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Research Article

Prognostic Significance of Microsatellite Instability in Turkish Patients with Stage II and III Colorectal Cancer

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Abstract

Objectives: Microsatellite instability (MSI) – a form of pervasive erratic expansion of microsatellites – can be identified in 15–20% of all patients with sporadic colorectal cancer (CRC). To gain further insight into the prognostic value of MSI in CRC, we sought to investigate this issue in a nonselected sample of Turkish patients seen in daily practice and to establish whether the MSI status is associated with survival outcomes. We specifically focused on patients with stage 2 and stage 3 CRC because they are a heterogeneous group in need of an improved clinical management.

Methods: A total of 81 patients were enrolled into the study. MSI analysis was performed a dedicated platform and classified into three types, as follows: microsatellite stability (MSS), low microsatellite instability (MSI-L), and high microsatellite instability (MSI-H). Disease-free survival (DFS) and overall survival (OS) served as the main outcome measures.

Results: Patients with MSI-H had a significantly higher frequency of right colon tumors compared with those with MSI-L/MSS. Moreover, no cases of rectal tumor were observed in the former group (p=0.002). As a result, the use of radiotherapy was limited to patients with MSI-L/MSS (p=0.02). Patients with MSI-H did not differ from those with MSI-L/MSS both in terms of DFS and OS.

Conclusion: Our study demonstrates that Turkish patients with proximal colon cancer more frequently have MSI-H compared to those with distal colon cancer. However, the MSI status did not have a significant impact on survival outcomes.

Keywords: Colorectal cancer, microsatellite instability, survival, tumor sidedness

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Colorectal cancer (CRC) is the third most commonly diagnosed malignancy worldwide and currently ranks as the fourth leading cause of cancer-related death.[1,2] Clinically, disease stage remains the most widely accepted prognostic determinant and is commonly used to guide treatment decision.[3] While patients with stage 1 CRC are treated with surgery alone,[4] curative tumor resection followed by adjuvant chemotherapy is the standard of care for patients who have evidence of nodal involvement (stage 3).[5] Currently, the clinical benefits of adjuvant che-

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motherapy for patients with stage 2 disease – who have no evidence of nodal spread – remain controversial.[6,7] Accordingly, stage 2 and 3 CRC is presently conceptualized as comprising a heterogeneous group of patients who can carry regional lymph node involvement and/or micrometastases.[8,9] In this scenario, an improved prognostic stratification is paramount to inform clinicians of expected outcomes and make treatment of stage 2 and 3 CRC more personalized, while reducing unnecessary morbidity.[8]

Microsatellite instability (MSI) – which reflects the somatic destabilization of short tandem repeated genetic motifs – is believed to reflect defective DNA mismatch repair.[10] This form of pervasive erratic expansion of microsatellites can be identified in 15-20% of all patients with sporadic CRC.[11–13] According to its frequency, MSI can be distinguished into three types, as follows: microsatellite stability (MSS), low microsatellite instability (MSI-L), and high microsatellite instability (MSI-H).[10] In general, the presence of MSI-H in CRC is associated with poor differentiation, tumor location in the proximal colon, and abundant cancer-infiltrating lymphocytes.[11–13] Moreover, MSI-H (*versus* MSI-L/MSS) has a lower metastatic potential and seems to have a tendency towards a better prognosis.[11–13]

To gain further insight into the prognostic value of MSI in CRC, we sought to investigate this issue in a nonselected sample of Turkish patients seen in daily practice and to establish whether MSI-H is associated with survival outcomes. We specifically focused on patients with stage 2 and stage 3 CRC because they are a heterogeneous group in need of an improved clinical management.[8]

Methods

Study Patients

The study sample included 81 patients (45 men and 36 women) who were diagnosed with stage 2 or 3 sporadic primary CRC at the Uludag University Medical Center (Bursa, Turkey), between January 2000 and December 2012. All participants underwent surgical excision of the primary tumor and none of them had distant metastases. The histological diagnosis was adenocarcinoma in all participants. The following data were retrieved from clinical records: age, sex, tumor location, use of elective surgery, tumor stage, grade, use and type of chemotherapy, number of chemotherapy cycles, use of radiotherapy, tumor recurrence, number of deaths, and non-cancer mortality. Ethics approval was granted by the local Institutional Review Board (approval number: 2012-25/24). Owing to the retrospective nature of the study, the need for informed consent was waived.

MSI Analysis

DNA was extracted from formalin-fixed, paraffin-embedded tumor and normal tissue specimens using the QIAamp Fast DNA Tissue Kit (Qiagen, Hilden, Germany). MSI analysis was performed a dedicated platform (MSI Analysis System; Promega, Madison, WI, USA) as previously described.[14] The system comprises five nearly monomorphic mononucleotide markers (BAT-25, BAT-26, NR-21, NR-24, and MONO-27) for assessment of the MSI status and two polymorphic pentanucleotide repeat markers (Penta C and Penta D) for sample identification. All loci were amplified by PCR and products were analyzed using an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). We interpreted microsatellite instability at ≥2 mononucleotide loci as MSI-H, instability at a single mononucleotide locus as MSI-L, and no instability at any of the loci tested as MSS.[15]

Outcome Measures

Disease-free survival (DFS) and overall survival (OS) served as the main outcome measures.

DFS was calculated as the time elapsed from diagnosis to the date of cancer recurrence or death from any cause, whereas OS was defined as the interval from diagnosis to the date of death from any cause.

Statistical Analysis

Continuous variables were expressed as means±standard deviations and compared with the independent sample Student's *t*-test. Categorical variables are given as counts and percentages and proportions were compared between groups using the chi-square test or the Fisher's exact test, as appropriate. Patients with MSI-L and MSS were grouped together for the purpose of analysis. Event-free survival curves were plotted with the Kaplan-Meier method and intergroup comparisons (MSI-H *versus* MSI-L/MSS) were performed with the log-rank test. Multivariable Cox proportional hazards regression analyses were carried out to elucidate independent associations with the survival endpoints. All variables with a p value<0.1 on univariable analysis were entered into the multivariable model. The development and timing of recurrences were included in the model as time-dependent covariates to minimize their confounding effect. All calculations were carried out in SPSS version 22.0 (IBM, Armonk, NY, USA). Statistical significance was determined by a two-tailed p value<0.05.

Results

Table 1 depicts the general characteristics of the 81 study patients with CRC (stage 2, n=20; stage 3, n=61) before and after stratification for the MSI status. MSI-H, MSI-L, and

Table 1. General characteristics of patients with stage 2 and 3 colorectal cancer

	Entire cohort (n=81, 100%)	MSI-H (n=12, 14.8%)	MSI-L/MSS (n=69, 85.2%)	p
Age, years (mean±SD)	61.7±11.0	56.6±13.2	62.5±10.4	0.08
Men, n (%)	45 (55.6)	4 (33.3)	41 (59.4)	0.09
Tumor location, n (%)				
Rectum	23 (28.4)	0 (0)	23 (33.3)	**0.002**
Right colon	26 (32.1)	9 (75.0)	17 (24.6)	
Left colon	32 (39.5)	3 (25.0)	29 (42.0)	
Elective surgery, n (%)	65 (80.2)	10 (83.3)	55 (79.7)	0.77
Stage, n (%)				
2	20 (24.7)	3 (25.0)	17 (24.6)	0.97
3	61 (75.3)	9 (75.0)	52 (75.4)	
Tumor grade 2/3, n (%)	54 (66.7)	7 (58.3)	47 (68.1)	0.50
Chemo, n (%)				
5-FU/capacitabine	29 (35.8)	5 (41.7)	24 (34.8)	0.83
Folfox/Xelox	51 (63.0)	7 (58.3)	44 (63.8)	
None	1 (1.2)	0	1 (1.4)	
Number of chemo cycles, (mean±SD)	5.2±1.3	5.1±1.7	5.2±1.2	0.91
Radiotherapy, n (%)	22 (27.2)	0 (0)	22 (31.9)	**0.02**
Tumor recurrence, n (%)	17 (21.0)	3 (25.0)	14 (20.3)	0.77
Number of deaths, n (%)	28 (34.6)	3 (25.0)	25 (36.2)	0.45
Non-cancer mortality	8 (28.6)	1 (33.3)	7 (28.0)	0.84

MSI-H: High microsatellite instability; MSI-L: Low microsatellite instability; MSS: Microsatellite stability; SD: Standard deviation; chemo: Chemotherapy; 5-FU: 5-fluorouracil. Data are given as counts (percentages) unless otherwise indicated. Significant p values are marked in bold.

MSS were identified in 12, 1, and 68 participants, respectively. Therefore, the single patient with MSI-L was grouped together with MSS for the purpose of analysis. The MSI-H (n=12) and MSI-L/MSS (n=69) groups did not differ significantly in terms of age, sex, use of elective surgery, tumor stage, grade, use of chemotherapy, number of chemotherapy cycles, tumor recurrence, number of deaths, or non-cancer mortality (Table 1). However, patients with MSI-H had a significantly higher frequency of right colon tumors compared with those with MSI-L/MSS. Moreover, no cases of rectal tumor were observed in the former group (p=0.002). As a result, the use of radiotherapy was limited to patients with MSI-L/MSS (p=0.02). Patients with MSI-H did not differ from those with MSI-L/MSS both in terms of DFS (p=0.76, log-rank test; Fig. 1) and OS (p=0.49, log-rank test; Fig. 2). The results of multivariable Cox regression analysis did not identify any independent predictor of DFS in our cohort. However, tumor recurrence was found to independently predict OS (hazard ratio: 46.28; 95% confidence interval: 16.58-129.20, p<0.001).

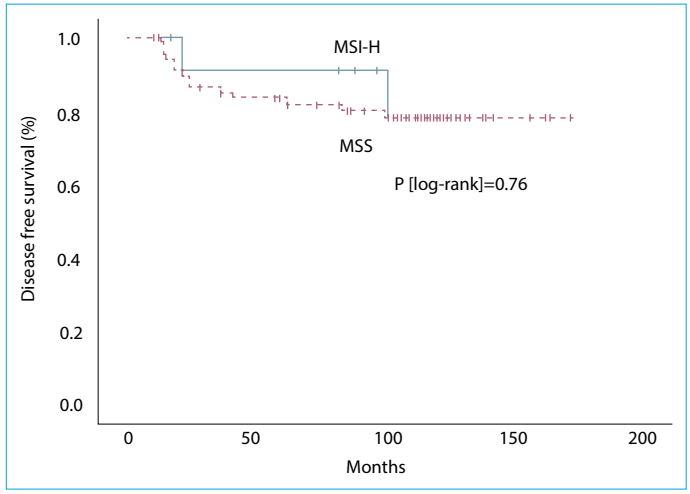

Figure 1. Disease-free survival in patients with stage 2 and 3 colorectal cancer according to the microsatellite instability status (MSI-H *versus* MSI-L/MSS).

Discussion

In this study comprising patients with sporadic CRC from Turkey, the presence of MSI-H was associated with a higher frequency of right colon tumors compared with those with MSI-L/MSS. Because there were no cases of rectal tumor in patients with MSI-H, the use of radiotherapy was limited to the MSI-L/MSS group. Notably, we found no significant differences in terms of both DFS and OS between the MSI-H and MSI-L/MSS groups. These results suggest that MSI may be associated with lesion sidedness in Turkish patients with CRC, although this biomarker was not identified as an independent predictor of survival endpoints.

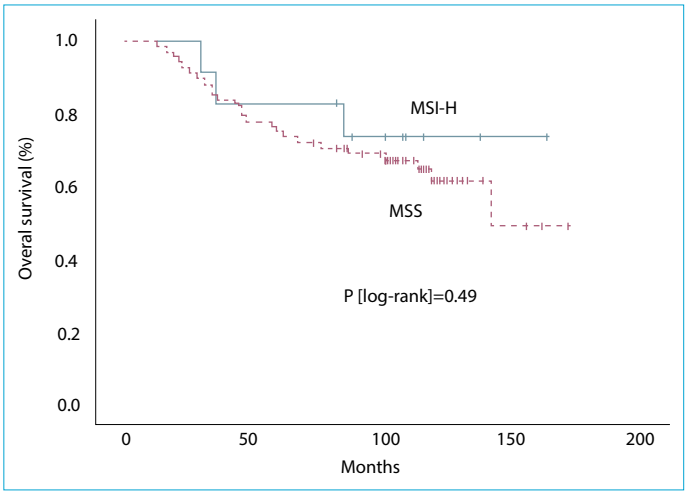

Figure 2. Overall survival in patients with stage 2 and 3 colorectal cancer according to the microsatellite instability status (MSI-H *versus* MSI-L/MSS).

The prevalence of MSI-H in our cohort (14.8%) is in line with that previously reported for sporadic CRC.[11–13] In addition, MSI-H was predominantly present in tumors of the right colon. The MSI status has been repeatedly associated with CRC sidedness[16–18] and our findings confirm that tumors showing MSI-H are more commonly found in the proximal colon. Clinical and genomic features of right and left-sided CRC are known to differ considerably,[18] and the MSI-H status may be a molecular hallmark of proximal tumors where it can play a pathophysiological role.[16]

Apart from CRC sidedness, neither MSI-H was associated with any other clinicopathological risk factor nor was identified as a predictor of survival endpoints in our study. The prognostic significance of MSI-H remains a matter of ongoing debate in the published literature.[11–13] While MSI-H may be associated with features that are generally correlated with poor prognosis (including right-sided CRC),[16–18] the marked genetic instability of tumor cells with MSI-H may make them more prone to apoptosis–ultimately counterbalancing risk or acting as a buffer against the unfavorable prognostic significance of tumor location.[11–13] These findings may offer an explanation as to why we did not identify any correlation between the MSI status and patient survival in our patients with CRC. Moreover, the data presented here show that–as expected–tumor recurrence was the main independent predictor of OS.

Several limitations of our study merit comment. First, its single-center design may have limited the external validity of our findings. Second, although we did not find a prognostic effect of the MSI status in terms of DFS and OS, it is possible that the sample size may not have been sufficiently large to identify intergroup differences and, for that reason, larger prospective cohorts are needed. Even so, we found that disease recurrence had a decisive influence on OS. Therefore, for Turkish patients with CRC, disease recurrence could have had an effect on OS that could mitigate the prognostic significance of MSI. While we were able to confirm the well-known association between MSI-H and tumors arising in the right colon,[16–18] it is possible that the number of participants was not sufficiently large to detect significant differences in relation to other variables, and larger studies are necessary to address this issue. Finally, we analyzed the role of the MSI status as a predictor of survival in patients with stage 2 and stage 3 CRC. An analysis in patients with metastatic disease would also have been interesting; however, our study focused on stage 2/3 because these patients are in special need of predictive biomarkers that may guide election for specific treatment options.[8]

In summary, our study demonstrates that Turkish patients with proximal colon cancer more frequently have MSI-H compared to those with distal colon cancer. However, the MSI status did not have a significant impact on survival outcomes.

Disclosures

Ethics Committee Approval: The study was in accordance with the ethical standards of the with the 1964 Declaration of Helsinki and approved by the clinical research ethics committee of Bursa Uludag University Faculty of Medicine (approval number: 2012-25/24). Owing to the retrospective nature of the study, the need for informed consent was waived.

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Conflict of Interest: None declared.

Authorship Contributions: Concept – A.D.; Design – A.D.; Supervision – N.A.; Materials – M.H., E.H.; Data collection &/or processing – M.H., E.H.; Analysis and/or interpretation – A.B.S., B.O.; Literature search – E.C., O.I., S.O.O; Writing – A.D., S.O.S.; Critical review – M.K., E.C.,T.E.

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