Microsatellite Instability in Lung Adenocarcinoma

Akciğer Adenokarsinomlarında Mikrosatellit İnstabilite

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ABSTRACT

Background: Lung cancer is the most common cause of cancer-related mortality worldwide. Microsatellite instability has been shown as a prognostic factor in many tumor types in recent years. In this study, we investigated the incidence and prognostic effects of MSI in lung adenocarcinomas.
Methods: Thirty-four lung resection cases diagnosed as adenocarcinoma between 2011 and 2016 were included in the study. MLH1,MSH2,MSH6 and PMS2 antibody, which are Mismatch repair proteins(MMR) were applied immunohistochemically to all cases and the relationship between MMR protein expression and clinical parameters was investigated.
Results: As a result of immunohistochemical study, MLH1 expression was seen in 31 (91.2%), MSH 2 and MSH 6 expressions were seen in 32 cases (94.1%) and PMS2 expression was seen in 27 cases (79.4%). Loss of expression of MLH1, MSH2, MSH6 and PMS2 were detected in 3 (8.8%), 2 (5.9%), 2 (5.9%) and 7 (20.6%) patients, respectively. With these findings, 27 tumors (79.4%) expressing all MMR proteins were accepted as microsatellite stable (MSS), and 7 tumors (20.6%) were accepted as microsatellite unstable (MSI). Between the MSS and MSI groups, lymphovascular invasion (p=0.549), lymph node metastasis (p=0.442), presence of metastasis (p=0.289), pathological T stage (p=0.412) and clinical stage (p=0.10) were statistically significant no difference. The 5-year survival rate was 42% in MSI group and 40% in MSS group (p=0.875).
Conclusion: As a result of the study, no prognostic relationship was found between MSI and pathologic and clinical stages of lung adenocarcinomas, presence of lymph node and distant metastasis, presence of lymphovascular invasion and no effect on overall survival.

Keywords: Lung, adenocarcinoma, microsatellite instability

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ÖZET

Giriş: Akciğer kanseri tüm dünyada kansere bağlı ölümler arasında ilk sırada yer almaktadır. Bu nedenle akciğer kanserli hastaların prognozunu etkileyebilecek moleküler çalışmalar oldukça önem taşımaktadır. Mikrosatellit instabilite (MSI)'de son yıllarda pek çok tümör tipinde prognostik bir faktör olarak gösterilmektedir. Biz de bu çalışmamızda akciğer adenokarsinomlarında MSI sıklığı ve prognostik etkisini araştırdık.
Gereç ve Yöntem:Çalışmaya 2011 ile 2016 tarihleri arasında adenokarsinom tanısı alan 34 akciğer rezeksiyon olgusu dahil edildi. Tüm olgulara immünhistokimyasal olarak Mismatch repair proteinleri(MMR) olan MLH1, MSH2,MSH6 ve PMS2 antikoru uygulandı ve MMR protein ekspresyonları ile klinik parametreler arasındaki ilişki araştırıldı.
Bulgular: Yapılan immünhistokimyasal çalışma sonucunda, MLH1 ekspresyonu 31 olguda (%91.2), MSH2 ve MSH6 ekspresyonları 32'şer olguda (%94.1) ve PMS2 ekspresyonu da 27 olguda (%79.4) görüldü. MLH1, MSH2, MSH6 ve PMS2 ekspresyon kaybı ise sırasıyla 3 (%8.8), 2 (%5.9), 2 (%5.9) ve 7 (%20.6) olguda saptandı. Bu bulgularla tüm MMR proteinlerini eksprese eden 27 tümör (%79.4) mikrosatellit stabil (MSS), 7 tümör (%20,6) ise mikrosatellit instabil (MSI) olarak kabul edildi. MSS ve MSI gruplar arasında, lenfovasküler invazyon (p=0.549), lenf nodu metastazı (p=0.442), metastaz varlığı (p=0.289), patolojik T (p=0.412) evresi ve klinik evre(p=0.10) açısından istatistiksel olarak anlamlı bir fark bulunmadı. 5 yıllık yaşam oranı MSI hasta grubunda %42, MSS hasta grubunda ise %40 olarak saptandı (p=0.875).
Sonuç: Çalışma sonucunda, akciğer adenokarsinomlarında MSI ile hastaların patolojik ve klinik evreleri, lenf nodu ve uzak organ metastaz varlığı ve lenfovasküler invazyon varlığı arasında prognostik bir ilişki saptanmadı ve MSI'nin genel sağkalım üzerine etkisi olmadığı görüldü.

Anahtar Sözcükler: Akciğer, adenokarsinom, mikrosatellit instabilite

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INTRODUCTION

Lung cancer ranks first among cancer-related deaths worldwide, with the majority of cases (80%) being non-small cell lung cancers (NSCLC) (1-3). Among NSCLC, adenocarcinoma is the most common histological subtype (1,2). Recently, considerable attention has been given to various clinical, morphological, immunohistochemical, and molecular features that can guide the treatment of lung adenocarcinomas, leading to personalized treatment regimens. Microsatellite instability (MSI) is one of the molecular methods that has been investigated for its prognostic impact in several tumor types in recent years.

Microsatellites are simple and short nucleotide repeats scattered throughout the human genome. They are predominantly found in non-coding DNA regions (3-8). During DNA replication, the lengths of microsatellite alleles can change through insertion or deletion, but these errors are typically corrected by DNA mismatch repair (MMR) genes under normal conditions. However, mutations in MMR genes can lead to unrepairable errors in the gene, resulting in the formation of repeated nucleotide sequences, known as microsatellites. MutL Protein Homolog 1 (MLH1), MutS Protein Homolog 2 (MSH2), MutS Protein Homolog 6 (MSH6), and Postmeiotic Segregation Increased 2 (PMS2) are essential proteins involved in the MMR system, and inactivation of these proteins leads to microsatellite instability (MSI) (3,4). MSI has been suggested as an independent prognostic factor in various cancer types, particularly colon and endometrial cancer, in recent years. In this study, our aim was to determine the frequency of MSI in lung adenocarcinomas and investigate its prognostic impact.

MATERIALS and METHODS

A total of 34 cases of lung adenocarcinoma diagnosed in the Department of Pathology, Baskent University, between January 2011 and May 2016 were included in the study. Clinical information regarding the patients was obtained from medical records and the hospital system. This study was approved by the Baskent University Medical and Health Sciences Research Board (Project No: KA19/272) and supported by the Baskent University Research Fund.

The resection materials belonging to the patients were histopathologically re-evaluated, and the dominant patterns of the cases were determined according to the World Health Organization (WHO) 2015 classification (1). After selecting paraffin blocks that best represented the tumor histological type for each case, 3 µm thick sections were obtained from the paraffin blocks coated with poly-l-lysine on slides. The immunohistochemical staining process was performed automatically using the "DAKO Omnis" instrument and EnVisionFlex IHC staining kits. The sections prepared from the relevant blocks were incubated in a 60°C oven for 60 minutes. Deparaffinization of the tissues was performed by applying "Clearify (Dako)" solution in the machine at 25°C for 1 minute. Subsequently, antigen retrieval was carried out to recover the antigens in the tissues. For MLH1, MSH2, MSH6, and PMS2 antibodies, the tissues were boiled in EDTA buffer (EnVFlex HRS, High pH) at 97°C for 30 minutes. After the washing process, the tissues were incubated with MSH2 antibody (Monoclonal Mouse, RTU, clone FE11, Dako), MSH6 antibody (Monoclonal Rabbit, RTU, clone EP49, Dako), MLH1 antibody (Monoclonal Mouse, RTU, clone ES05, Dako), and PMS2 antibody (Monoclonal Rabbit, RTU, clone EP51, Dako) for 30 minutes. Subsequently, to prevent background staining, the peroxidase solution belonging to the kit (EnV FLEX Peroxidase-Blocking Reagent, Dako) was applied to the tissues treated with the primary antibody and incubated for 3 minutes. After the washing process, the EnVFlex/HRP solution was applied to the sections and incubated for 20 minutes. The sections were then washed with distilled water after dropping the working solution containing chromogen and enzyme substrate (EnVFlex Substrate Working Solution, Dako) and incubating at room temperature for 5 minutes. In the final step, counterstaining was performed with hematoxylin for 5 minutes, followed by passing through alcohol and xylene stages, and then the slides were covered.

The nuclear staining observed in tumor cells on immunohistochemical staining was considered positive. Accordingly, tumors expressing all four MMR proteins were classified as microsatellite stable (MSS). Tumors showing loss of expression in at least one MMR protein were classified as microsatellite instability (MSI). Subsequently, the relationship between MMR protein expression status and clinical parameters was investigated.

The statistical analysis of the data was performed using the SPSS 15.0 software package. The obtained descriptive results were expressed as mean±standard deviation, percentages, and range values.

The relationship between categorical groups was investigated using the chi-square test, and the relationship between other variables was examined using ANOVA test. The average survival times of the patients were evaluated using the Kaplan-Meier method. A significance level of $p<0.05$ was considered statistically significant.

RESULTS

General Information

Out of a total of 34 cases of lung resection, 28 (82.4%) were male and 6 (17.6%) were female. The mean age was 61.73±10.42. Among the patients, 28 (82.4%) had a history of smoking, and out of these patients, 18 (64.2%) had quit smoking. The smokers had a mean smoking history of 37.03±11.37 pack-years. The remaining 6 patients (17.6%) had never smoked in their lifetime. In terms of tumor location, tumors were found in the right lung of 24 patients (70.6%) and in the left lung of 10 patients (29.4%). Lobectomy was performed in 30 patients (88.2%) surgically, while 4 patients (11.8%) underwent pneumonectomy.

Morphologically, among the tumors, 18 (52.9%) were classified as acinar, 10 (29.4%) as solid, 3 (8.8%) as papillary, 2 (5.9%) as mucinous, and 1 (2.9%) as predominantly lepidic pattern. Lymphovascular invasion was observed in 16 tumors (47.1%), while it was not present in 18 patients (52.9%). Among the total of 22 patients (64.2%), there were no lymph node metastases. Mediastinal lymph node metastasis was observed in 7 patients (20.6%), and peribronchial lymph node metastasis was seen in 5 patients (14.7%). Additionally, brain metastasis was detected in 2 tumors (5.9%).

Pathological staging revealed that 9 patients (26.5%) were classified as pT1a, 8 (23.5%) as pT1b, 3 (8.8%) as pT2a, 5 (14.7%) as pT2b, and 9 (26.5%) as pT3. In our study, we divided a total of 25 patients (73.5%) with pT1a, pT1b, pT2a, and pT2b into an early pT stage group, and 9 patients (26.5%) with pT3 into an advanced pT stage group. According to the clinical staging, 11 patients (32.4%) were classified as Stage 1a, 3 patients (8.8%) as Stage 1b, 4 patients (11.8%) as Stage 2a, 8 patients (23.5%) as Stage 2b, 7 patients (20.6%) as Stage 3a, and 1 patient (2.9%) as Stage 4. In our study, we categorized a total of 26 patients (76.5%) with Stage 1a, 1b, 2a, and 2b as the early-stage tumor group, and 8 patients (23.5%) with Stage 3a and 4 as the advanced-stage tumor group. Among the patients, 6 (17.6%) received neoadjuvant chemotherapy (CT), 20 (58.8%) received adjuvant CT, and 6 (17.6%) received postoperative radiotherapy (RT) treatment.

Immunohistochemical Findings

MLH1 expression was observed in 31 cases (91.2%), MSH2 expression in 32 cases (94.1%), MSH6 expression in 32 cases (94.1%), and PMS2 expression in 27 cases (79.4%). Loss of MLH1, MSH2, MSH6, and PMS2 expression was detected in 3 cases (8.8%), 2 cases (5.9%), 2 cases (5.9%), and 7 cases (20.6%), respectively. One MMR protein expression loss was found in 4 tumors, two MMR protein expression losses in 1 tumor, and loss of expression in all MMR proteins in 2 tumors. The remaining 27 tumors showed expression of all MMR proteins (Figure 1). Therefore, 27 tumors (79.4%) expressing all MMR proteins were classified as MSS, and 7 tumors (20.6%) showing loss of at least one MMR protein expression were classified as MSI. Accordingly, we determined the frequency of MSI in the included lung adenocarcinomas as 20.6%. PMS2 was the most frequently lost MMR protein, and we observed negative expression of PMS2 in all cases classified as MSI.

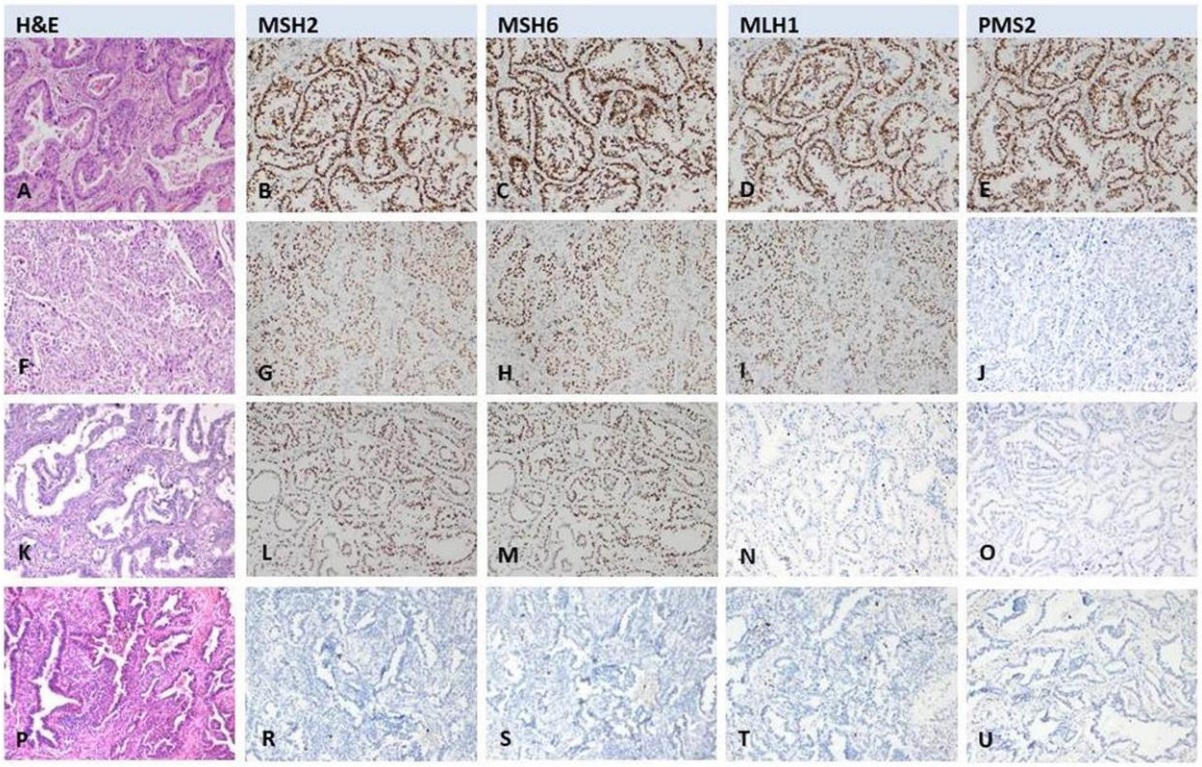

Figure 1. Microscopic appearance of MMR protein expressions in lung adenocarcinomas. Hematoxylin and eosin (H&E) stained microscopic view of lung adenocarcinoma in an acinar pattern (A), immunohistochemical expression of MSH2 (B), MSH6 (C), MLH1 (D), PMS2 (E) in the tumor shown in A. H&E stained microscopic view of lung adenocarcinoma in a solid pattern (F), immunohistochemical expression of MSH2 (G), MSH6 (H), MLH1 (I), loss of PMS2 expression (J) in the tumor shown in F. H&E stained microscopic view of lung adenocarcinoma in an acinar pattern (K), immunohistochemical expression of MSH2 (L), MSH6 (M), loss of MLH1 expression (N), loss of PMS2 expression (O) in the tumor shown in K. H&E stained microscopic view of lung adenocarcinoma in an acinar pattern (P), loss of immunohistochemical expression of MSH2 (R), MSH6 (S), MLH1 (T), PMS2 (U) in the tumor shown in P (magnification x100).

General Information of MSS Cases

Out of the 27 patients with MSS, 24 (88.9%) were male and 3 (11.1%) were female. The mean age was 61.25±10.07. Among the patients, 24 (88.9%) had a history of smoking and had smoked an average of 37.58±11.17 pack-years. The remaining 3 patients (11.1%) had never smoked. Morphologically, 13 tumors (48.1%) were classified as acinar, 9 tumors (33.3%) as solid, 2 tumors (7.4%) as papillary, 2 tumors (7.4%) as mucinous, and 1 tumor (3.7%) as lepidic dominant. Lymphovascular invasion was present in 12 tumors (44.4%). Among the tumors, 17 (63%) did not show lymph node metastasis. Five tumors (18.5%) had peribronchial-hilar lymph node metastasis, and another 5 tumors (18.5%) had mediastinal lymph node metastasis. Additionally, only 1 tumor (3.7%) showed brain metastasis. Among the patients, 19 (70.4%) were in early pathological stage, and 8 (29.6%) were in advanced pathological stage. In terms of clinical staging, 19 patients (70.4%) were in early stage, and 8 patients (29.6%) were in advanced stage. Five patients (18.5%) received neoadjuvant CT, 16 patients (59.3%) received adjuvant CT, and 5 patients (18.5%) received postoperative RT.

General Information of MSI Cases

Out of the 7 patients with MSI, 4 (57.1%) were male and 3 (42.9%) were female. The mean age was 63.57±12.38. Among the patients, 4 (57.1%) had a history of smoking and had smoked an average of 33.75±13.76 pack-years. The remaining 3 patients (42.9%) had never smoked. Morphologically, 5 tumors (71.4%) were classified as acinar, 1 tumor (14.3%) as solid, and 1 tumor (14.3%) as papillary dominant. Lymphovascular invasion was present in 4 tumors (57.1%). Among the tumors, 5 (71.4%) did not show lymph node metastasis, while 2 tumors (28.6%) had mediastinal lymph node metastasis. Additionally, only 1 tumor (14.3%) showed brain metastasis. Among the patients, 6 (85.7%) were in early pathological stage, and 1 (14.3%) was in advanced pathological stage. In terms of clinical staging, all patients were in early stage. One patient (14.3%) received neoadjuvant CT, 4 patients (57.1%) received adjuvant CT, and 1 patient (14.3%) received postoperative RT.

Relationship between MSI and MSS groups

A total of 34 patients were divided into two groups, MSS and MSI, based on their MMR protein expression. There were no statistically significant differences between the groups in terms of patient age (p=0.609) and smoking duration (p=0.543). Although the frequency of smoking history was higher in MSS patients compared to MSI patients, there was a borderline statistical significance in terms of smoking status between the two groups (p=0.05). There was no statistically significant difference between the groups in terms of receiving neoadjuvant CT (p=0.793). Similarly, there were no statistically significant differences between the groups in terms of tumor histopathological subtype (p=0.675), lymphovascular invasion (p=0.549), lymph node metastasis (p=0.442), and presence of metastasis (p=0.289). Although MSI patients had an earlier pathological tumor stage and clinical stage compared to MSS patients, there were no statistically significant differences between the two groups in terms of pathological tumor stage or clinical stage (p=0.412, p=0.10). The clinical and pathological findings of MSI and MSS patients are shown in Table 1.

Table 1: The relationship between microsatellite instability (MSI) status and clinicopathological data

		MSS	MSI	*p*
Age (mean)		61.25±10.07	63.57±12.38	*p*=0.609
Sex				
Female	6	3 (%11.1)	3 (%42.9)	*p*=0.05
Male	28	24 (%88.9)	4 (%57.1)	
Smoking history				
Present	28	24 (%88.9)	4 (%57.1)	*p*=0.05
Absent	6	3 (%11.1)	3 (%42.9)	
Dominant Histologic Pattern				
Asinar	18	13 (%48.1)	5 (71.4)	
Solid	10	9 (%33.3)	1 (%14.3)	
Papillary	3	2 (%7.4)	1 (%14.3)	*p*=0.675
Mucionous	2	2 (%7.4)	0 (%0)	
Lepidic	1	1 (%3.7)	0 (%0)	
Lymphovascular Invasion				
Present	16	12 (%44.4)	4 (%57.1)	*p*=0.549
Absent	18	15 (%55.6)	3 (%42.9)	
Lymph Node Metastasis				
Present	12	10 (%37)	2 (%28.6)	*p*=0.442
Absent	22	17 (%63)	5 (%71.4)	
Pathological Tumor Stage				
pT1a	9	7 (%25.9)	2 (%28.6)	
pT1b	8	7 (%25.9)	1 (%14.3)	
pT2a	3	1 (%3.7)	2 (%28.6)	*p*=0.412
pT2b	6	5 (%14.7)	1 (%14.3)	
pT3a	10	9 (%26.5)	1 (%14.3)	
Clinical Stage				
Stage 1a	11	8 (%29.6)	3 (%42.9)	
Stage 1b	3	2 (%7.4)	1 (%14.3)	
Stage 2a	4	4 (%14.8)	0 (%0)	*p*=0.10
Stage 2b	8	5 (%18.5)	3 (%42.9)	
Stage 3a	7	7 (%25.9)	0 (%0)	
Stage 4	1	1 (%3.7)	0 (%0)	

Abbreviations: MSI: Microsatellite Unstable, MSS: Microsatellite Stable

Follow-up and Survival

The average follow-up period for the total of 34 patients included in the study is 42±28.54 months. Out of these patients, 12 (35.3%) are still alive, while the remaining 22 (64.7%) have passed away. Among the 22 deceased patients, 18 (81.8%) were MSS and 4 (18.2%) were MSI. The overall survival duration for MSI patients is 35±24.8 months, while it is 43.8±29.5 months for MSS patients. Kaplan-Meier analysis revealed a 5-year survival rate of 42% for the MSI group and 40% for the MSS group, indicating that MSI status did not have a significant impact on overall survival (p=0.875) (Figure 2).

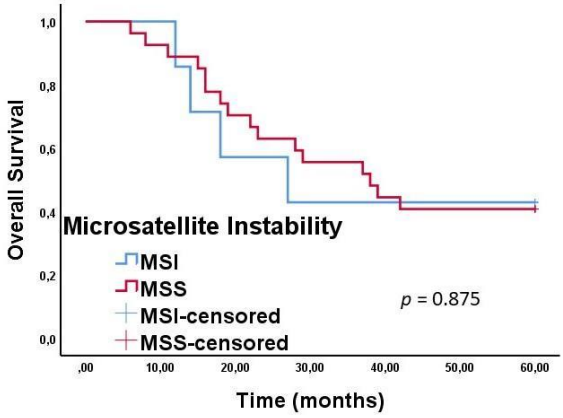

Figure 2. Kaplan-Meier survival curve. According to the Kaplan-Meier log-rank test, there was no significant difference in overall survival between the MSS and MSI patient groups (p=0.875).

DISCUSSION

Adenocarcinomas are the most common histological subtype of lung cancer and exhibit a wide range of clinical, radiological, molecular, and pathological features (1). In recent years, there have been significant developments in the classification and molecular characteristics of adenocarcinomas. In 2011, the International Association for the Study of Lung Cancer (IASLC), American Thoracic Society (ATS), and European Respiratory Society (ERS) proposed a new classification system and a grading system based on predominant histological patterns for adenocarcinomas (2). This classification was also adopted by the World Health Organization (WHO) in 2015 (1). According to this classification, lung adenocarcinomas are categorized as invasive adenocarcinoma, minimally invasive adenocarcinoma, and in situ adenocarcinoma. Invasive adenocarcinomas are further divided into subtypes based on the predominant histological pattern: lepidic, acinar, papillary, micropapillary, solid, and invasive mucinous adenocarcinoma. Lepidic pattern is associated with well-differentiated tumors, while acinar and papillary patterns are indicative of moderately differentiated tumors, and micropapillary and solid patterns are associated with poorly differentiated tumors (1,2). In recent years, numerous molecular studies have been conducted to guide the treatment of adenocarcinomas. Molecular alterations such as epidermal growth factor receptor (EGFR) mutations, anaplastic lymphoma kinase (ALK) rearrangements, ROS proto-oncogene 1, receptor tyrosine kinase (ROS1) rearrangements, B-raf oncogene (BRAF) mutations, and programmed death-ligand 1 (PD-L1) expressions have been extensively studied (1,2). MSI has emerged as one of the most studied molecular methods due to its prognostic and treatment-guiding effects in various tumor types.

DNA MMR genes are one of the most important mechanisms utilized by cells to repair DNA damage. Although there are several specific DNA enzymes in the MMR system, there are four core genes: MLH1, MSH2, MSH6, and PMS2. MSH2 and MSH6 form a heterodimer and are responsible for binding to the site of DNA mismatch. MLH1 and PMS2 also form a heterodimer and are responsible for excising and resynthesizing the correct DNA chain (4,5). When there is a mutation in one of these genes that encode the DNA repair system, DNA MMR deficiency occurs, leading to the accumulation of mismatched DNA sequences called microsatellites. This condition is referred to as MSI. MMR protein dysfunction causing MSI can result from germline mutations or spontaneous hypermutation alterations. Lynch syndrome is a genetic disease characterized by germline mutations in MMR genes and follows an autosomal dominant inheritance pattern. It is associated with various organ cancers, including colon and endometrial cancers. Other cancers such as kidney, ovary, stomach, bladder, pancreas, and lung can also occur in Lynch syndrome (4-6). After the identification of MMR genes, numerous studies have been conducted on colorectal cancers, revealing different clinical and histological features of MSI tumors and a much better clinical outcome (7-12). Following its impact on colorectal cancers, MSI has been investigated in various tumor types. Due to the advanced stage of diagnosis and relatively low survival rates in lung cancers, despite the advancements in treatment options, any molecular parameter that can affect prognosis is of significant clinical importance. Therefore, MSI has become a frequent topic of research in lung cancers. Detecting MMR protein losses causing MSI and developing treatment protocols to regulate the activities of these proteins could provide hope for patients with lung tumors with poor prognosis. Consequently, studies investigating MMR proteins to identify MSI in lung adenocarcinomas contribute significantly to the literature.

MSI detection is most commonly performed using polymerase chain reaction (PCR) and immunohistochemistry (IHC) methods. PCR is used to identify repetitive microsatellites, while IHC is used to evaluate MMR protein expression (4,5). Although PCR has been considered the gold standard for detecting MSI, recent studies have shown that when all four MMR protein expressions are evaluated together, the sensitivity of IHC is as high as PCR (9,10,13). In addition, the ease of application, shorter processing time, and simultaneous detection of all affected MMR genes are advantages of IHC over PCR (9,11). In our study, we utilized immunohistochemical methods to detect MSI.

Literature review reveals that although the number of studies is limited, MSI has been investigated in lung cancers, and the reported frequency of MSI ranges widely from 0% to 40% (3,14,15,16,17,18). In 1994, Shridhar et al. conducted MSI studies using PCR method in 38 NSCLC cases and detected MSI in 13 patients (34%). All MSI-positive patients were found to be in stage T1. Among the included patients, 21 had adenocarcinoma, and MSI was detected in 7 of them (33.3%) (18). Similarly, Kim et al. also studied 38 NSCLC cases in 1998 and detected MSI in 13 cases (34%) using PCR method. Among these cases, 15 were adenocarcinomas, and 6 cases (40%) were considered as MSI tumors among adenocarcinomas (16). In a study conducted by Xinarianos et al. in 2000, MLH1 and MSH2 were evaluated in 150 NSCLC cases, including 49 adenocarcinomas, using IHC method. As a result, loss of MLH1 expression was observed in 8 out of 150 patients (5.3%), and among the 49 adenocarcinoma cases, 2 (4%) showed loss of MLH1 expression (19). All cases with loss of MLH1 expression were smokers. Loss of MSH2 expression was observed in only 1 out of 49 adenocarcinoma cases (2%). There was no statistically significant relationship between MLH1 or MSH2 expressions and prognostic parameters of the patients (19). In another study published in 2000, Chang et al. studied a total of 68 NSCLC cases, and 25 of these cases were adenocarcinomas. MSI was detected in a total of 28 cases (41.2%) using PCR method. Immunohistochemically, MLH1 expression was evaluated in 64 patients, and among these cases, 26 patients had tumors that were determined to have MSI by PCR method. Among these 26 cases, loss of MLH1 expression was observed in 20 patients (76.9%). Despite the detection of MSI by PCR, 6 cases with MLH1 expression were found, possibly indicating loss of other MMR genes in these 6 cases. There was no significant difference in terms of survival between MSI and MSS groups (15). In a study conducted by Wang et al. in 2003 on 77 NSCLC cases, loss of MLH1 and MSH2 expression was detected in 40 cases (51.9%) and 14 cases (18.2%), respectively, using immunohistochemistry (20). In 2006, Kanellis et al. studied MSH2 expression in 42 lung fine-needle aspiration fluids using IHC method. Among the cases, 13 were adenocarcinomas, and loss of MSH2 expression was observed in 6 cases (46%) (21).

One of the largest studies conducted on adenocarcinomas is the MSI study by Warth et al. in 2016, which included 480 lung adenocarcinomas (3). In this study, Warth et al. detected MSI in 4 cases (0.8%) using the PCR method. All MSI cases were in Stage 1 and had a history of smoking. All MSI-positive patients were classified as pT1 or pT2, and none of them had lymph node or distant organ metastasis. There was no significant difference in overall survival between MSI and MSS patients. In addition to PCR, immunohistochemical staining for MLH1, MSH2, MSH6, and PMS2 was performed, and loss of MLH1 and PMS2 expression was observed in 3 out of 4 patients with MSI detected by PCR. The remaining case did not show loss of expression in any of the MMR proteins. As a result, one MSI case detected by PCR could not be identified by immunohistochemical method (3). Similarly to Warth et al., a significant portion of the literature reports very low MSI rates (0-2%) in lung adenocarcinomas (5-8, 22, 23). The likely reasons for the discrepancy in MSI frequency in the literature are patient selection, the number of cases evaluated, evaluation methods, MSI acceptance criteria, and ethnic and/or geographic variations.

In our study, we evaluated MMR protein expressions and MSI in lung adenocarcinomas using the immunohistochemical method, and we found an MSI frequency of 20.6%. Similar to the study conducted by Warth et al., which is one of the largest studies on MSI in the literature, and the studies by Shridhar et al., our study also showed that MSI cases had lower pathological and clinical stages compared to MSS cases. However, due to the limited number of cases in our study, this finding was not statistically significant (3,18). Consistent with some other studies in the literature, no statistically significant difference in overall survival was found between MSI and MSS lung adenocarcinomas in our study (15,19).

Our study aimed to investigate MSI in a limited number of lung adenocarcinoma cases using immunohistochemical methods and determine whether MSI has a prognostic impact in lung adenocarcinomas. The study results did not show a statistically significant difference in patient prognosis between the MSI and MSS groups. However, the limited number of cases included in our study is the most important limitation.

CONCLUSION

In lung adenocarcinomas, MSI detection by immunohistochemical evaluation of MMR protein expression loss, reveal a certain proportion of MSI cases. This finding suggests the potential development of treatment regimens based on regulating the activities of MMR proteins in adenocarcinomas, which are often diagnosed at advanced stages and have a poor prognosis. However, more extensive studies with a larger number of cases are needed to elucidate the prognostic effects of MMR protein expression and MSI in lung adenocarcinomas.

Conflict of interest
No conflict of interest was declared by the authors.

REFERENCES

1. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. Tumours of the lung. WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart, 4th edition, Lyon, IARC, 2015; 51-63
2. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y et al. International association for the study of lung cancer/American Thoracic Society/European Respiratory Society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol. 2011;6(2):244-85.
3. Warth A, Körner S, Penzel R, Muley T, Dienemann H, Schirmacher P. et al. Microsatellite instability in pulmonary adenocarcinomas: a comprehensive study of 480 cases. Virchows Arch. 2016;468(3):313-9.
4. Baretti M, Le DT. DNA mismatch repair in cancer. Pharmacol Ther. 2018;189:45-62.
5. Zhao P, Li L, Jiang X, Li Q. Mismatch repair deficiency/microsatellite instability-high as a predictor for anti-PD-1/PD-L1 immunotherapy efficacy. J Hematol Oncol. 2019;12(1):1-14.
6. Shia J, Holck S, Depetris G, Greenson JK, Klimstra DS. Lynch Syndrome-associated neoplasms: A discussion on histopathology and immunohistochemistry. Fam Cancer. 2013;12(2):241-60.

7. Cortes-Ciriano I, Lee S, Park WY, Kim TM, Park PJ. A molecular portrait of microsatellite instability across multiple cancers. Nat Commun. 2017;8:15180:1-12.
8. Bonneville R, Krook MA, Kautto EA, Miya J, Wing MR, Chen HZ. et al Landscape of microsatellite instability across 39 cancer types. JCO Precis Oncol. 2017;1-21.
9. Shia J. Evolving approach and clinical significance of detecting DNA mismatch repair deficiency in colorectal carcinoma. Semin Diagn Pathol. 2015;32(5):352-61.
10. Southey MC, Jenkins MA, Mead L, Whitty J, Trivett M, Tesoriero AA et al. Use of molecular tumor characteristics to prioritize mismatch repair gene testing in early-onset colorectal cancer. J Clin Oncol. 2005; 23(27): 6524-32.
11. Lindor NM, Burgart LJ, Leontovich O, Goldberg RM, Cunningham JM, Sargent DJ et al. Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. J Clin Oncol. 2002;20(4):1043-8.
12. Chen W, Swanson BJ, Frankel WL. Molecular genetics of microsatellite-unstable colorectal cancer for pathologists. Diagn Pathol. 2017;12(1):24.
13. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P et al. Screening for the Lynch Syndrome (Hereditary nonpolyposis colorectal cancer). N Engl J Med. 2005;352(18):1851-60.
14. Ryberg D, Lindstedt BA, Zienolddiny S, Haugen A. A hereditary genetic marker closely associated with microsatellite instability in lung cancer. Cancer Res. 1995;55(18):3996-9.
15. Chang JW, Chen YC, Chen CY, Chen JT, Chen SK, Wang YC. Correlation of genetic instability with mismatch repair protein expression and p53 mutations in non-small cell lung cancer. Clin Cancer Res. 2000;6(5):1639-46.
16. Kim CH, Yoo CG, Han SK, Shim YS, Kim YW. Genetic instability of microsatellite sequences in non-small cell lung cancers. Lung Cancer. 1998;21(1):21-5.
17. Mao L, Lee DJ, Tockman MS, Erozan YS, Askin F, Sidransky D. Microsatellite alterations as clonal markers for the detection of human cancer. Proc Natl Acad Sci USA. 1994;91(21):9871-5.
18. Shridhar V, Siegfried J, Hunt J, del Mar Alonso M, Smith DI. Genetic instability of microsatellite sequences in many non-small cell lung carcinomas. Cancer Res. 1994;54(8):2084-7.
19. Xinarianos G, Liloglou T, Prime W, Maloney P, Callaghan J, Fielding P. et al. hMLH1 and hMSH2 expression correlates with allelic imbalance on chromosome 3p in non-small cell lung carcinomas. Cancer Res. 2000;60(15):4216-21.
20. Wang YC, Lu YP, Tseng RC, Lin RK, Chang JW, Chen JT. et al. Inactivation of hMLH1 and hMSH2 by promoter methylation in primary non-small cell lung tumors and matched sputum samples. J Clin Invest. 2003;111(6):887-95.
21. Kanellis G, Chatzistamou L, Koutselini H, Politi E, Gouliamos A, Vlahos L. et al. Expression of DNA mismatch repair gene MSH2 in cytological material from lung cancer patients. Diagn Cytopathol. 2006;34(7):463-6.
22. Dudley JC, Lin MT, Le DT, Eshleman JR. Microsatellite instability as a biomarker for PD-1 blockade. Clin Cancer Res. 2016;22(4):813-20.
23. Takamochi K, Takahashi F, Suehara Y, Sato E, Kohsaka S, Hayashi T. et al. DNA mismatch repair deficiency in surgically resected lung adenocarcinoma: Microsatellite instability analysis using the Promega Panel. Lung Cancer. 2017;110-26-31.