ÖZGÜN ARAŞTIRMA ORIGINAL RESEARCH

Med J SDU / SDÜ Tıp Fak Derg ▶ 2021:28(3):487-497 doi: 10.17343/sdutfd.866797

THE IMMUNOHISTOCHEMICAL EXPRESSIONS OF MISMATCH REPAIR GENES MLH1, PMS2, MSH6, MSH2 IN GASTRIC CANCER; A TISSUE MICROARRAY STUDY

HATALI EŞLEŞME GENLERİNDEN MLH1, PMS2, MSH6, MSH2'İN MİDE KANSERLERİNDE İMMÜNHİSTOKİMYASAL EKSPRESYONU; BİR DOKU MİKROARRAY ÇALIŞMASI

Gamze ERKILINÇ[1], Nermin KARAHAN[1], Şirin BAŞPINAR[1], Zümrüt Arda KAYMAK[2], Şehnaz EVRİMLER[3]

[1] Süleyman Demirel University, Faculty of Medicine, Department of Pathology, ISPARTA
[2] Süleyman Demirel University, Faculty of Medicine, Department of Radiation Oncology, ISPARTA
[3] Süleyman Demirel University, Faculty of Medicine, Department of Radiology, ISPARTA

Cite this article as: Erkılınç G, Karahan N, Başpınar Ş, Kaymak ZA, Evrimler Ş. The Immunohistochemical Expressions of Mismatch Repair Genes MLH1, PMS2, MSH6, MSH2 in Gastric Cancer; A Tissue Microarray Study. Med J SDU 2021; 28(3): 487-497.

Öz

Amaç
Mide kanserinde MLH1, PMS2, MSH6, MSH2'in immünhistokimyasal ekspresyonları ile klinikopatolojik parametrelerin arasındaki ilişkiyi değerlendirmeyi amaçladık.

Gereç ve Yöntem
Yüzüç primer mide adenokarsinom ve tümörsüz 27 mide mukozasına ait doku mikroarray (DMA) kesitlerine immünhistokimyasal uygulama yapıldı. Tüm markerlar nükleer boyanma açısından değerlendirildi. Markerlardan herhangi birinde negatiflik eksiklik olarak kabul edildi. Daha sonra hatalı eşleşme genlerinde eksiklik var (dMMR) ve hatalı eşleşme genleri sağlam (pMMR) olmak üzere 2 alt grup arasında karşılaştırma yapıldı.

Bulgular
Histopatolojik olarak intestinal ve intestinal olmayan alt tiplerinden, MSH2'nin intestinal grupta intestinal olmayan gruba göre ekspresyonunda anlamlı kayıp gözlendi. PMS2 ekspresyonu taşlı yüzük hücreli karsinomda diğer alt tiplere göre anlamlı olarak yüksekti. Ayrıca MLH1 ve PMS2 ekspresyonlarının kaybının orta/kötü diferansiye tümörlerde, iyi diferansiye tümörlere göre daha yüksek olduğunu gözlemledik. MLH1, MSH6, PMS2 ekspresyon kaybı ve dMMR olan olgularda pMMR'li olgulara göre perinöral invazyon anlamlı şekilde daha yüksekti. Kemoterapi/radyoterapi alan ve almayan gruplar karşılaştırıldığında dMMR ve pMMR arasında anlamlı fark yoktu. MLH1, MSH2, MSH6, PMS2 ekspresyonları ile sağkalım arasında anlamlı ilişki bulunmadı.

Sonuç
Perinöral invazyon ile MLH1, MSH6 ve PMS2 ekspresyon kaybı arasında anlamlı ilişki bulduk. PMS2 ekspresyonu taşlı yüzük hücreli karsinomda diğer alt tiplere göre anlamlı olarak yüksekti.

Anahtar Kelimeler: Mide adenokarsinom, MLH1, PMS2, MSH6, MSH2

Abstract

Objective
We aimed to evaluate the correlation between the immunohistochemical expressions of MLH1, PMS2,

İletişim kurulacak yazar/Corresponding author: gamzecirak@gmail.com
Müracaat tarihi/Application Date: 23.01.2021 • Kabul tarihi/Accepted Date: 07.09.2021
ORCID IDs of the authors: G.E: 0000-0003-4704-7415; N.K: 0000-0003-0883-4037; Ş.B: 0000-0002-4947-7569; Z.A.K: 0000-0002-7284-008X; Ş.E: 0000-0002-9907-0011

MSH6, MSH2 and clinicopathological parameters in gastric carcinoma.

Matherials and Methods
Immunohistochemistry was performed on the tissue microarray (TMA) sections of 103 primary gastric adenocarcinoma and 27 gastric mucosal tissue samples without tumor. All markers were evaluated for the presence of nuclear staining. Negative expression in any of the markers was accepted as a deficiency. Then, the comparison was made between the two subgroups as; deficient mismatch repair (dMMR) and proficient mismatch repair (pMMR).

Results
The histopathological subtypes as intestinal and non-intestinal, the intestinal group showed significant deficient expression of MSH2 compared with the non-intestinal group. PMS2 expression was significantly higher in the other subtypes than signet ring cell carcinoma. Also, we observed that the loss of MLH1 and PMS2 expressions were higher in moderately/poor differantiated tumors than the well differantiated ones. Perineural invasion was significantly higher in patients with loss of MLH1, MSH6, PMS2 expression and dMMR compared to patients with pMMR. There was no significant difference between dMMR and pMMR when compared the groups who received chemotherapy/ radiotherapy and who did not. There was not found significant relationship between MLH1, MSH2, MSH6, PMS2 expressions and survival.

Conclusion
We found a significant relationship between perineural invasion and the loss of expression of MLH1, MSH6 and PMS2. PMS2 expression was also significantly higher in the other subtypes of GC than signet ring cell carcinomas.

Keywords: Gastric adenocarcinoma, MLH1, PMS2, MSH6, MSH2

Introduction

Gastric cancer (GC) is one of the major health problems which is the leading third cause of cancer-related deaths (1). Although the incidence has been declining, it is still the fourth most common cancer (2). GC may be sporadic, familial or hereditary. Most of them are sporadic, but 5-10% have APC (antigen-presenting cell promotor 1B) mutation. Hereditary GC with germline mutations like CDH1 (cadherin 1) or CTNNA1 (catenin alfa) may be observed (3). Many genetic mutations are detected in GC, but their role is undetermined (4). Four subtypes have been identified in the molecular level by genomic analysis. These are microsatellite instability (MSI), Epstein Barr Virus (EBV) positivity, chromosomal instability, and genomic stability (3).

Microsatellite instability, a subtype of genetic instability, is the sequence repeating in different lengths (5). The mismatch repair (MMR) genes associated with Lynch Sendrome (LS) consists Human mutL homolog 1 (MLH1), human mutS homolog 2 (MSH2), human mutS homolog 6 (MSH6), postmeiotic segregation increased 2 (PMS2), and epithelial cellular adhesion molecule (EPCAM). The MSI phenotype was found to be related with better survival in stage II GC (10). 2-4% of colorectal carcinomas are associated with LS (6-9). In addition to colon cancers, endometrium, small intestine, ureter and renal pelvis related tumors were more common in LS cases compared to normal population (8). The same was observed in pancreas, ovary and stomach (8).

The molecular classification of GC is needed urgently. These classifications will play an important role in the diagnosis and treatment of the GC. DNA MMR genes (MLH1, PMS2, MSH2, MSH6), which are MSI genes can be evaluated by the immunohistochemical methods (3).

The purpose of our study was to evaluate the correlation between the immunohistochemical expressions of MLH1, PMS2, MSH6, MSH2 and clinicopathological parameters which may affect the treatment and prognosis of GC.

Materials and Methods

Patients And Tissue Samples

We studied samples of 103 primary gastric adenocarcinoma patients who underwent CT imaging and gastrectomy between 2007-2019 in our hospital. Enough number of patients from both gender were included to the study. The resection materials from GC patients that had sufficient clinical and pathological data were re-evaluated. Non-tumoral materials from patients were assigned as control group. Non tumoral patients were numbered and 27 patients were selected using computerized randomization method.

4-5 µm-thick sections of Hematoxylin &Eosin stained

slides obtained from the formalin-fixed blocks of all of the tissue samples were re-evaluated and optimal blocks were selected. All of the cases were histopathologically confirmed as gastric adenocarcinoma according to the World Health Organisation (WHO) classifications of tumors of Digestive System 2019 version (11). The gastric adenocarcinoma primary tumor (pT) was determined using the College of American Pathologists (CAP) 2017 version (12).

Gastric adenocarcinoma cases were re-evaluated retrospectively for histopathological subtype, tumor localization, lymphovascular invasion, perineural invasion, lymph node metastasis, treatment effects and pT stage according to TNM classification. Also, slides stained for C erb-B2 were re-evaluated. Clinical findings such as age, gender, metastasis, disease-free survival, overall survival, adjuvant therapy, recurrence were analyzed via the hospital's information system.

According to the radiological evaluation tumor localizations were classified into three groups: 1; fundus, cardia, 2; greater and lesser curvature, corpus, 3; antrum, pylorus. Tumor diameter was divided into groups according to ≤5 cm and >5 cm in diameter.

The clinicopathological features of GC cases were summarized in Table 1.

Ethics committee approval was obtained for the study by report no. 199285 on 03.12.2019 at Suleyman Demirel University Local Ethics Committee.

Immunohistochemistry

The tissue microarray (TMA) blocks were constructed from the selected paraffin blocks manually. Briefly, suitable areas were marked on standard H&E stained slides. To increase representative focus due to tumour heterogeneity from each case two representative 2 mm diameter tissue cores were punched from the original block and inserted into a new recipient paraffin block manually. The resection materials of the patients that underwent gastrectomy for non-tumoral conditions were assigned as control group. In addition, normal colon mucosa was used as a positive control. A manual TMA Builder (Labvision, Thermo Fisher Scientific) instrument was used for the construction of these TMA's. The sections with a thickness of 4 µm of TMA blocks were used for immunohistochemistry by streptavidin biotin peroxidase tecnique for MLH1, PMS2, MSH6, MSH2. The sections were deparaffinized in xylene and dehydrated in descending dilutions of ethanol. Antigen retrieval was achieved by heat treatment at 98°C in citrate buffer (pH= 6.0) for 20 minute. The immunostaining was performed using DAKO Omnis Autostainer™ (Santa Clara, USA). Endogenous peroxidase activity was blocked by 20 minute of incubation with 0.3% hydrogen peroxidase. Slides were tested with MSH6 (Dako, FLEX Monoclonal Rabbit, Anti-Human, clone EP49, the cellular staining pattern is predominantly nuclear, ready to use, catolog number: IR086), MSH 2 (Dako, FLEX Monoclonal Mouse, Anti-Human, clone FE11, the cellular staining pattern is predominantly nuclear, ready to use, catolog number: IR085), PMS2 (Dako, FLEX Monoclonal Rabbit, Anti-Human, Clone EP51, the cellular staining pattern is predominantly nuclear, ready to use, catolog number: IR087), MLH1 (Dako, FLEX Monoclonal Mouse, Anti-Human, clone ES05, the cellular staining pattern is predominantly nuclear, ready to use, catolog number: IR079).

Evaluation Of Immunohistochemical Staining

All immunohistochemically stained slides were evaluated by pathologists, blinded to the clinical and histopathological findings. Positive staining for MLH1, MSH2, MSH6, PMS2 was determined according to the presence of nuclear staining regardless of the percentage. Negative staining was identified as loss of expression in all of the tumor cells (13).

The comparison analysis was made for the expression of each of the 4 immunohistochemical stains. Negative expression in any of MLH1, PMS2, MSH6, MSH2 markers was accepted as a deficiency. Then, the comparison was made between the two subgroups, deficient mismatch repair (dMMR) and proficient mismatch repair (pMMR).

Statistical Analysis

Statistical analysis was performed using SPSS 21 (Armonk, New York). The normality of continuous data was tested using Kolmogorow smirnov test or Shapiro-Wilk test. Continious data were compared using independent sample t test or Mann-Whitney-U test. Categoric data were compared using Pearson Chi-square test. Fisher's exact test was used when expected value problem occurred. A p value<0.05 was regarded as statistical significant. Kaplan Meier analysis was used for survival analysis. Log rank test was used for comparison of survival data.

Results

The cases of GC, 33 (32%) were under 60 years old and 70 (%68) were over 60 years old. Most of the patients were male (64.1% vs 35.9%). The distrubition of the tumors localisation were as follows: fundus and cardia: 28.3%, greater, lesser curvature and corpus:

27.2%, antrum and pylorus: 16.5%. In %81 of cases GC shows LVI and %65 were PNI. Intestinal type tumor was observed in %70.9 of GC. Cer B2 score was 0 (%64.1) in most of the cases (Table 1).

When expressions of MLH1, MSH2, MSH6, PMS2 in gastric adenocarcinoma cases were evaluated;

MLH1 expression: All of the nonneoplastic gastric tissue showed dense MLH1 nuclear expression (Figure 1A). Gastric adenocarcinomas with positive and negative MLH1 immunoexpression were shown in Figures 1B and 1C, respectively.

There was a significant relationship between MLH1 expression and perineural invasion and diferantiation of the tumors (p=0.01, p=0.005, respectively) (Table 2). MLH1 negativity was higher in moderately/poorly differentiated adenocarcinomas and also tumors with perineural invasion.

Considering other clinicopathological parameters of GC cases, no statistically significant correlation was found between MLH1 expression and the variables such as gender, age, tumor localisation, lymphovascular invasion, histopathologic subtype, Cerb B2 score and TNM of GC group (p>0.05) (Table 2).

MSH2 expression: All of the nonneoplastic gastric tissue showed dense MSH2 nuclear expression (Figure 2A). Gastric adenocarcinomas with positive and negative MSH2 immunoexpression were shown in Figures 2B and 2C, respectively.

There was a significant relationship between MSH2 expression and histologic type of GC (p=0.02) (Table 2). MSH2 negativity was significantly higher in intestinal type gastric carcinoma compared to other histologic types (p=0.02).

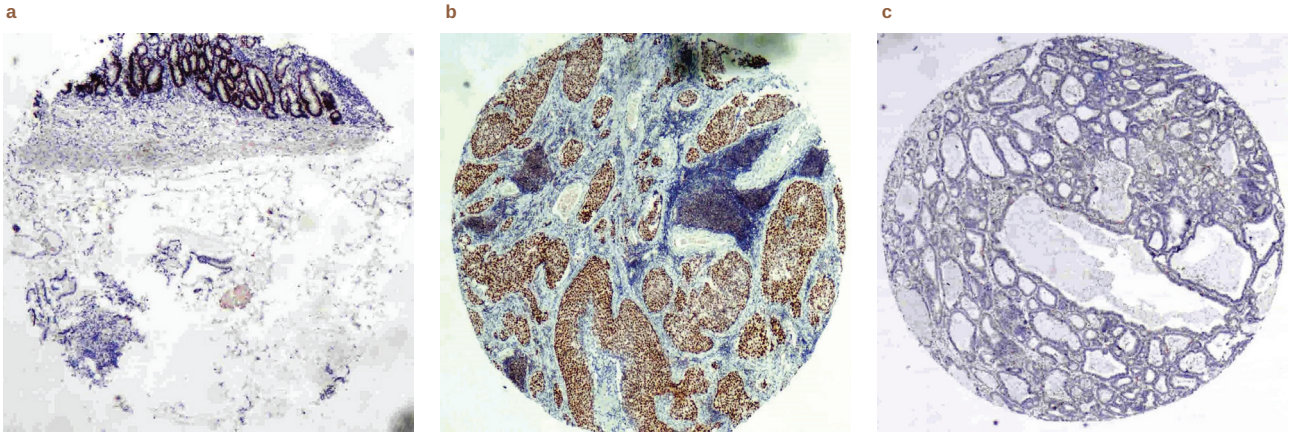

Figure 1
MLH1 immunoreactivity; Non-neoplastic gastric tissue diffuse nuclear positive
(A), gastric adenocarcinoma strong nuclear positive (B), gastric adenocarcinoma negative (C) (x40)

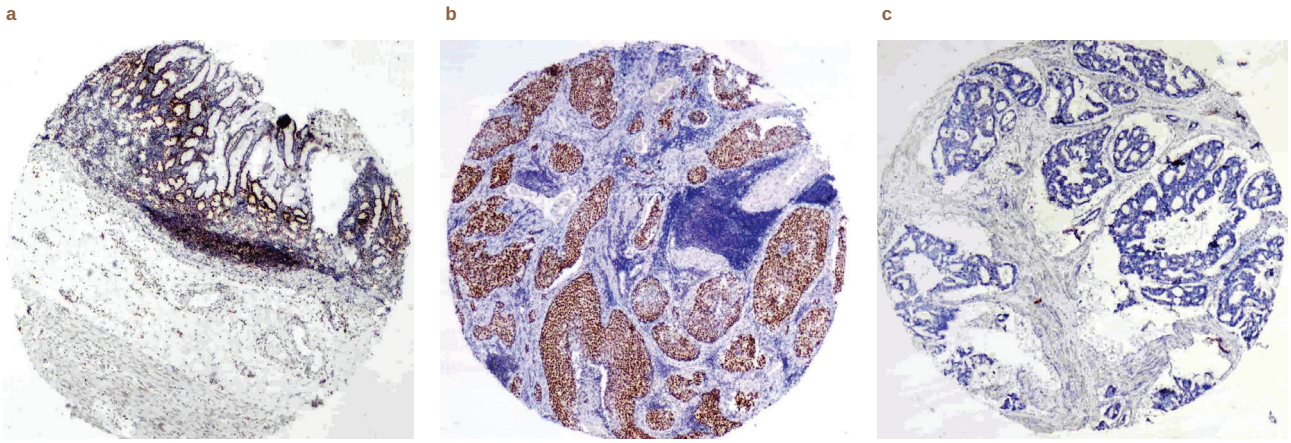

Figure 2
MSH2 immunoreactivity; Non-neoplastic gastric tissue diffuse nuclear positive
(A), gastric adenocarcinoma strong nuclear positive (B), gastric adenocarcinoma negative (C) (x40)

Considering other clinicopathological parameters of GC cases, no statistically significant correlation was found between MSH2 expression and the other variables (p>0.05) (Table 2).

MSH6 expression: All of the nonneoplastic gastric tissue showed dense MSH6 expression (Figure 3A). Gastric adenocarcinomas with positive and negative MSH6 immunoexpression were shown in Figures 3B and 3C, respectively.

Table 1 Clinicopathological features of GC cases

	n(%)		n(%)
Age		**Cerb B2**	
≤60	33 (32)	**Score 0**	66 (64.1)
>60	70 (68)	**Score 1**	4 (3.9)
Gender		**Score 2**	2 (1.9)
Female	37 (35.9)	**Score 3**	12 (11.7)
Male	66 (64.1)	**pT**	
Tumours localisation		**1**	6 (5.8)
Group 1	58 (56.3)	**2**	10 (9.7)
Group 2	28 (27.2)	**3**	60 (58.3)
Group 3	17 (16.5)	**4**	27 (26.2)
Lymphovascular Invasion (LVI)		**pN**	
Positive	81 (78.6)	**0**	15 (14.6)
Negative	22 (21.4)	**1**	14 (13.6)
Perineural Invasion (PNI)		**2**	35 (34)
Positive	65 (63.1)	**3**	39 (37.9)
Negative	38 (36.9)	**pM**	
Histopathologic subtype		**0**	75 (72.8)
İntestinal	73 (70.9)	**1**	27 (26.2)
The other	30 (29.1)		

pT: Primary tumour, pN: Regional lymph nodes, pM: Distant metastasis

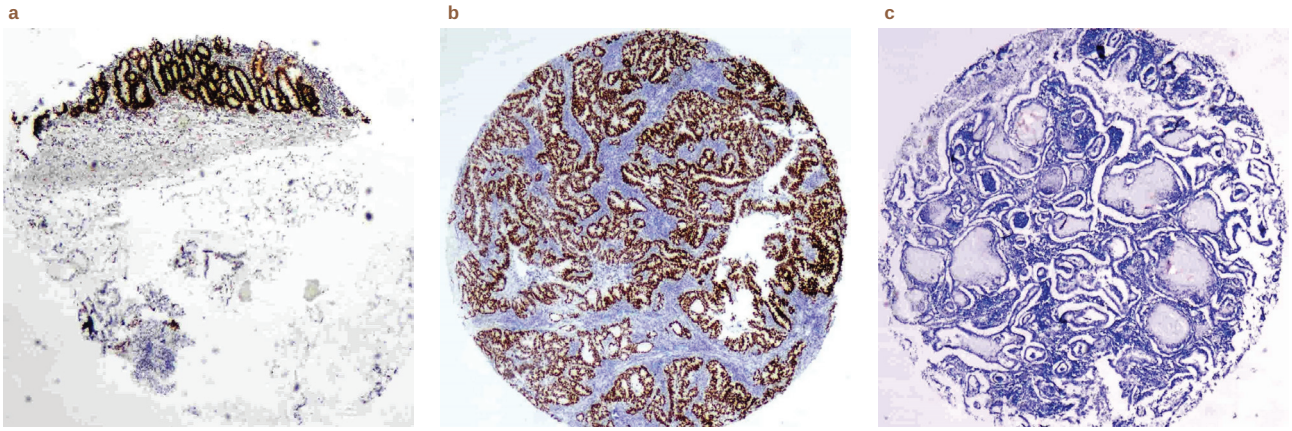

Figure 3
MSH6 immunoreactivity; Non-neoplastic gastric tissue diffuse nuclear positive (A), gastric adenocarcinoma strong nuclear positive (B), gastric adenocarcinoma negative (C) (x40)

Table 2 The distribution of MLH1, PMS2, MSH6, MSH2 expressions according to the clinicopathological features in GC cases

	MLH1 expression			MSH2 expression			MSH6 expression			PMS2 expression		
	Negative (n%)	Positive (n%)	P	Negative (n%)	Positive (n%)	P	Negative (n%)	Positive (n%)	P	Negative (n%)	Positive (n%)	P
Gender												
Female	12 (32.4)	25 (37.9)	0.58	10 (32.3)	27 (37.5)	0.61	5 (31.3)	32 (36.8)	0.67	12 (31.6)	25 (38.5)	0.48
Male	25 (67.6)	41 (62.1)		21 (67.7)	45 (62.5)		11 (68.8)	55 (63.2)		26 (68.4)	40 (61.5)	
Age	64±13	65±13	0.70	61±11	66±14	0.12	59±14	65±13	0.91	65±13	64±13	0.85
Tumor Localisation												
Group 1	8 (21.6)	9 (13.6)	0.272	7 (22.6)	10 (13.9)	0.508	3 (18.8)	14 (16.1)	0.711	8 (21.1)	9 (13.8)	0.194
Group 2	17 (45.9)	41 (62.1)		17 (54.8)	41 (56.9)		10 (62.5)	48 (55.2)		17 (44.7)	41 (63.1)	
Group 3	12 (32.4)	16 (24.2)		7 (22.6)	21 (29.2)		3 (18.8)	25 (28.7)		13 (34.2	15 (23.1)	
LVI												
Negative	6 (16.2)	16(24.2)	0.34	8 (25.8)	14(19.2)	0.47	2 (12.5)	20 (23)	0.51	5 (13.2)	17(26.2)	0.12
Positive	31 (83.8)	50(75.8)		23 (74.2)	58(80.6)		14 (87.5)	67 (77)		33 (86.8)	48(73.8)	
PNI												
Negative	8 (21.6)	30(45.5)	**0.01**	10 (32.3)	28(38.9)	0.52	2 (12.5)	36(41.4)	**0.02**	7 (18.4)	31(47.7)	**0.003**
Positive	29 (78.4)	36(54.5)		21 (67.7)	44(61.1)		14 (87.5)	51(58.6)		31 (81.6)	34(52.3)	
Histology												
Intestinal	25 (67.6)	48(51.6)	0.09	23 (74.2)	50(50.5)	**0.02**	12 (75)	61(53.5)	0.10	24 (63.2)	49(53.3)	0.30
Other	12 (32.4)	45(48.4)		8 (25.8)	49(49.5)		4 (25)	53(46.5)		14 (36.8)	43(47.7)	
SRCC	10 (27.0)	14 (15.1)	0.112	6 (19.4)	25 (80.6)	0.883	2 (12.5)	22 (19.3)	0.512	12(31.6)	12 (13.0)	**0.013**
Other	27 (73.0)	79 (84.9)		25 (80.6)	81 (81.8)		14 (87.5)	92 (80.7)		26 (68.4)	80 (87.0)	
Cerb B2												
Score 0	27(87.1)	39 (73.6)		16 (66.2)	50 (83.3)		10 (90.9)	56 (76.7)		27 (87.1)	39 (73.6)	
Score 1	1 (3.2)	3 (5.7)	0.458	3 (12.5)	1 (1.7)	0.08	0 (0)	4 (5.5)	0.409	1 (3.2)	3 (5.7)	0.192
Score 2	0 (0)	2 (3.8)		0 (0)	2 (3.3)		0 (0)	2 (2.7)		0 (0)	2 (3.8)	
Score 3	3 (9.7)	9 (17)		5 (20.8)	7 (11.7)		1 (9.1)	11 (15.1)		3 (9.7)	9 (17)	
Differantiation												
Well	7 (18.9)	31 (47.0)	**0.005**	14 (45.2)	24 (33.3)	0.254	5 (31.3)	33 (37.9)	0.611	6 (15.8)	32 (49.2)	**0.001**
Moderately-Poor	30 (81.1)	35 (53.0)		17 (54.8)	48 (66.7)		11 (68.8)	54 (62.1)		32 (84.2)	33 (50.8)	
pT												
1	1 (2.7)	5 (7.6)		2 (6.5)	4 (5.6)		0(0)	6 (6.9)		0(0)	6 (9.2)	
2	3 (8.1)	7 (10.6)	0.55	3 (9.7)	7 (9.7)	0.32	2 (12.5)	8 (9.2)	0.78	3 (7.9)	7 (10.8)	0.17
3	24 (64.9)	36(54.5		21(67.7)	39(54.2)		12 (75)	48(55.2)		26(68.4)	34(52.3)	
4	9 (24.3)	18(27.3)		5 (16.1)	22(30.6)		2 (12.5)	25(28.2)		9(23.7)	18(27.7)	
pN												
0	5 (13.5)	10(15.2)		6 (19.4)	9 (12.5)		2 (12.6)	13(14.9)		4 (10.5)	11(16.9)	
1	7 (18.9)	7 (10.6)	0.63	3 (9.7)	11(15.3)	0.47	1 (6.3)	13(14.9)	0.78	6 (15.8)	8 (12.3)	0.87
2	13 (35.1)	22(33.3)		12 (38.7)	23(31.9)		6 (37.5)	29(33.3)		15 (39.5)	20(30.8)	
3	12 (32.4)	27(40.9)		10 (32.3)	29(49.3)		7 (43.8)	32(36.8)		13 (34.2)	26(40)	
pM												
0	29 (80.6)	46(69.7)	0.23	22 (71)	53(74.6)	0.69	13 (81.3)	62(72.1)	0.55	29(78.4)	46(70.8)	0.40
1	7 (19.4)	20(30.3)		9 (29)	18(25.4)		3 (18.8)	24(27.9)		8 (21.6)	19(29.2)	

MLH1: MutL Protein Homolog 1, MSH 2: Muts Protein Homolog 2, MSH6: (Muts Protein Homolog 6,
PMS2: Postmeiotic Segregation Increased 2, SRCC: Signet ring cell carcinoma

There was a significant relationship between MSH6 expression and perineural invasion positivity (p=0.02) (Table 2). MSH6 negativity was significantly higher in patients with perineural invasion compared to patients without perineural invasion.

Considering other clinicopathological parameters of GC cases, no statistically significant correlation was found between MSH6 expression and the other variables (p>0.05) (Table 2).

PMS2 expression: All of the nonneoplastic gastric tissue showed dense PMS2 expression (Figure 4A). Gastric adenocarcinomas with positive and negative PMS2 immunoexpression were shown in Figures 4B and 4C, respectively.

There was a significant relationship between PMS2 expression and perineural invasion, differantiation and histopathologic subtype of GC (p=0.003, p=0.001, p=0.013; respectively) (Table 2). Loss of PMS2 expression was significantly higher in patients with perineural invasion and higher in moderately/ poorly differentiated carcinoma compared to well

differentiated carcinoma. PMS2 expression was significantly higher in other histologic types compared to SRCC.

Considering other clinicopathological parameters of GC cases, no statistically significant correlation was found between PMS2 expression and the other variables (p>0.05) (Table 2).

The relation of dMMR, pMMR expressions with the clinicopathological features of GC cases shows that perineural invasion was significantly higher in patients with dMMR compared to patients with pMMR (p=0,019). There was no statistically significant difference between dMMR and pMMR groups regarding as gender, tumor diameter, localisation, differentiation, histopathology, tumor type, Cerb-B2 score, LVI, TNM classification and treatment effect of adjuvant radiotherapy and chemotherapy (p>0.05) (Table 3).

MLH1, PMS2, MSH6, MSH2 expressions and postoperative survival of GC patients shows that no significant difference was found in MLH1, MSH2, MSH6 and PMS2 positive and negative patients in terms of overall survival. Although better survival was observed in dMMR patients no significant difference was found between dMMR and pMMR groups (p>0.05) (Figure 5, 6). MLH1, MSH2, MSH6 and PMS2 had no effect on overall survival (HR: 3.8, 1.3, 1.6, 1.7 respectively, p>0.05) (Table 4).

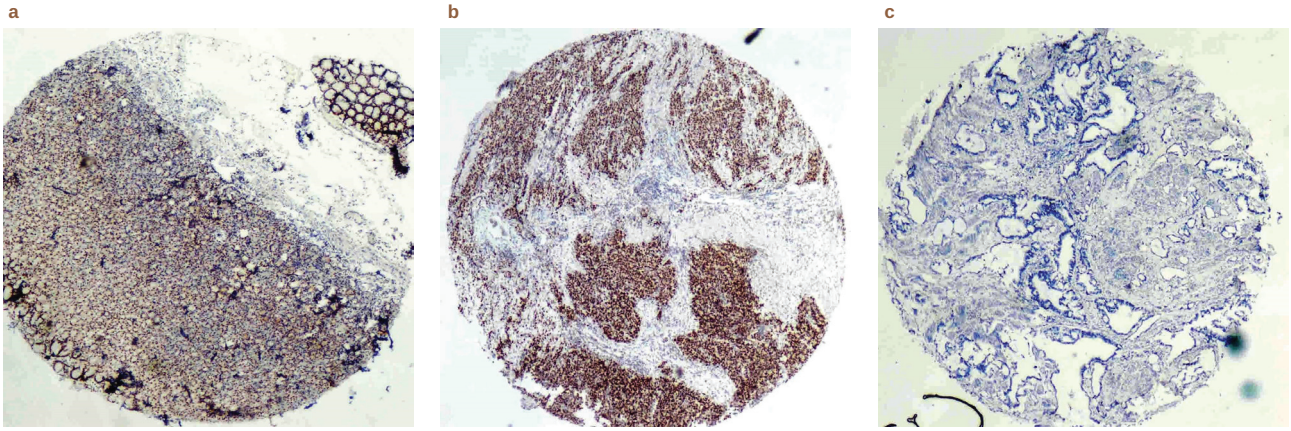

Figure 4
PMS2 immunoreactivity; Non-neoplastic gastric tissue diffuse nuclear positive (A), gastric adenocarcinoma strong nuclear positive (B), gastric adenocarcinoma negative (C) (x40)

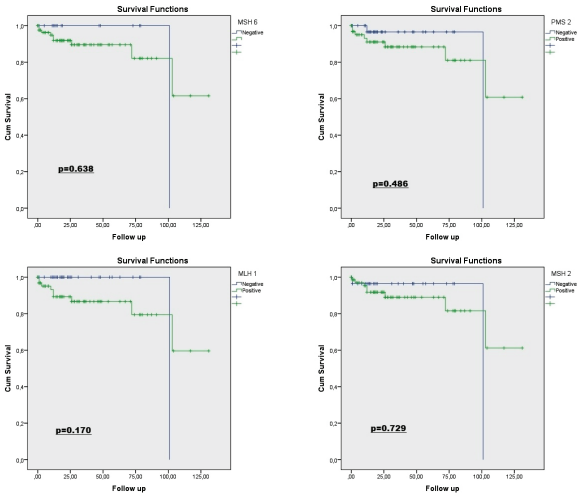

Figure 5
Kaplan Meier Survival analysis in patients with showing MLH1, MSH2, MSH6, PMS2 positivity and negativity

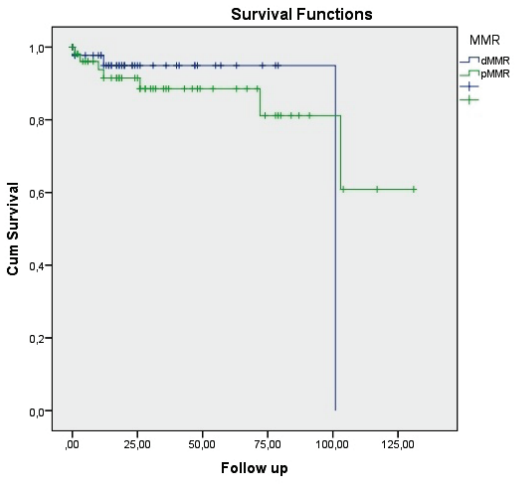

Figure 6
Kaplan Meier Survival analysis of GC patients with dMMR and pMMR positivity and negativity

Table 3 The distribution of MLH1, PMS2, MSH6, MSH2 expressions according to the clinicopathological features in GC cases

	dMMR	pMMR	P		dMMR	pMMR	P
Gender				**LVI**			
Female	17 (35.4)	20 (36.4)	0.920	**No**	10 (20.8)	12 (21.8)	0.903
Male	31 (64.6)	35 (63.6)		**Yes**	38 (79.2)	43 (78.2)	
Tumor Diameter				**PNI**			
≤ 5 cm	12 (25.0)	19 (34.5)	0.292	**No**	12 (25.0)	26 (47.3)	**0.019**
>5cm	36 (75.0)	36 (65.5)		**Yes**	36 (75.0)	29 (52.7)	
Tumor localisation				**pT**			
Group 1	11 (22.9)	6 (10.9)	0.174	**1**	2 (4.2)	4 (7.3)	0.494
Group 2	23 (47.9)	35 (63.6)		**2**	3 (6.3)	7 (12.7)	
Group 3	14 (29.2)	14 (25.5)		**3**	31 (64.6)	29 (52.7)	
				4	12 (25.0)	15 (27.3)	
Differentiation				**pN**			
Well	14 (29.2)	24 (43.6)	0.163	**0**	7 (14.6)	8 (14.5)	0.574
Moderately	18 (37.5)	12 (21.8)		**1**	8 (16.7)	6 (10.9)	
Poorly	16 (33.3)	19 (34.5)		**2**	18 (37.5)	17 (30.9)	
				3	15 (31.3)	24 (43.6)	
Histopathology				**pM**			
SRCC	13 (27.1)	11 (13.4)	0.053	**0**	35 (74.5)	40 (72.7)	0.843
Other	35 (72.9)	71 (86.6)		**1**	13 (25.5)	15 (27.3)	
Type of tumor							
İntestinal	33 (68.8)	40 (54.8)	0.499	**Radiotherapy**	38 (79.2)	42 (76.4)	0.733
Papillary	0 (0)	2 (3.6)		**No**	10 (20.8)	13 (23.6)	
Signet Ring Cell	13 (27.1)	11 (20.0)		**Yes**			
Mucinous	2 (4.2)	2 (3.6)					
Cerb-B2							
Skor 0	31 (79.5)	35 (77.8)	0.623	**Chemotherapy**	27 (56.3)	34 (61.8)	0.566
Skor 1	3 (7.7)	1 (2.2)		**No**	21 (43.8)	21 (38.2)	
Skor 2	0 (0)	2 (4.4)		**Yes**			
Skor 3	5 (12.8)	7 (15.6)					

SRCC: Signet ring cell carcinoma

Table 4 Cox regression analysis in predicting the overall survival of GC patients

Overall survival			
	HR	**95% CI**	**P**
MLH1 expression			
Negative **Positive**	Reference 3.8	0.48-31.1	0.134
MSH2 expression **Negative** **Positive**	 Reference 1.3	 0.27-6.36	 0.734
MSH6 expression **Negative** **Positive**	 Reference 1.6	 0.20-13.1	 0.620
PMS2 expression **Negative** **expression Positive**	 Reference 1.7	 0.35-8.49	 0.472
PNI **Negative** **Positive**	 Reference 0.8	 0.23-2.81	 0.751

MLH1: MutL Protein Homolog 1, MSH 2: Muts Protein Homolog 2, MSH6: (Muts Protein Homolog 6, PMS2: Postmeiotic Segregation Increased 2

Discussion

Multiple alterations of oncogenes and tumor suppressor genes plays a role in the development of gastric adenocarcinoma (14). Despite that, the progression mechanism of the multiple gene mutations to the carcinogenesis in GC has not been fully understood (14). MMR genes are necessary for the genome to be copied accurately in cell proliferation and if there is a deficiency, mutation risk increases by 100 times compared with a healthy cell (14, 15). Base mismatch, genomic repair defects caused by MMRS (mistmach repair genes) mutation can lead to an increase in the genome instability which can be observed in gastric, endometrial, and ovarian cancers (16).

MLH1 and MSH2, the microsatellite instability genes, were found higher in both female and male patients with GC. No significant correlation has been identified with MSH6 (17, 18). MLH1, MSH2, PMS2, MSH6 mutations were found to demonstrate dominancy in male patients with Lynch syndrome in another study (19). MSI was detected in 15-33% of sporadic GC (2, 20). There are many studies in the literature showing that MSI was higher in older patients (2, 21, 22). In our study there was no significant difference between loss of MLH1, MSH2, PMS2, MSH6 expressions and age and gender of the GC patients. We also didn't find a significant difference in dMMR and pMMR groups between the age and gender of the patients.

Yamamoto et al. (23) found that gastric tumors with MSI, advanced age, female gender, distal localization were associated with good prognosis (23, 24). In our study, we didn't observe a significant relationship between the tumor localization and loss of expressions of MLH1, MSH2, PMS2, MSH6.

Arai et al. (25) demonstrated that poorly-differentiated tumors were less common than the well-differentiated tumors among the GC in the early stage with MSI, but there was no significant difference among the GC in advanced stage (25). We didn't find a significant relationship between tumor stage (pT stage) and loss of expression of MLH1, MSH2, PMS2, MSH6.

Hirotsu et al. (26) observed positive expression for MLH1, MSH2, PMS2, MSH6 in the signet cell gastric adenocarcinoma when compared with the non-signet cell tumors. In another study, they found the percentage of signet cell carcinoma with dMMR as 33% (27). We did not find any significant difference in terms of histopathology between the patients

with dMMR and pMMR. But only PMS2 expression was significantly higher in other histologic subtypes compared to in signet ring cell carcinoma.

Arai et al. (25) suggested that MSI may be associated with specific histological subtypes and that GC with MSI may be originated from differentiated type carcinomas causing histological diversity during tumor progression. In our study, we didn't find a significant difference in dMMR-pMMR between the well, moderately, poorly differentiated tumors. Moderately/ poorly differantiated tumors demonstrated a significant loss of expression of MLH1, PMS2 compared with the others. However, such a relation was not found regarding the expression of MSH2, MSH6.

In another study, no significant relationship was found between MLH1 expression and stage of lymph node metastasis in the MSI+ tumors by PCR method (28). Likewise, we observed no significant difference among metastatic lymph node stages and the expression of MLH1, MSH2, MSH6, PMS2, and dMMR-pMMR groups.

There is limited data in the literature investigating the relation between the immunohistochemical expression of MLH1, MSH2, MSH6, PMS2 and perineural invasion of gastric adenocarcinoma. We observed a significant correlation with the loss of MLH1, MSH6, PMS2 expressions and perineural invasion in the gastric adenocarcinoma cases, whereas there was no association with MSH2 and patients with perineural invasion compared to patients without perineural invasion was significantly higher in dMMR.

Seo et al. (2) found no significant difference in MSI between the lymphovascular invasion groups (positive, negative, unknown). Also, we didn't find a significant difference in the expression of MLH1, MSH2, MSH6, PMS2, and dMMR-pMMR between the positive and negative lymphovascular invasion groups.

Kim et al. (29) found that GC with peritoneal metastasis had higher pMMR than the GC with lung, bone, lymph node, liver, cranial, and other metastasis. In our study, there was no significant difference in the expressions of MLH1, MSH2, MSH6, PMS2, and dMMR-pMMR between the groups regarding distant metastasis.

Cerb B2 is a protooncogene in the epidermal growth factor receptor family showing an 8-56% alteration in the expression of GC (30). In the literature, there is limited data for the evaluation of CerbB2 score and MSI in GC. We found no significant difference between the CerbB2 score and dMMR-pMMR groups (0-3). The limitation of our study was that the evaluated tissue samples may have not represented all of the tumor, because of using the tissue microarray method. Therefore, we may have observed false negativity than expected in our study.

Conclusion

Gastric adenocarcinoma may present with different histopathological subtypes and clinical findings. There are many factors in a routine histopathological analysis that can affect prognosis. We found a significant relationship between perineural invasion and the loss of expression of MLH1, MSH6 and PMS2. Also, we observed that the loss of MLH1 and PMS2 expressions were higher in moderately/ poor differentiated tumors than the well differentiated ones, and PMS2 expression was also significantly higher in the other subtypes of GC than signet ring cell carcinomas. According to these findings we suggest that the mentioned genes may contribute to the differentiation and aggressive behaviour of GC. In addition, none of the MMR genes had an effect on overall survival and prognosis of GC in our study.

Acknomledgments: Ethics committee approval was obtained for the study by report no. 199285 on 03.12.2019 at Local Ethics Committee.

Conflict of Interest
The authors have no conflicts of interest to declare.

References

1. Bösch F, Todorova R, Link H, et.al. Molecular subtyping of gastric cancer with respect to the growth pattern of lymphnode metastases Journal of Cancer Research and Clinical Oncology 2019; 145(11), 2689-2697.
2. Seo HM, Chang YS, Joo SH, et.al. Clinicopathologic Characteristics and Outcomes of Gastric Cancers With the MSI-H Phenotype. Journal of Surgical Oncology 2009;99:143–147.
3. Maleki SS, Röcken C. Chromosomal Instability in Gastric Cancer Biology. Neoplasia 2017;19:412-420.
4. Thibodeau SN, French AJ, Roche PC, et al. Altered expression of hMSH2 and hMLH1 in tumors with microsatellite instability and genetic alterations in mismatch repair genes. Cancer Res 1996; 56:4836–4840.
5. Yamamoto H, Adachi Y, Taniguchi H, et.al. Interrelationship between microsatellite instability and microRNA in gastrointestinal cancer. World J Gastroenterol 2012 June 14; 18(22): 2745-2755.
6. Ikenoue T, Arai M, Ishioka C, et al. Importance of gastric cancer for the diagnosis and surveillance of Japanese Lynch syndrome patients. Journal of Human Genetics 2019;64.12:1187-1194.
7. Ligtenberg MJ, Kuiper RP, Chan TL, et al. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3′ exons of TACSTD1. Nat Genet. 2009;41:112–117.

8. Peltomaki P. Update on Lynch syndrome genomics. Fam Cancer. 2016;15:385–393.
9. Cunningham JM, Kim CY, Christensen ER, et al. The frequency of hereditary defective mismatch repair in a prospective series of unselected colorectal carcinomas. Am J Hum Genet. 2001;69:780–790.
10. Beghelli S, de Manzoni G, Barbi S, et al. Microsatellite instability in gastric cancer is associated with better prognosis in only stage II cancers. Surgery 2006;139:347–356.
11. Fukayama M., Rugge M, Washington MK, World Health Organisation (WHO) Classification of Tumours. Digestive System Tumours. Fifth Edition 2019;59-110.
12. Protocol for the Examination of Specimens From Patients With Carcinoma of the Stomach Collage of American Pathologists. Includes pTNM requirements from the 8th Edition, AJCC Staging Manual June 2017.
13. Yuan L, Chi Y, Chen W, et al. Immunohistochemistry and microsatellite instability analysis in molecular subtyping of colorectal carcinoma based on mismatch repair competency. Int J Clin Exp Med. 2015;8(11):20988–21000.
14. Kitajima Y, Miyazaki K, Matsukura S, et. al. Loss of expression of DNA repair enzymes MGMT, hMLH1, and hMSH2 during tumor progression in gastric cancer. Gastric Cancer. 2003;6:86–95.
15. Prolla TA, Abuin A, Bradley A. DNA mismatch repair deficient mice in cancer research. Cancer Biology 1996;7:241–247.
16. Chen X, Li X, Liang H, Wei L, et.al. A new mutL homolog 1 c.1896+5G>A germline mutation detected in a Lynch syndrome associated lung and gastric double primary cancer patient. Mol Genet Genomic Med. 2019;00:787.
17. Karimi M, Salomé JV, Aravidis C, et al. A retrospective study of extracolonic, non-endometrial cancer in Swedish Lynch syndrome families. Karimi et al. Hereditary Cancer in Clinical Practice 2018;16:16.
18. Vasen HF, Blanco I, Aktan-Collan K, et al. Revised guidelines for the clinical management of lynch syndrome (HNPCC): recommendations by a group of European experts. Gut. 2013;62(6):812–823.
19. Barrow E, Robinson L, Alduaij W, et al. Cumulative lifetime incidence of extracolonic cancers in lynch syndrome: a report of 121 families with proven mutations. Clin Genet. 2009;75(2):141–149.
20. Chong J-M, Fukayama M, Hayashi Y, et al.: Microsatellite instability in the progression of gastric carcinoma. Cancer Res 1994;54:4595–4597.
21. Beghelli S, de Manzoni G, Barbi S, et al.: Microsatellite instability in gastric cancer is associated with better prognosis in only stage II cancers. Surgery 2006;139:347–356.
22. Neri S, Gardini A, Facchini A, et al.: Mismatch repair system and aging: Microsatellite instability in peripheral blood cells from differently aged participants. J Gerontol A Biol Sci Med Sci 2005;60:285–292.
23. Yamamoto H, Perez-Piteira J, Yoshida T, et.al. Gastric cancers of the microsatellite mutator phenotype display characteristic genetic and clinical features. Gastroenterology 1999;116:1348–1357.
24. Yamamoto H, Imai K. Microsatellite instability: an update. Arch Toxicol 2015.
25. Arai T, Sakurai U, Sawabe M, et.al. Frequent microsatellite instability in papillary and solid-type, poorly differentiated adenocarcinomas of the stomach. Gastric Cancer 2013;16:505–512.
26. Hirotsu Y, Mochizuki H, Amemiya K, et al. Deficiency of mismatch repair genes is less frequently observed in signet ring cell compared with non-signet ring cell gastric cancer. Medical Oncology 2019;36:23.
27. Shenying J, et al. The PD-1, PD-L1 expression and CD3 + T cell infiltration in relation to outcome in advanced gastric signet-ring cell carcinoma, representing a potential biomarker for immunotherapy. Oncotarget. 2017;8:38850–38862.
28. Kim KJ, Lee TH, Cho NY, et.al. Differential clinicopathologic features in microsatellite-unstable gastric cancers with and without MLH1 methylation. Human Pathology 2013;44.6:1055-1064
29. Kim HS, Shin SJ, Beom SH, et. al.Comprehensive expression profiles of gastric cancer molecular subtypes by immunohistochemistry: implications for individualized therapy. Oncotarget 2016(7):28.
30. Ross JS, McKenna BJ. The HER-2/neu oncogene in tumors of the gastrointestinal tract. Cancer Invest 2001;19:554-568.