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RESEARCH ARTICLE

Frequency and Clinical Impact of Microsatellite Instability in Colorectal Dysplasia Subgroups

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ABSTRACT

Aim: The risk of colorectal cancer development associated with low-grade dysplasia (LGD) and high-grade dysplasia (HGD) colon polyps at baseline polypectomy remains unclear. In this study, we investigated the role of microsatellite instability (MSI) in the formation and prognosis of dysplasia.

Method: In the study, 40 polyps diagnosed as HGD, and 40 polyps diagnosed as LGD were evaluated according to the revised Vienna criteria (2015) as a result of polypectomy. MSI analysis was performed by fragment analysis using five different primers.

Results: Three of the polyps diagnosed with LGD and eight of the polyps diagnosed with HGD turned into cancer during the follow-up period. The rate of MSI in sporadic colorectal dysplasia was determined as 36.3. A significant correlation was found between MSI status and polyp recurrence within the five-year follow-up period after polypectomy.

Conclusion: In our study, it was determined that LGD and HGDs with MSI can recur, but polypectomy may be effective in preventing cancer formation in cases of dysplasia with MSI.

Keywords: Colorectal polyps, low-grade dysplasia, high-grade dysplasia, microsatellite instability

Introduction

Colorectal cancers (CRCs) originate from initially benign polyps identified as high-grade dysplasia (HGD) and low-grade dysplasia (LGD).[1] Elimination of polyps by polypectomy was reported to reduce CRC incidence in the National Polyp Study cohort.[2] Although studies have examined CRC formation from HGD, CRC development from low-grade adenomas has to date not been evaluated. The reported risk of progression of HGD and CRC for LGD varies between 0.5-54%.[3]

Despite the location, size, and number of polyps being defined as risk factors for CRC, an understanding of the factors that affect the CRC risk of patients with different clinical outcomes is limited. Although the genetic basis of CRC is complex and heterogeneous, this cancer includes point mutations, abnormal gene fusion, and various somatic and germline gene mutations, such as epigenetic changes.[4] Sporadic CRC is known to result from polyps in the initial stages of CRC due to mutations in the adenomatous polyposis coli gene.[5]

The genetic basis of inherited forms of CRC is not clearly defined. The most common form of hereditary non-polyposis syndromes is hereditary non-polyposis colorectal cancer (HNPCC), accounting for approximately 2-3% of all CRCs.

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Among these, 30-60% of HNPCC patients have germline mutations that lead to the microsatellite instability (MSI) phenotype. However, approximately 10-25% of CRC arises via other molecular changes, such as MSI. MSI is caused by the loss of DNA mismatch repair activity found in 12% of sporadic CRCs. The prognostic impact of MSI is still controversial today. Several research groups have reported that CRC with MSI has a slightly better prognosis than colorectal tumors without MSI. Therefore, The National Comprehensive Cancer Network (NCCN) guideline does not recommend chemotherapy for MSI-positive CRC patients.[6] However, the status and prognostic significance of MSI in sporadic colon dysplasia are unclear.

An assessment of the prognosis for these patients is necessary. This study aimed to investigate the frequency and prognostic effect of MSI in LGD and HGD. This may help to define the role of these dysplasia types within the category of advanced adenoma to guide clinical management.

Materials and Methods

Patient Selection

In this retrospective study, 40 patients with LGD and 40 patients with HGD tissue who applied to the university hospital general surgery clinic between 1998 and 2016 were included. The CRC archive database at the university's department of general surgery (medical faculty) was used to collect the patients' clinical information and follow-up data.

Basic demographic, clinical, and lesion characteristics such as location, number, and size were analyzed. The polyp site was classified into three groups: right colon, left colon, and rectum. The polyp site of the proximal to mid-transverse colon was defined as the right colon, while the distal of the mid-transverse colon was defined as the left colon. Only sporadic colorectal polyps were included to create a homogenous study group. Patients who had a previous history of cancer, who had undergone surgery for cancer, and who received preoperative chemotherapy and/or radiation, were excluded from the study. Clinical follow-up after polypectomy was based on periodic clinical visits, the results of biochemical tests, imaging techniques, and surveillance colonoscopy results. Patients who had/had not experienced disease recurrence, based on at least five years of follow-up, were included. The combination of these clinical data allowed for the classification of patients as having either progressive or stable disease conditions. The time to recurrence of polyps and the disease-free interval was defined as the time from the date of polypectomy to the date of confirmed tumor relapse and the date of the last follow-up, respectively. For carcinoma formation, carcinomas arising in the same region as the polyp after polypectomy were evaluated.

Disease-free survival was defined as being alive without any evidence of recurrent disease as of the latest clinical follow-up. The median survival times and the median progression-free survival times were also calculated. The study was approved by the Bursa Uludag University Local Ethics Committee (approval number, 2012-3/12) and conformed to the ethical standards of the Helsinki Declaration.

DNA Isolation

Hematoxylin and eosin-stained slides cut from formalin-fixed paraffin-embedded (FFPE) tissue were evaluated by two expert pathologists. The normal tissue samples were most commonly an uninvolved proximal or distal resection margin. FFPE tissue sections were deparaffinized using xylene and 95% ethanol. According to the manufacturer's protocol, DNA was extracted from 40 HGD tissue samples, 40 LGD tissue samples, and 20 normal specimens using a DNA Extraction kit (Qiagen, Germantown, MD). The quality and concentration of isolated DNA in a 4 µL volume of all samples were measured using a Beckman Coulter DU-730 spectrophotometer (Beckman Coulter Inc., CA, US). The high-quality DNA samples with absorbance ratios between 1.9 and 2.1 were used for the subsequent analysis.

Microsatellite Instability Analysis

This study used 80 polyps DNA and respective 20 normal tissue DNA samples to independently study MSI status using five microsatellite markers [BAT25, BAT26, D2S123, D5S346, and D17S250 (the Bethesda panel)]. Forward primers were dye-labeled for automated high-throughput multiplex detection using capillary array electrophoresis (CEQ 8000XL; Beckman Coulter, Inc., Fullerton, CA). The differences in the polymerase chain reaction (PCR) product fragment lengths among different tissue categories were visualized using the CEQ software (Beckman Coulter, Inc.). The PCR products from the five amplified microsatellite regions in the tumor were compared with the normal epithelium reference. The patients whose polyp DNA showed alleles that were not present in the corresponding normal DNA were classified as MSI positive. If only one of the five markers showed MSI, the polyps were classified as MSI-low (MSI-L), and if two or more markers showed MSI, the polyps were classified as MSI-high (MSI-H). The results were visually evaluated by two independent reviewers.

Statistical Analysis

The significant differences among the study groups concerning the pathologic and clinical characteristics of MSI-H, MSI-L, and microsatellite stable (MSS) tumors were calculated using the chi-square test (c2) and Fisher's exact test. Progression-free survival curves were plotted using the Kaplan-Meier method. The log-rank test was used to assess the survival

differences between groups. Overall survival was defined as the intermediate time interval between sampling and the last follow-up. A chi-square (c2) and Fisher's exact tests were performed using the SPSS Statistics (v.16.00) software for Windows (IBM, Chicago, IL), and the Kaplan-Meier analysis and a log-rank test were performed using MedCalc (v.12.4.00 statistical software (Bvba, Ostend, Belgium). The 95% confidence intervals were calculated using associated estimated standard errors. A p-value <0.05 was considered significant.

Results

Patient Characteristics

This study included 43 female and 37 male patients, with ages ranging from 26-73 years (mean: 56±4.3 years); 30 polyps were within the right colon, and the remaining 50 polyps were in the left colon. Among them, 11 polyps were localized in the rectum. The mean polyp size was 2.3±0.3 cm (Table 1).

Table 1. Patients' clinicopathological features

Variables	LGD, (n=40)	HGD, (n=40)	p-value
Gender			0.102
Female	23 (57.5%)	20 (50%)	
Male	17 (42.5%)	20 (50%)	
Age	56.3	57.5	
Polyp Localizaiton			0.055
Rectum	9 (22.5%)	2 (5%)	
Sigmoid	14 (35%)	7 (17.5%)	
Descending colon	4 (10%)	14 (35%)	
Transverse colon	7 (17.5%)	2 (5%)	
Right colon and caecum	6 (15%)	15 (37.5)	
Polyp Size			0.054
<1 cm	12 (30%)	7 (17.5%)	
1-5 cm	17 (42.5%)	29 (72.5%)	
>1 cm	11 (27.5%)	4 (10%)	
Recurrence			0.081
Presence	29 (72.5%)	22 (55%)	
Absence	11 (27.5%)	18 (45%)	
Carcinogenesis			0.096
Presence	37 (92.5%)	32 (80%)	
Absence	3 (7.5%)	8 (20%)	

Bold values indicate statistical significance, p<0.05. A chi-square test and Fisher's exact test were used for statistical analysis. LGD: Low-grade dysplasia, HGD: High-grade dysplasia

Histopathologically, 40 lesions were HGD, and 40 lesions were LGD. The mean age was 56.3±3.4 years in the LGD patients and 57.5±5.6 years in the HGD patients. In 35% of the polyps diagnosed with LGD, the polyps were localized in the right colon, 42% in the left colon, and 23% in the rectum. Of the polyps diagnosed with HGD, 42% were in the right colon, 38% were in the left colon, and 20% were in the rectum. Additionally, 3 of the polyps with LGD and 8 of the polyps with HGD were diagnosed with CRC during the follow-up period. Polyp recurrence at the same site was determined in 8 of the polyps with LGD and in 21 of the polyps with HGD. There was no statistically significant difference between the HGD and LGD groups in terms of gender, age, location, or size of the adenoma (p>0.05). Furthermore, HGD was associated with a higher risk of polyp recurrence (p=0.021).

MSI Status in High- and Low-Grade Dysplasia

Of the 80 polyps, 51 (63.7%) colon polyps were MSS and the remaining 29 (36.3%) lesions were MSI. The frequency of positivity among the five markers determined by the Bethesda criteria was examined. Among the five markers, BAT25 was the most frequently observed positivity (n=29). BAT26 was positive in 10 patients, D17S256 in 9 patients, D2S123 in 8 patients, and D5S346 in 3 patients. The frequency of MSI was as follows: 17 of 80 (21.25%) polyps had MSI-H; 12 of 80 (15%) were MSI-L.

The frequencies of MSI among the two dysplasia groups were 41.4% (12/40) in patients with LGD and 58.6% (17/40) in patients with HGD. The distribution of the clinicopathological features of an MSI status, based on the different histological dysplasia groups, are shown in Table 2.

Differences between the MSS and MSI tumors were observed in terms of recurrence and the formation of carcinoma in the polyps. During the five-year follow-up period, 29 polyps (36.3%) recurred at the same site. MSI was detected in 69% of the polyps (n=20) with recurrence (p=0.001). Invasive cancer was determined in 11 (21.6%) cases, and all the cases diagnosed with cancer were MSS.

Discussion

This study examined the frequency and clinical relevance of MSI status in CRC polyps classified according to the Revised Vienna Criteria. The findings indicated that the frequency of MSI was 36.3% in sporadic colorectal polyps. MSI-H was detected more frequently in MSI-positive polyps (n=29) than MSI-L polyps (MSI: 58.6%, all patients: 21.25%).

There are limited published data in the literature showing the rate of MSI, particularly in sporadic colorectal polyps. Approximately 15% of all CRCs in Western countries constitute MSI-H CRCs.[7] However, according to existing

Table 2. The clinopathological features of the groups

Variables	LGD, (n=40)		HGD, (n=40)		p-value
	MSI, (n=12)	MSS, (n=28)	MSI, (n=17)	MSS, (n=23)	
Gender					0.716
Female	3 (7.5%)	10 (25%)	9 (22.5%)	15 (37.5%)	
Male	9 (22.5%)	18 (45.5%)	8 (20%)	8 (20%)	
Age	54	57	56	52	0.082
Localization					0.103
Rectum	2 (5%)	7 (17.5%)	1 (2.5%)	1 (2.5%)	
Sigmoid	5 (12.5%)	9 (22.5%)	3 (7.5%)	4 (10%)	
Descending colon	1 (2.5%)	3 (7.5%)	5 (12.5%)	9 (22.5%)	
Transverse colon	0 (0%)	7 (17.5%)	1 (2.5%)	1 (2.5%)	
Right colon and caecum	4 (10%)	2 (5%)	7 (17.5%)	8 (20%)	
Size					0.336
<1 cm	4 (10%)	8 (20%)	5 (12.5%)	2 (5%)	
1-5 cm	5 (12.5%)	12 (30%)	12 (30%)	17 (42.5%)	
>5 cm	3 (7.5%)	8 (20%)	0 (0%)	4 (10%)	
Recurrence					0.001
Presence	6 (15%)	5 (12.5%)	14 (35%)	4 (10%)	
Absence	6 (15%)	23 (57.5)	3 (7.5%)	19 (47.5%)	
Carcinogenesis					0.332
Presence	0 (0%)	3 (7.5%)	0 (0%)	8 (20%)	
Absence	12 (30%)	25 (62.5%)	17 (42.5%)	15 (37.5%)	

Bold values indicate statistical significance, $p<0.05$. A chi-square test and Fisher's exact test were used for statistical analysis. LGD: Low-grade dysplasia, HGD: High-grade dysplasia, MSI: Microsatellite instability, MSS: Microsatellite stable

studies reported by our group and others, a relatively high frequency of MSI-H has been consistently observed in Turkish patients with CRC.[8,9] We hypothesize that the high frequency of MSI-H CRCs in Turkey is mainly based on the low prevalence of genetic mutations in CRCs, and because there are ethnic differences in the major molecular alterations associated with CRCs.

Classically, the development of CRC is characterized by the adenoma-carcinoma sequence.[10] Throughout this sequence, the normal epithelium acquires sequential genetic and epigenetic mutations in specific oncogenes, or tumor suppressor genes, becomes a hyperproliferative mucosa, and subsequently gives rise to a benign adenoma that changes into a carcinoma.[11] Studies indicating the specific stage that MSI is in during this sequencing are unclear. MSI status has a pivotal role in treatment decisions for stage II CRC.[12,13] The NCCN guideline does not recommend chemotherapy for these patients, based on the good prognosis linked to patients with stage II CRC accompanied by MSI-H.[6] However, the reason for their good prognosis remains unclear.[14] Additionally, the effect of MSI status on the prognosis of polyps is unknown. Therefore, MSI has several problems that limit its use as a practical prognostic factor across all stages of CRC. Studies have reported contradicting results indicating that MSI was not statistically correlated with prognosis.[15,16] This result may be explained by the fact that MSI CRCs have distinctive clinical features and are associated with both good and poor outcomes. Many studies have reported proximal colon tumors as being MSI-H.[17,18] The relationship between the location of the polyps and MSI status is unknown. In our study, no significant difference was found between MSI positivity and polyp localization. However, this situation should be re-examined by increasing the number of polyps with tumor formation. Our results show that MSI status is associated with polyp recurrence. However, all polyps that turned into cancer were MSS.

Conclusion

In our study, we examined 40 LGD and 40 HGD colorectal polyps classified according to the Revised Vienna Criteria. The results indicated that 7.5% of polyps diagnosed with LGD and 20% of polyps diagnosed with HGD turned into cancer. Cancer rates after polypectomy for colon polyps with HGD are approximately 30% in the literature. However, the conversion rate of LGD polyps to cancer is unknown. Similar to recent studies, age, gender, polyp size, morphology, pathology, and polyp site did not differ between patients with formation of cancer. In a meta-analysis study conducted by Saini, patients with HGD in polyps experienced a 1.84-fold risk of developing advanced adenoma compared to those without HGD. Two meta-analyses have shown that the presence of HGD was slightly associated with future advanced adenoma. Upon multivariate analysis, the presence of HGD was not found to confer the recurrence of metachronous adenoma. The natural history of colonic adenoma thus remains elusive.

Adenoma-carcinoma sequencing is a widely accepted technique for investigating CRC development. Our study confirmed that 13.75% of patients with colorectal polyps developed cancer during the five-year follow-up period. However, the MSI status of the polyps appears to have an impact on recurrence rather than the development of invasive cancer. Particularly in MSI polyps diagnosed with HGD, recurrence after polypectomy was observed. Further clinical studies are warranted for determining the relationship between polyp recurrence, cancer development, and the MSI status of polyps.

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Ethics

Ethics Committee Approval: The study was approved by the Bursa Uludag University Local Ethics Committee (approval number, 2012-3/12) and conformed to the ethical standards of the Helsinki Declaration.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.A.A., M.E., Ç.T., Design: S.A.A., M.E., Ç.T., Data Collection or Processing: S.G., Analysis or Interpretation: N.U., Ö.Y., E.Ö., Literature Search: T.Y., E.Ö., S.G., Ö.I., Writing: S.A.A., Ö.I.

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