SSHS, 2025; 7(1), 17-21
DOI: 10.55895/sshs.1607710
Sabuncuoglu Serefeddin Health Sciences (SSHS)
e-ISSN: 3062-3731

INVESTIGATION OF THE FREQUENCY OF MICROSATELLITE INSTABILITY FROM PATHOLOGICAL TISSUE SAMPLES OF PATIENTS WITH GASTRIC CANCER AND ITS EFFECT ON DISEASE PROGNOSIS AND TREATMENT

MİDE KANSERİ HASTALARINA AİT PATOLOJİK DOKU ÖRNEKLERİNDE MİKROSATELLİT İNSTABİLİTE SIKLIĞININ ARAŞTIRILMASI VE HASTALIK PROGNOZU İLE TEDAVİ ÜZERİNE ETKİSİ

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Research Article
Received: 26.12.2024 **Accepted:** 27.03.2025
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Abstract
Gastric cancers continues to be one of the most important health problems in our society with high morbidity and mortality rates. Based on genomic characterization, gastric cancer has recently been defined as a heterogeneous disease consisting of different subtypes, each with unique molecular features and specific clinics. In this study, we tried to investigate the frequency of microsatellite instability in gastric cancers and its effects on the disease. In our study, microsatellite instability results of the patients were seen as microsatellite stable (MSS) in 45 (90%) patients, microsatellite instability high (MSI-H) in 2 (4%) patients and microsatellite instability low (MSI-L) in 3 (6%) patients. It was observed that microsatellite stability was an independent risk factor for mortality. (p=0.035). As a result, it was seen that microsatellite stability was a negative risk factor for mortality in gastric cancer, however, it was concluded that microsatellite instability should be evaluated together with other risk factors that may have an effect on the disease.

Keywords: Gastric cancer, Microsatellite instability, Morbidity, Mortality, Treatment.

Öz
Mide kanserleri hem lokal ileri evrelerde hem de metastatik evrelerde sınırlı tedavi seçeneklerinin olduğu, en agresif seyirli malignitelerden birisidir. Genomik karakterizasyona bağlı olarak, mide kanserleri son zamanlarda her biri kendine özgü moleküler özelliklere ve spesifik kliniklere sahip farklı alt tiplerden oluşan heterojen bir hastalık olarak tanımlanmıştır. Bu çalışmada mide kanserlerinde mikrosatelit instabilitesinin sıklığını ve hastalık üzerindeki etkilerini araştırmaya çalıştık. Çalışmamızda hastaların mikrosatelit instabilitesi sonuçlarına bakıldığında 45 (%90) hastada mikrosatelit stabil (MSS), 2 (%4) hastada mikrosatelit instabilite yüksek (MSI-H), 3 (%6) hastada ise mikrosatelit instabilite düşük (MSI-L) olarak görüldü. Mikrosatelit stabil olmanın mortalite açısından bağımsız bir risk faktörü olduğu görüldü (p=0,035). Sonuç olarak, mikrosatelit stabilitesinin mide kanserinde mortalite açısından negatif bir risk faktörü olduğu görüldü, ancak mikrosatelit instabilitesinin hastalığa etkisi olabilecek diğer risk faktörleriyle birlikte değerlendirilmesi gerektiği sonucuna varıldı.

Anahtar Kelimeler: Mide kanseri, Mikrosatellit instabilite, Morbidite, Mortalite, Tedavi.

1. Introduction

Gastric cancer is one of the most aggressive malignancies with limited treatment options in both locally advanced and metastatic stages (Ratti, 2018).

For this reason, many treatment regimens have been tried and researched over the years. For many years, cisplatin and 5-FU/capecitabine with or without epirubicin have been the standard treatment in the perioperative period for gastric cancer. More recently, the taxane-containing FLOT regimen (Docetaxel, Oxaliplatin, Leucovorin and 5-FU) has been shown to be superior in terms of histological response, relapse-free survival and overall survival (Al-Batran, 2019).

The MSI-H phenotype, which results from inactivation of the DNA missmatch repair (MMR) system due to somatic hypermethylation of the MLH1 gene promoter or germline mutations in MLH1, MSH2, MSH6 and PMS2, has recently received much attention because of the marked immunogenicity of MSI-H cancers and their apparent response to immune checkpoint blockade (Kloor, 2016). It has been observed that the frequency of microsatellite instability is not taken into consideration especially in gastric cancer cases seen in our country. Therefore, in this study, we aimed to evaluate the frequency of microsatellite instability in gastric cancer, its effect together with demographic factors, and its effect on treatment and mortality.

2. Material and Methods

2.1 Working Group

Our study is a prospective cross-sectional study. It was conducted between 01.06.2021-30.09.2022 in patients followed up with the diagnosis of stomach cancer in the Medical Oncology Department of Atatürk University Research Hospital. Patients who underwent MSI examination at the Ataturk University Research Hospital Genetics Laboratory and those who signed an informed consent form were accepted into the study. Patients who did not sign the informed consent form were not accepted into the study. Demographic characteristics of the patients included in the study (age, gender, systemic disease, smoking history, alcohol use history), gastrectomy history, tumor localization, metastasis status, number of treatments and treatment protocols, progression-free survival time, and exitus status were recorded from patient files and the hospital electronic information system.

2.2 MSI Detection

After the patients received pathological diagnosis, MSI study was performed on pathological tissue samples at the Medical Genetics Laboratory of Atatürk University Research Hospital. In this study, specific regions containing microsatellite sequences were amplified as short DNA fragments with the kit used on the EasyPGX® platform, and then stability/instability status was determined for each marker with specific probes after 16 denaturation and hybridization steps. Analysis was performed by comparing individual samples and positive controls for each marker (Table 1).

Table 1. Markers for MSI detection

Marker	Gen	Chromosome
BAT25	cKIT	4 (4q12)
BAT26	MSH2	2 (2p21-p16.3)
NR21	SLC7A8	14 (14q11.2)
NR22	STT3A	11 (11q24.2)
NR24	ZNF2	2 (2q11.1)
NR27	BIRC3	11 (11q22.2)
CAT25	CASP2	7 (7q24)
MONO27	MAP4K3	2 (2p22.1)

2.3 Statistical Analysis

Statistical analyses were performed using SPSS version 15 software. The conformity of variables to normal distribution was examined using visual (histogram and probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive analyses were given using median and interquartile range for variables that were not normally distributed and mean ± standard deviation for variables that were normally distributed. Mann Whitney U test was used to evaluate differences between groups in continuous variables. Categorical variables such as diabetes, hypertension, tumor size, tumor location, disease stage, history of gastrectomy, number of treatments received, and microsatellite instability were expressed as numbers and percentages. The presence of differences between groups in terms of these frequencies was compared using Chi-square or Fisher tests, as appropriate. The effects of microsatellite instability and stage on survival were examined using the log rank test. Survival rates were calculated using Kaplan-Meier survival analysis. A separate log rank analysis was used to calculate the effects of microsatellite instability on survival, adjusting for disease stage. Type-1 error levels below 5% were interpreted as statistically significant.

3. Results and Discussion

A total of 50 gastric cancer patients were included in our study. 37 (74%) of the patients were male and 13 (26%) were female, the mean age was 60±10 and the median was 62 (minimum-maximum; 32-82). Demographic characteristics of the patients, smoking, alcohol use and additional systemic disease status such as diabetes mellitus and hypertension are presented in Table 2.

After the patients were diagnosed with pathological gastric cancer, the disease was staged according to

their pathological features and PET-CT images. In the staging, 30 (60%) of the patients were determined to have stage-4 (end-stage) metastatic gastric cancer.

Table 2. Demographics

Age, years, median (minimum–maximum)	62 (32-82)
Female, n (%)	13 (26)
Male, n (%)	37 (74)
Systemic Diseases, n (%)	
Diabetes Mellitus, n (%)	7 (14)
Hypertension, n (%)	8 (16)
Coronary artery disease, n (%)	4 (8)
Atrial fibrillation, n (%)	1 (2)
Chronic obstructive pulmonary disease, n (%)	2 (4)
Smoking, n (%)	
Yes	32 (64)
No	16 (36)
Alcohol, n (%)	
Yes	11 (22)
No	39 (78)

There were no stage 1 patients among the patients. In the distribution of the patients according to the regions where they metastasized in their PET-CTs, 49 (98%) patients were observed to have lymph node metastasis. In terms of the frequency of metastasis, the lymph nodes were the most common site of metastasis. This was followed by the liver in 19 (38%). Other metastasis sites were the peritoneum, lung, adrenal gland, bone, brain, and ovaries.

The follow-up period of the patients was 12±2 months (minimum-maximum; 1 month-22 months). During the follow-up period, progression was observed in 25 patients (50%). It was determined that 20 patients (40%) died. It was observed that the disease progressed in all 20 patients who died. Factors affecting progression and mortality are shown in Tables 3 and 4.

The distribution of 50 gastric cancer patients MSI results was as follows: 45 (90%) were seen as microsatellite stable (MSS). Microsatellite instability high (MSI-H) was determined in 2 (4%) patients and microsatellite instability low (MSI-L) was determined in 3 (6%) patients.

When the relationship between progression status and MSI was evaluated, patients were divided into two groups with MSS and without MSS, and the effect of the two groups on progression was not found to be statistically significant (log Rank value = 0.071). When the same comparison was made in terms of survey, the mortality of the non-MSS group was found to be significantly lower. (log Rank p value = 0.022)

The log Rank test was also performed to evaluate the effect of the disease stage on mortality, and it was seen that mortality increased as the disease stage increased (log Rank p value = 0.033). Thereupon, a new log Rank model was created to understand whether the disease stage changed the effect of MSI stability on mortality, and it was found that the non-MSS group was associated with mortality independently of the disease stage (log Rank p = 0.035).

When we evaluated the current results, no significant relationship was seen between MSI and prognosis. When we look at the relationship between MSI and mortality, it was seen that having MSS was associated with mortality.

As in other studies (Halling, 2019), In our study, low level MSI was observed in gastric cancers, and the current situation was thought to be low due to the small number of samples in our study or because the molecular results of patients with advanced stage disease resulted in a high rate of MSS.

When we investigated the effect of the current MSI status on disease prognosis, it was observed that MSI had no effect on disease prognosis. It has been seen that there are studies that support and do not support this situation (An, 2012; Choi, 2014).

The strength of our study was that there were limited studies on MSI and stomach cancer in our country. It was observed that the studies were generally conducted in Asian societies where stomach cancer is very common. There was no study that could show the level of MSI in stomach cancers in our country. Therefore, our study was a study that could evaluate the relationship between stomach cancer and MSI. In addition, we examined other factors that may have an

effect on morbidity and mortality in gastric cancer and evaluated whether there is a relationship between these factors and MSI.

Table 3. Factors that effect disease progresion

Progression	**No**	**Yes**	***P* value**
Age, years, mean ± standard deviation	59±10	62±10	0.091
Smoking, n (%)	15 (%60)	17 (%68)	0.556
Alcohol, n (%)	7 (%28)	4 (%16)	0.306
Hypertension, n (%)	3 (%12)	5 (%20)	0.702
Progression	**No**	**Yes**	***P* value**
Diabetes mellitus, n (%)	5 (%16)	3 (%12)	1.0
Tumor size, median cm (min-max)	5 (2-12)	5 (3-12)	0.628
Intragastric localization of the tumor, n (%)			
Corpus	9 (%36)	8 (%32)	0.622
Cardia	11 (%44)	9 (%36)	
Antrum	5 (%20)	8 (%32)	
Gastrectomy, n (%)	9 (%36)	7 (%28)	0.544
Disease stage, n (%)			
2	6 (%24)	2 (%8)	0.180
3	7 (%28)	5 (%20)	
4	12 (%48)	18 (%72)	
Number of chemotherapy, n (%)			
1	13 (%52)	6 (%24)	0.044
2	11 (%44)	11 (%44)	
3	1 (%4)	5 (%20)	

Table 4. Factors that effect mortalitiy

Mortality	**Alive**	**Ex**	***P* value**
Age	60±10	61±10	0.751
Smoking, n (%)	20 (%66.7)	12 (%60)	0.630
Alcohol, n (%)	9 (%30)	2 (%10)	0.163
Hypertension, n (%)	4 (%13)	4 (%20)	0.697
Diabetes mellitus, n (%)	5 (%16.7)	2 (%20)	0.687
Tumor size, median cm (min-max)	5 (2-12)	5 (3-12)	0.613
Intragastric localization of the tumor, n (%)			
Corpus	11 (%36.7)	6 (%30)	0.834
Cardia	12 (%40)	8 (%40)	
Antrum	7 (%23.3)	6 (%30)	
Gastrectomy, n (%)	11 (%36.7)	5 (%25)	0.386
Disease stage, n (%)			
2	2 (%23.3)	1 (%5)	0.015
3	10 (%33.3)	2 (%10)	
4	13 (%43.3)	17 (%85)	
Number of chemotherapy, n (%)			0.047
1	14 (&47.7)	5 (%25)	
2	14 (%46.7)	8 (%40)	
3	2 (%6.7)	4 (%20)	

4. Conclusion

MSI is a factor that guides prognosis and treatment, especially in colon cancer, and in our study we wanted to evaluate whether MSI can guide prognosis, mortality and treatment for stomach cancer. We also aimed to see the rates at which MSI is seen in stomach cancers in our center. In this way, we evaluated whether there is a finding that can guide targeted treatments that can be effective in the follow-up and treatment of stomach cancer, which still causes serious mortality in the world and in our country. In addition, studies are needed on this subject with larger populations of patients with stomach cancer. We believe that studying MSI as a genetic marker in patients diagnosed with stomach cancer and investigating the effect of MSI on the prognosis, mortality and treatment of stomach cancer in more detail in future studies will enable the emergence of new modalities that can change the course of the disease and may be useful in the treatment of stomach cancer.

Conflicts of interest: No conflict of interest

Funding Statement: This research received no grant from any funding agency, commercial or not-for-profit sectors.

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