	8 (53.3)	0.659‡
Left column	2 (33.3)	7 (46.7)	
Classification			
Significance unknown	-	1 (11.1)	1.000‡
Pathogenic / Possibly pathogenic	4 (100)	8 (88.9)	

*, Column percentages are indicated; ‡, Fisher Exact Test

Table 6. Relationship between Genetic Mutation Status and Overall Survival of Patients with Colon Cancer with Distant Organ Metastasis

Variables	Overall Survival		
	Alive (n/%)	Dead (n/%)	p values
APC			
No	-	-	-
Yes	6 (100)	19 (100)	
MUTYH			
No	-	-	-
Yes	6 (100)	19 (100)	
RAD50			
No	-	-	-
Yes	6 (100)	19 (100)	
MEN1			
No	-	1 (5.3)	1.000‡
Yes	6 (100)	18 (94.7)	
ATM			
No	2 (33.3)	2 (10.5)	0.234‡
Yes	4 (66.7)	17 (89.5)	
PALB2			
No	-	-	-
Yes	6 (100)	19 (100)	
NSH2			
No	-	1 (5.3)	1.000‡
Yes	6 (100)	18 (94.7)	
BRCA1			
No	-	-	-
Yes	6 (100)	19 (100)	
BRCA2			
No	-	-	-
Yes	6 (100)	19 (100)	
MLH1			
No	1 (16.7)	-	0.240‡
Yes	5 (83.3)	19 (100)	
BRIP1			
No	1 (16.7)	-	0.240‡
Yes	5 (83.3)	19 (100)	
TP53			
No	2 (33.3)	7 (36.8)	1.000‡
Yes	4 (66.7)	12 (63.2)	
PTEN			
No	-	2 (10.5)	1.000‡
Yes	6 (100)	17 (89.5)	
BARD1			
No	-	1 (5.3)	1.000‡
Yes	6 (100)	18 (94.7)	
MSH6			
No	2 (33.3)	1 (5.3)	0.133‡
Yes	4 (66.7)	18 (94.7)	
PMS2			
No	-	1 (5.3)	1.000‡
Yes	6 (100)	18 (94.7)	

Table 6. Continued

Variables	Overall Survival		
	Alive (n/%)	Dead (n/%)	p values
NBN			
No	1 (16.7)	-	0.240‡
Yes	5 (83.3)	19 (100)	
FAM175A			
No	1 (16.7)	-	0.240‡
Yes	5 (83.3)	19 (100)	

*, Column percentages are indicated; ‡, Fisher Exact Test

Discussion

CRC, about 56% of whom die of cancer, is the third most common cancer worldwide (Riihimaki et al.,2012). Both the frequency of CRC and its risks should be well defined in order to reduce the mortality rate from CRC. There are many habits or traits that increase the likelihood of developing colorectal cancer or polyp and are considered risk factors. Risk factors for CRC include age (Levin et al., 2008), inflammatory bowel disease (patients with ulcerative colitis have an 3.7% increased risk (Eaden et al., 2001) while people suffering from Crohn's disease have a 2.5% increased risk of developing CRC (Canavan et al., 2006) positive family history of CRC in relatives under the age of fifty (Johns et al., 2001), a sedentary lifestyle associated with obesity (Martinez-Useros et al., 2016), unhealthy eating habits(Willet et al., 2005).

Mutations may occur in tumor suppressor genes, oncogenes, and genes involved in DNA repair mechanisms in the initiation of CRC (Fear on et al., 1990). CRC can be classified as sporadic, hereditary, or familial. Sporadic cancers account for 70% of all CRC and are heterogeneous, as mutations in genes different from the molecular pathogenesis of sporadic cancer are targeted. CRC begins as a benign adenomatous intestinal polyp and turns into adenocarcinoma that can metastasize to distant organs. Each step in such gradual progression of CRC known as "multi-step tumorigenesis" is related to specific genetic changes in oncogenes or tumor suppressors (Fear on et al., 2011; Ferron et al., 1990; Markowitz et al., 2009). The development of polyps begins with a mutation in a tumor suppressor gene known as adenomatous polyposis coli (APC). Mutations in *KRAS, TP53*, and *DCC* follow the *APC* mutation. Hereditary polyposis cancer primarily includes familial adenomatous polyposis (FAP), while hereditary non-polyposis colorectal cancer (HNPCC) is associated with mutations in DNA repair mechanisms. Lynch syndrome is the most common cause of HNPCC. Inherited mutations in genes encoding DNA repair proteins such as *MLH1, PMS1, MSH2, PMS2* and *MLH6* are responsible for this syndrome (Lynch et al., 2003; Umar et al., 2004).

Although the genes involved in the development of CRC are well defined, there has not been much study on the genes that play a role in the development of distant organ metastasis and affect survival. Fang et al. (Fang et al., 2014) identified six genes (*ADCY2, ADCY9, APC, GNB5, KRAS,* and *LRP6*) associated with CRC metastasis. *APC, KRAS*, and β-catenin mutations

Table 7. Examination of the Frequency Distributions, Percentages and the Relationship with Cancer Stage of Patients with Distant Organ Metastasis and Colon Cancer without Distant Organ Metastasis According to Genetic Mutation Status

Variables	Stage			
	Stage II (n/%)	Stage III (n/%)	Stage IV (n/%)	p value
APC				
No	1 (16.7) [a, b]	2 (40) v	- [a]	0.014†
Yes	5 (83.3) [a, b]	3 (60) [b]	23 (100) [a]	
MUTHY				
No	1 (16.7) [a, b]	2 (40) [b]	- a	0.014†
Yes	5 (83.3) [a, b]	3 (60) [b]	23 (100) [a]	
NSH2				
No	- [a, b]	2 (40) [b]	- [a]	0.016†
Yes	6 (100) [a, b]	3 (60) [b]	23 (100) [a]	

*, Column percentages are indicated; †, Pearson Exact Chi-Square Test; Superscripts a and b show the difference between groups. The distributions with the same letter are similar.

are important in the development of adenoma, and *PIK3CA* and *TP53* mutations are especially important in the progression to invasive CRC (Lee et al., 2017). *KRAS* mutations are associated with metastasis, poor prognosis, and lower survival (Chen et al., 2014; Li et al., 2015) Recent studies have reported a reduced overall survival in *BRAF*-mutated CRC (41.1 vs 18.2 months) (Caymanian et al., 2018). In another study, it was observed that the loss of *PTEN* in primary tumors in patients with metastatic CRC was significantly associated with an increased risk of death and poor survival (Atria et al., 2013) Decrease in *BARD1* expression has been found to cause worse staging and therefore poor prognosis (Spurn et al., 2011). *TP53* mutations were reported in previous studies to be associated with poor prognosis in various types of cancer, including CRC (Oliver et al., 2010). *TP53* mutation rate increased to 80% in patients with metastatic CRC (Brannon et al., 2014). A *TP53* mutation gradually increases from early stage tumors to metastasis (Yeager et al., 2018). Therefore, *TP53* mutations play a role, especially in the late stage of tumorigenesis (Nakayama et al., 2019). Chang et al., (2016) showed that the frequency of NRAS mutation increased in patients with lung metastasis and that NRAS and TP53 were positively associated with tumor stage and prognosis. In our study, the presence of APC and MUTYH genetic mutations is 3.083 times greater in patients with colon cancer with distant organ metastasis compared to patients with colon cancer without distant organ metastasis. Similarly, patients with colon cancer with distant organ metastases exhibited 6.364 times more *TP53* genetic mutations than patients with colon cancer without distant organ metastases. In previous studies, the relationships between APC and *TP53* mutations and *CRC* metastasis have been shown, but as far as we know, this is the first study reporting that MUTYH affects CRC metastasis. In addition, in our study, we determined a relationship between APC, MUTYH, and NSH2 and tumor stage. As the stage of the tumor increased, the presence of APC, MUTYH, and NSH2

mutations also increased. Therefore, APC and MUTYH have a bad effect on overall survival as they both increase distant organ metastasis and are associated with high stage, and TP53 is increased in distant organ metastasis. When Cox regression analysis was performed in determining prognostic and predictive values, we found that age had a significant effect in determining the survival time for the colon cancer patient group with distant organ metastasis.

In conclusion, we show that APC, MUTYH, and TP53 mutations are associated with distant organ metastasis. We also found a relationship between APC, MUTYH, and NSH2 and tumor stage. We found that age had a significant effect in determining the survival time for the colon cancer patient group with distant organ metastasis. The results of our study should be supported by larger studies.

Author Contribution Statement

YO and YO the idea for research. MC, YO and MD were the major contributors in literature search writing the manuscript, and spelling and grammar check. HS, EC and NG managed collecting tissues. MI, Referred the patients NB and DK conducting the DNA analysis and NGS. All authors read and approved the final manuscript.

Acknowledgements

Ethics approval

The institutional human study review committees of the Erinyes University 2019/794 approved this retrospective case-control study.

Consent to participate

This study was conducted in accordance with the tenets of the Declaration of Helsinki.

Consent for publication

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version

Availability of data and material not applicable

Conflicts of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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