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Clinicopathological Characteristics and Prognostic Significance of Microsatellite Instability in Patients with Metastatic Colorectal Cancer

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Abstract

Purpose. To evaluate the clinicopathological characteristics and prognostic relevance of microsatellite instability (MSI) in patients with metastatic colorectal cancer (mCRC).
Materials and methods. This retrospective study included 60 patients with histologically confirmed mCRC treated at the National Center of Oncology, Azerbaijan Republic, between 2020 and 2024. MSI status was assessed using immunohistochemistry (IHC) and polymerase chain reaction (PCR). Clinical, demographic, and molecular features – including KRAS, BRAF, and NRAS mutations – were analyzed.
Results. High microsatellite instability (MSI-H) was detected in 43.3% of patients. MSI-H tumors were more frequently associated with younger age, right-sided localization, and female sex, although these differences were not statistically significant. KRAS mutations were present in 86.7% of patients, while BRAF and NRAS mutations were not observed.
Conclusion. MSI is a clinically meaningful biomarker in mCRC and may guide therapeutic decision-making, especially regarding immune checkpoint inhibitor therapy. Larger studies are needed to validate these findings and further clarify the predictive and prognostic role of MSI in metastatic settings.
Keywords: microsatellite instability, metastatic colorectal cancer, MSI-H, KRAS, immunotherapy, mismatch repair, biomarker, molecular profiling

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Клинико-патологические характеристики и прогностическое значение микросателлитной нестабильности у пациентов с метастатическим колоректальным раком

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Заявление о согласии на участие в исследовании. Информированное согласие было получено от всех участников исследования.
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Резюме

Цель. Оценить клиникопатологические характеристики и прогностическую значимость микросателлитной нестабильности (микросателлитная нестабильность, MSI) у пациентов с метастатическим колоректальным раком (mCRC).
Материалы и методы. В данное ретроспективное исследование были включены 60 пациентов с гистологически подтвержденным метастатическим колоректальным раком, проходивших лечение в Национальном онкологическом центре Азербайджанской Республики в период с 2020 по 2024 год. Статус микросателлитной нестабильности (MSI) оценивался с помощью иммуногистохимии (IHC) и полимеразной цепной реакции (PCR). Были проанализированы клинические, демографические и молекулярные характеристики, включая мутации KRAS, BRAF и NRAS.
Результаты. Высокая микросателлитная нестабильность (MSI-H) была выявлена у 43,3% пациентов. Опухоли с MSI-H чаще ассоциировались с более молодым возрастом, правосторонней локализацией и женским полом, хотя эти различия не были статистически значимыми. Мутации KRAS присутствовали у 86,7% пациентов, в то время как мутации BRAF и NRAS не наблюдались.
Заключение. MSI является клинически значимым биомаркером при метастатическом колоректальном раке и может помочь в принятии терапевтических решений, особенно в отношении терапии ингибиторами контрольных точек иммунитета. Необходимы более масштабные исследования для подтверждения этих результатов и дальнейшего уточнения прогностической роли MSI при метастатическом раке.
Ключевые слова: микросателлитная нестабильность, метастатический колоректальный рак, MSI-H, KRAS, иммунотерапия, репарация несовпадений, биомаркер, молекулярное профилирование

INTRODUCTION

Colorectal cancer (CRC) remains one of the leading causes of cancer morbidity and mortality worldwide. According to the World Health Organization, approximately 1.9 million new cases of CRC are diagnosed annually, with more than 900,000 deaths attributed to the disease each year [1, 2]. In developed countries, CRC ranks as the third most common malignancy among men and the second among women [2]. Although the incidence is relatively lower in developing countries, it continues to rise, likely due to changing lifestyle habits, dietary patterns, and increasing life expectancy [3].

In recent years, microsatellite instability (MSI) has emerged as a key molecular mechanism involved in the pathogenesis of CRC. MSI is a genetic phenomenon resulting from deficiencies in the DNA mismatch repair (MMR) system [4]. These deficiencies lead to the accumulation of insertion or deletion mutations within microsatellite regions, thereby contributing to tumorigenesis [5].

Approximately 15% of all CRC cases exhibit high levels of MSI (MSI-High), making it a relevant diagnostic, prognostic, and therapeutic biomarker [6]. MSI-H tumors are associated with distinct clinicopathological features, including localization, histological characteristics, and immune microenvironment [7]. Furthermore, these tumors often display a hypermutated phenotype, which contributes to their sensitivity to immune checkpoint blockade therapy [8, 9].

The biological basis of MSI lies in MMR protein dysfunction, which impairs the correction of DNA replication errors occurring within repetitive sequences. Microsatellites – short tandem repeats – are commonly located in noncoding DNA but can also be found in coding regions of genes involved in cell cycle regulation, apoptosis, and DNA repair [10, 11]. Loss of function in key MMR proteins such as MLH1, MSH2, MSH6, and PMS2 results in the development of MSI and subsequent accumulation of mutations in critical genes, including TGFBR2, BAX, and ACVR2A [12, 13]. These alterations promote unchecked proliferation, evasion of apoptosis, and increased tumor aggressiveness [14].

MSI status can be assessed using two main approaches: immunohistochemistry (IHC) for MMR protein expression, and polymerase chain reaction (PCR)-based analysis of microsatellite markers. IHC offers a reliable and cost-effective method for detecting MMR deficiency by identifying the absence of protein expression in tumor tissue [15, 16]. The PCR-based Bethesda panel, recommended by the U.S. National Cancer Institute (NCI), includes five standard microsatellite markers (BAT-25, BAT-26, D2S123, D5S346, and D17S250), where length alterations between tumor and normal DNA indicate MSI-H [17].

MSI status not only provides insights into the molecular pathogenesis of CRC but also plays a pivotal role in prognosis and therapeutic decision-making. In early-stage CRC, MSI-H is typically associated with a favorable prognosis and lower risk of recurrence [6, 18, 19]. However, in metastatic CRC (mCRC), the clinical significance of MSI is more complex. Several studies suggest that MSI-H tumors may be less responsive to conventional 5-fluorouracil (5-FU)-based chemotherapy [20]. On the other hand, MSI-H mCRC exhibits a high tumor mutational burden and increased infiltration by cytotoxic T lymphocytes, making these tumors excellent candidates for immune checkpoint inhibitors such as anti-PD-1/PD-L1 antibodies [9, 21, 22].

This study aims to investigate the clinicopathological characteristics and potential prognostic implications of MSI in patients with metastatic colorectal cancer.

■ MATERIALS AND METHODS

This retrospective study was conducted at the National Center of Oncology of the Republic of Azerbaijan and included 60 patients with histologically confirmed metastatic colorectal cancer (mCRC), treated between 2020 and 2024. Patients were selected based on the availability of archived tumor tissue samples suitable for immunohistochemical and molecular genetic analysis.

Among the enrolled patients, 30 were male (50%) and 26 were female (43.3%). The mean age at diagnosis was 64.6 years. Based on tumor localization, 68.3% of cases (n=41) were located in the left colon, and 21.7% (n=13) in the right colon; in the remaining cases, precise anatomical localization could not be established.

Histopathological evaluation confirmed adenocarcinoma in all patients. To assess mismatch repair (MMR) status, immunohistochemical staining was performed using antibodies against MLH1, MSH2, MSH6, and PMS2 proteins. Loss of expression of one or more MMR proteins was interpreted as deficient MMR (dMMR), corresponding to high-level microsatellite instability (MSI-H).

MSI testing was additionally performed by PCR analysis using the standard Bethesda panel, which includes five microsatellite markers: BAT-25, BAT-26, D2S123, D5S346, and D17S250, according to the guidelines of the U.S. National Cancer Institute (NCI). The presence of instability in ≥2 markers was classified as MSI-H, instability in one marker as MSI-L, and absence of instability as microsatellite stable (MSS).

Furthermore, the mutational status of the KRAS, NRAS, and BRAF genes was assessed. KRAS mutations (including G12D) were detected in 11 patients. Mutations in BRAF and NRAS were rare or absent in the studied cohort.

Data analysis was performed using SPSS software (version XX). The associations between MSI status and clinicopathological parameters were evaluated using the chi-square (χ^2) test, Fisher's exact test, and the Mann – Whitney U test for nonparametric variables. A p-value <0.05 was considered statistically significant.

■ RESULTS

A total of 60 patients with metastatic colorectal cancer (mCRC) were included in the study. The mean age was 64.6 years. Of these, 30 patients (50.0%) were male and 26 (43.3%) were female.

High microsatellite instability (MSI-H) was identified in 26 patients (43.3%), while 13 patients (21.7%) had microsatellite stable (MSS) tumors. In the remaining cases, MSI status could not be determined due to technical limitations in processing archived tumor material.

In the majority of cases (65.0%), tumors were located in the left colon (n=39), while right-sided tumors were observed in 13 patients (21.7%). All tumors were histologically classified as adenocarcinomas, with the predominant histological grade being grade 2 (G2). Among patients with MSI-H tumors, right-sided localization was more common (19.2%), whereas all MSS tumors were left-sided.

Molecular analysis revealed KRAS mutations, including G12D, in 52 patients (86.7%), indicating a high prevalence of RAS pathway activation in this mCRC cohort. No mutations in BRAF or NRAS were detected, which may be attributed to the limited sample size or technical constraints of mutation testing.

Patients with MSI-H tumors tended to be younger (mean age: 61.0 years) compared to those with MSS tumors (mean age: 81.8 years), although this difference did not reach statistical significance (p=0.6762, Mann – Whitney U test). A higher proportion of females was observed in the MSI-H group (53.8%), whereas males predominated in the MSS group; however, the sex difference was also not statistically significant (p=0.9098, χ^2 test). Likewise, no significant association was found between MSI status and tumor localization (p=0.4911, χ^2 test).

Despite the limited clinical response data, several patients with MSI-H tumors were candidates for immune checkpoint inhibitor therapy. Considering the known association between MSI-H and high tumor mutational burden, such patients represent a population potentially responsive to PD-1/PD-L1 blockade.

■ DISCUSSION

The findings of this study underscore the clinical relevance of microsatellite instability (MSI) as a molecular biomarker in metastatic colorectal cancer (mCRC). In our cohort, the prevalence of MSI-H was 43.3%, which is higher than commonly reported rates of 10–20% across all CRC stages. This discrepancy may reflect a selection bias favoring patients with known or suspected molecular profiles.

Although previous studies have shown associations between MSI-H tumors and younger age, female sex, and right-sided localization, our results did not reveal statistically significant differences in these variables between MSI-H and MSS groups. These observations may be attributed to the limited sample size and retrospective nature of the study.

The remarkably high frequency of KRAS mutations (86.7%) in our patient cohort is consistent with the known role of RAS pathway activation in CRC. This finding also highlights a major therapeutic implication, as patients harboring KRAS mutations are generally not candidates for anti-EGFR therapies. The absence of BRAF and NRAS mutations may result from technical limitations or population-specific mutation profiles.

MSI status was evaluated using both immunohistochemistry and PCR-based molecular assays. The concordance between these methods reinforces their value for routine MSI testing and patient stratification. Importantly, MSI-H tumors have been shown to exhibit increased immunogenicity due to a high mutational burden and infiltration by tumor-infiltrating lymphocytes, making them strong candidates for immunotherapy with immune checkpoint inhibitors.

Although we did not perform formal survival analysis or treatment response evaluation, several patients with MSI-H tumors received PD-1 inhibitors based on their molecular profile. This aligns with current clinical practice, where MSI-H serves as a key criterion for recommending immunotherapy in advanced CRC.

In summary, while this study confirms established biological trends, the lack of statistically significant associations highlights the need for larger, prospective studies to validate the prognostic and predictive value of MSI in real-world clinical settings.

■ CONCLUSIONS

Microsatellite instability (MSI) represents a clinically significant biomarker in metastatic colorectal cancer (mCRC). In our study, MSI-H tumors were more frequently observed

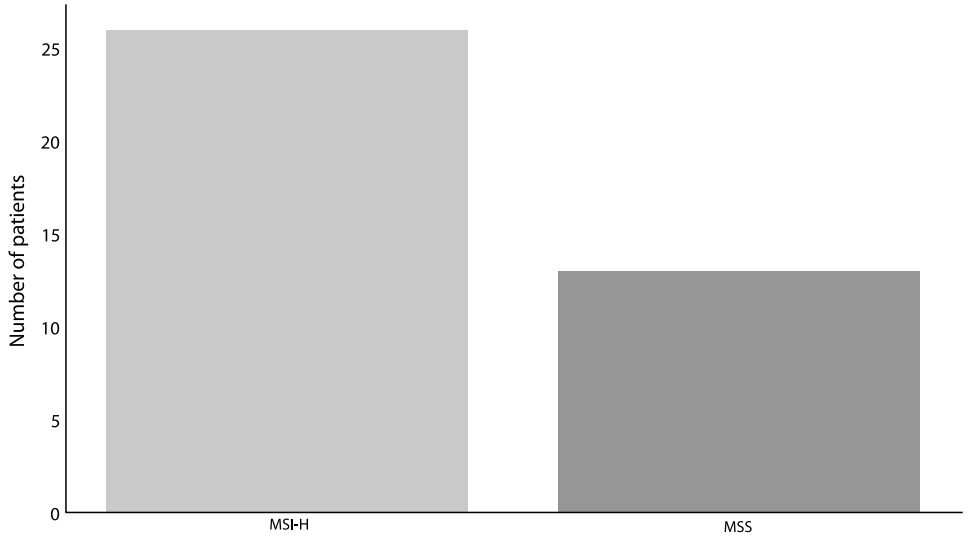

Fig. 1. Distribution of patients by MSI status

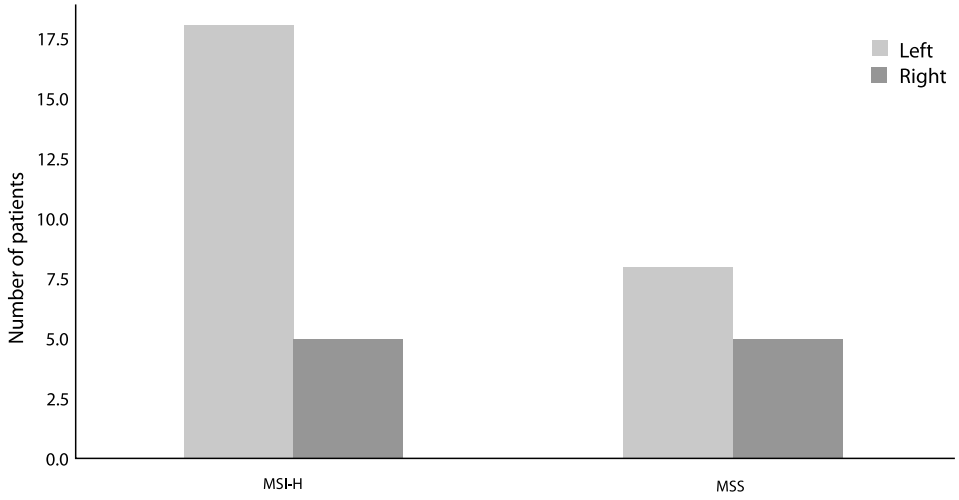

Fig. 2. Tumor localization by MSI group

in younger patients and those with right-sided localization, and showed a higher proportion of female patients, although these trends did not reach statistical significance.

The high prevalence of KRAS mutations limits the applicability of anti-EGFR therapy for the majority of patients in this cohort. Nonetheless, MSI-H status identifies a subset of patients who may benefit from immune checkpoint blockade, highlighting the importance of molecular profiling in routine clinical practice.

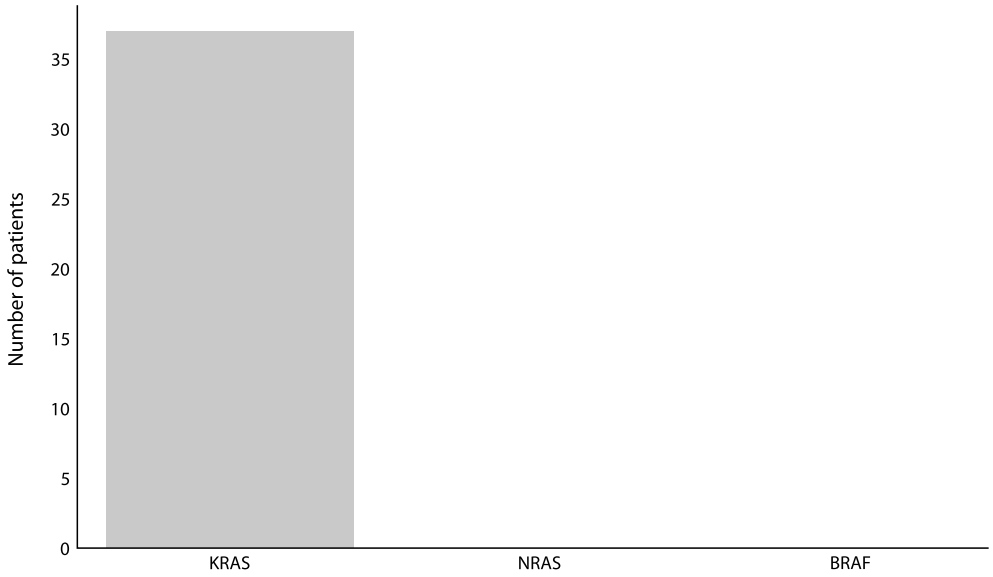

Fig. 3. Frequency of KRAS, NRAS, and BRAF mutations

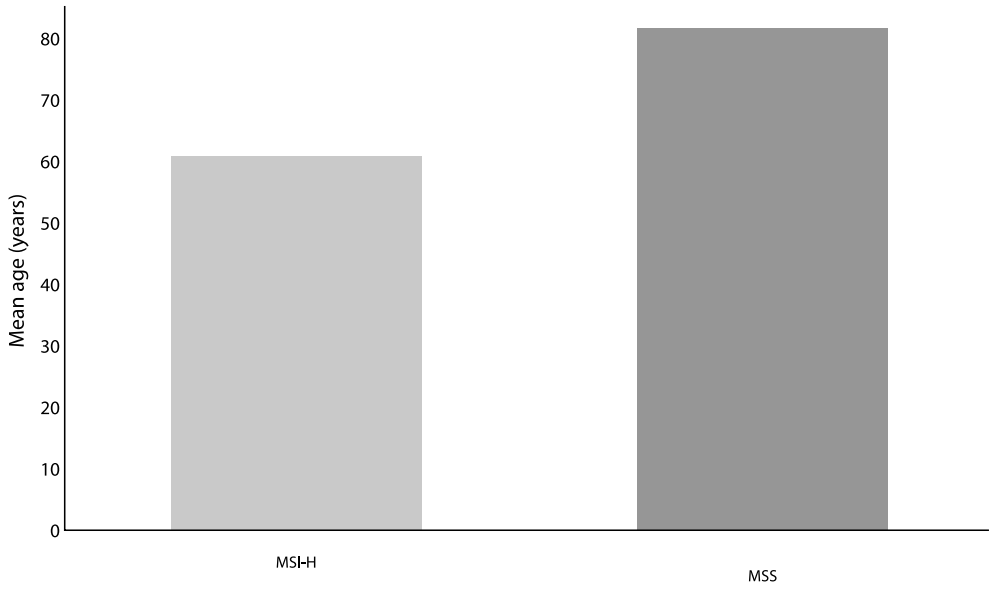

Fig. 4. Mean age by MSI group

Our findings emphasize the relevance of MSI testing in guiding personalized treatment strategies for mCRC. Further large-scale, prospective studies are needed to validate these observations and clarify the prognostic and predictive role of MSI in metastatic disease.

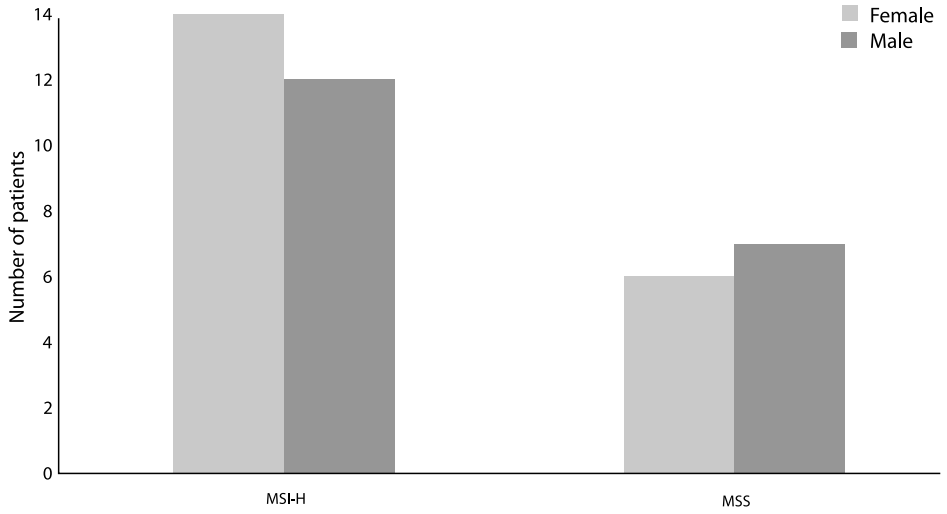

Fig. 5. Distribution by gender

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