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Original Article

Immunohistochemical and genetic features of mucinous and signet-ring cell carcinomas of the stomach, colon and rectum: a comparative study

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Abstract: Gastric and colorectal cancers are prevalent and fatal cancers worldwide. Although mucinous adenocarcinoma (MAC) and signet-ring cell carcinoma (SRCC) are relatively uncommon, they are of critical importance because of poor prognosis. In Turkey, studies on MSI and other molecular characteristics in mucinous adenocarcinoma (MAC) and signet-ring cell carcinoma (SRCC) of stomach and colon have not been conducted. The present study aimed to investigate the similarities and differences between gastric/colorectal MACs and SRCCs. A total of 590 patients with gastric carcinoma and 1075 patients with colorectal carcinoma, in whom pathologic diagnosis was made within a period of 8 years in our hospital, were retrospectively evaluated. Tissue blocks and slides obtained from the pathology archive were used for immunohistochemical and genetic studies and for microscopic re-evaluation according to the WHO criteria. Data from a total of 135 patients, of whom 78 had been diagnosed with MAC and 57 had been diagnosed with SRCC, were analyzed. MAC patients were significantly older than those with SRCCs. While colorectal localization was more common among MACs, SRCC patients mostly showed gastric localization. Macroscopically, ulceroinfiltrative type was the most prevalent in both groups followed by fungating type in MAC and infiltrative type in SRCC. When compared with SRCC group, MAC group was associated with higher tumor invasion stage, lower rate of patients with infiltrative growth pattern and perineural invasion, and less frequent lymph node invasion. More effective approaches will be developed in the treatment and prevention of cancer along with more data about the incidence, pathogenesis, prognostic factors, and clinical course of cancers.

Keywords: Gastrıc, colorectal, mucinous, signet ring cell, BRAF, MSI

Introduction

Gastric and colorectal cancers are among the most prevalent cancers worldwide and are at the top among the causes of cancer-related deaths [1]. Adenocarcinoma is the leading type of gastric and colorectal cancer. Mucinous adenocarcinoma (MAC), which is characterized by extracellular mucin accumulation, is relatively less prevalent. Sometimes mucin accumulates in the intracellular compartment resulting in signet-ring cell morphology [2]. According to the World Health Organization Classification, MAC is defined as the tumor in which extracellular mucin accounts for more than 50% of the mucin, whereas signet-ring cell carcinoma (SRCC) is defined as a tumor in which more than 50% of the cells contain intracellular mucin [3]. MAC and SRCC are important because they are known to be associated with poor prognosis [4, 5].

Multiple genetic and environmental factors play a role in the etiology of gastric and colorectal cancers [6, 7]. It is known that some genetic mutations play a role in colorectal cancers. A defect in the human mutS homolog 2 (hMSH2) and human mutL homolog 1 (hMLH1) genes, which are among the DNA mismatch repair genes, leads to genomic instability and risk of mutation [7]. In addition, tumor suppressor gene mutations (e.g. p53 pathway inactivation with TP53 mutation) and oncogene pathway activation (e.g. BRAF mutation) as well are considered to be associated with colorectal cancer [8].

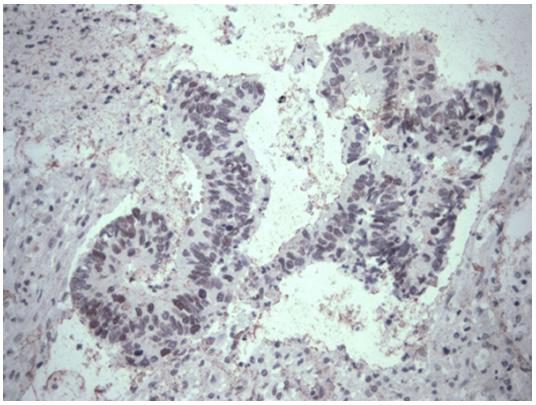

Figure 1. Right colon MAC; MLH-1 nuclear expression.

Similarities and differences between MAC and SRCC, which are characterized by mucin accumulation, have been the topic of investigations [9-11]. In the present study, we also aimed to investigate the similar and different clinical, pathological, prognostic, immunohistochemical and genetic characteristics of gastric and colorectal MAC and SRCC.

Materials and methods

Patients

A total of 590 gastric carcinoma patients and 1075 colorectal carcinoma patients with available pathologic diagnosis, who had undergone gastric or colon resection within a period of 8 years in our hospital, were retrospectively evaluated. Patients' demographic characteristics, clinical data, and tumor-related data were retrieved from the hospital records. Tissue blocks and slides obtained from the pathology archive were used for microscopic reevaluation, and immunohistochemical and genetic study. After reevaluating the blocks and the slides obtained from the pathology archive according to the WHO criteria, data from a total of 135 patients (78 patients with MAC and 57 patients with SRCC) were included in the analysis. Of overall MACs and SRCs, 75 had gastric and 60 had colorectal localization.

Microscopic evaluation

In each case, histologic type of tumor according to the WHO classification [3] was confirmed by examining a minimum of 5 and a maximum of 23 tumor-containing tissue samples under a light microscope.

Preparation of blocks for tissue microarray (TMA)

Blocks containing tumor and normal mucosa were selected from each case and used for the TMA procedure. Four core biopsies (3 with tumor and 1 with normal mucosa) were taken from each paraffin block using a punch biopsy technique. A tissue block containing tumor was also selected for BRAF mutation study.

Immunohistochemical analysis

Sections 3 μm in thickness, which had been obtained from the paraffin blocks for the analysis, were transferred to the slides and used after deparaffinization and rehydration procedures. Immunohistochemical staining was done using p53 primary antibody (DO-7: SC-47698, Santa Cruz®) as well as ready to use MSH-2 (clone 14, Zymed®) and MLH-1 (clone 14, Zymed®) primary antibodies. Nuclear staining of the epithelium of colon mucosa and the lymphocytes in lamina propria with MLH-1 and MSH-2 primary antibodies was considered as a positive control. Presence of nuclear staining in the carcinoma parenchymal cells was considered positive (**Figure 1**), whereas its absence was considered negative. For the evaluation of p53 expression, strong, diffuse nuclear staining was considered positive, whereas its absence/patchy staining was considered negative (**Figure 2A**, **2B**).

Genetic study for BRAF mutation

BRAF mutation was investigated by polymerase chain reaction (PCR). Macherey-Nagel Nucleo-Spin Tissue (Catalog No: 740952.50) kit was used for DNA extraction. Primary tumor DNA was amplified using the primers 5'-CTTTACTTACTACACCTCAG and 5'-TAACTCAGCAGCATCTCAGG in exon 15. Piko Thermal Cycler-Finnzymes Instruments was used for PCR cycles. One hundred and fifty base pair products were visualized by agarose gel (1%) electrophoresis.

Statistical analysis

SPSS 20.0 for Windows program was used for statistical analysis. Descriptive statistics were presented as numbers and percentages for categorical variables and as mean ± standard deviation and median for numerical variables. Chi-square test was used for the comparison of

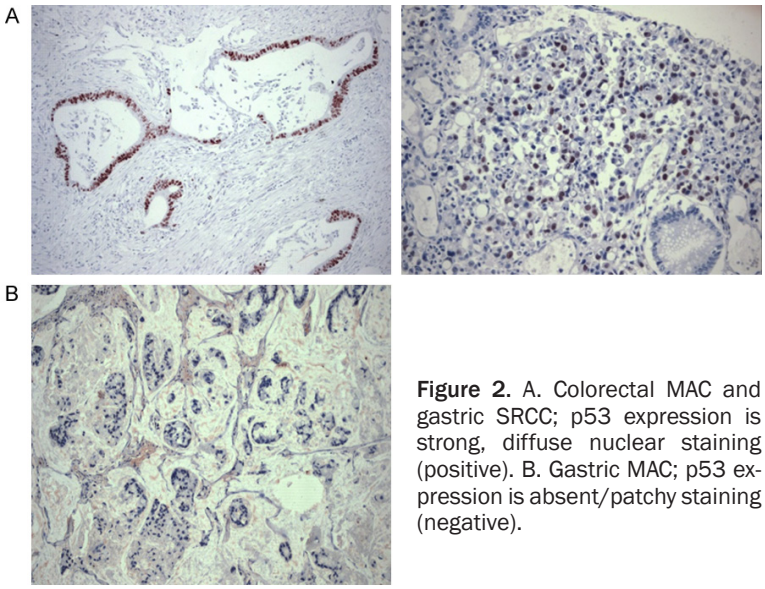

Figure 2. A. Colorectal MAC and gastric SRCC; p53 expression is strong, diffuse nuclear staining (positive). B. Gastric MAC; p53 expression is absent/patchy staining (negative).

Table 1. General characteristics of the mucinous adenocarcinoma (MAC) and signet-ring cell carcinoma (SRCC) groups

	MAC (n = 78) n (%)	SRCC (n = 57) n (%)	P
Gender			
Male	47 (60.3)	32 (56.1)	0.632
Female	31 (39.7)	25 (43.9)	
Age group, years			
≤ 50	11 (14.1)	24 (42.1)	0.001
> 50	67 (85.9)	33 (57.9)	
Localization			
Gastric	20 (25.6)	55 (96.5)	0.001
Colorectal	58 (74.4)	2 (3.5)	
Tumor size, cm			
≤ 5	15 (19.2)	10 (17.5)	0.803
> 5	63 (80.8)	47 (82.5)	
Macroscopic type			
Early	0 (0.0)	3 (5.3)	
Fungating	20 (25.6)	1 (1.8)	0.001
Infiltrative	10 (12.8)	17 (29.8)	
Ulceroinfiltrative	48 (61.5)	36 (63.2)	

independent categorical variables. The level of statistical significance was set at $P < 0.05$.

Results

In our hospital, MAC was detected in 3.4% (n = 20) and SRCC was detected in 9.3% (n = 55) of the 590 gastric carcinoma patients within a period of 8 years. Of the 1075 colorectal cancer patients, 5.4% (n = 58) had MAC and 0.2% (n = 2) had SRCC. Overall, 135 patients with MAC (n = 78) and SRCC (n = 57) were evaluated. The mean age of MAC patients was 62.7 ± 12.5 years (median 64 years) and that of SRCC patients was 55.8 ± 13.8 years (median 53 years). General characteristics of the MAC and SRCC groups are presented in **Table 1**.

Patients with MAC were older than the patients with SRCC. While colorectal localization was more common in MAC patients, gastric localization was more common in SRCC patients. Macroscopically, ulceroinfiltrative type was the leading type in both groups followed by fungating type in the MAC group and by infiltrative type in the SRCC group.

Pathologic prognostic features of the tumors are shown in **Table 2**.

Stage of tumor invasion was higher, the percentage of patients with infiltrative growth pattern and perineural invasion was lower, and lymph node invasion was less common in the MAC group in comparison to that of the SRCC group.

There was no difference between the MAC and SRCC groups in terms of immunohistochemical and genetic features (**Table 3**).

As for localization, 55.6% (n = 75) of the tumors were gastric, and 44.4% (n = 60) of the tumors were colorectal. General characteristics of the gastric and colorectal tumor groups are demonstrated in **Table 4**.

While SRCC was more common in the gastric region, MAC was more prevalent in the colorectal region. Ulceroinfiltrative type was the leading in both localizations followed by infiltrative

Table 2. Pathologic prognostic features of tumors in the mucinous adenocarcinoma and signet ring cell carcinoma groups

	MAC (n = 78) n (%)	SRCC (n = 57) n (%)	P
Depth of Tumor Invasion			
T0	0 (0.0)	0 (0.0)	
T1	1 (1.3)	5 (8.8)	0.001
T2	11 (14.1)	21 (36.8)	
T3	53 (67.9)	25 (43.9)	
T4	13 (16.7)	6 (10.5)	
Lymph Node Invasion			
N0	24 (30.8)	10 (17.5)	
N1	22 (28.2)	22 (38.6)	0.007
N2	27 (34.6)	12 (21.1)	
N3	5 (6.4)	13 (22.8)	
Growth Pattern			
Expansive	21 (26.9)	2 (3.5)	
Infiltrative	41 (52.6)	52 (91.2)	0.001
Expansive-Infiltrative	16 (20.5)	3 (5.3)	
Lymphatic Invasion			
Yes	63 (80.8)	50 (87.7)	0.280
No	15 (19.2)	7 (12.3)	
Vascular Invasion			
Yes	18 (23.1)	22 (38.6)	0.051
No	60 (76.9)	35 (61.4)	
Perineural Invasion			
Yes	40 (51.3)	51 (89.5)	0.001
No	38 (48.7)	6 (10.5)	

type in gastric tumors and by fungating type in colorectal cancers.

Pathologic prognostic features of tumors in the gastric and colorectal groups are shown in **Table 5**.

When compared with gastric cancers, colorectal cancers were associated with greaterdepth of tumor invasion, lower lymph node involvement, and less frequent lymphatic invasion, vascular invasion, and perineural invasion.

There was no difference between the tumors in the gastric or colorectal localization in terms of immunohistochemical and genetic features (**Table 6**).

Discussion

MAC and SRCCs, which account for a small proportion of gastric and colorectal cancers, are important because of poorer prognosis as compared to classical adenocarcinomas. MAC accounts for 3-10% of gastric cancers [12] and 10-15% of colorectal cancers [13]. In recent years, the incidence of SRCC has been reported to increase while the incidence of gastric cancer has been decreasing worldwide. SRCC accounts for 8-30% of gastric cancers [14] and for nearly 1% of colorectal cancers [15]. Chang et al. [16] reported that signet ring cell differentiation is more common in early-onset (≤ 40 years) colorectal cancers when compared to colorectal cancers in the 40 > year age group (13% vs. 1%). In the present study, the prevalence of MAC was 3.4% and the prevalence of SRCC was 9.3% among gastric carcinomas, whereas the prevalences of MAC and SRCC were found to be 5.4% and 0.2%, respectively among colorectal cancers. Studies emphasize that gastrointestinal MACs and SRCCs show diverse characteristics [17-20]. SRCC is reported to be associated with poorer prognosis as compared to MAC [17, 19, 21]. The factors that influence prognosis in gastrointestinal cancers include patient age, tumor type, stage, size and localization, depth of invasion, presence of lymphovascular and perineural invasion, histologic differentiation, and oncogene expression [22-25]. The present study investigated some of these prognostic factors in MAC and SRCC.

In the present study, no difference was found between the MAC and SRCC patients in terms of gender distribution, with male predominance in both groups. Likewise, Bozkaya et al. [19] found no difference between gastric MAC and SRCC patients in terms of gender with a high rate of male patients in both groups. Kang et al. [17] found the male/female ratio to be 47/53 in MACs and 51/49 in SRCCs. Jiang et al. [20] reported a higher ratio of male patients in gastric MACs in comparison to that in SRCCs. In the present study, the percentage of patients aged > 50 years was also found to be higher in MACs when compared with that of SRCCs (85.9% vs. 57.9%, P = 0.001). Kang et al. [17]

Table 3. Immunohistochemical and genetic features of mucinous adenocarcinoma and signet-ring cell carcinoma groups

	MAC (n = 78) n (%)	SRCC (n = 57) n (%)	P
MLH-1 expression			
Yes	60 (76.9)	38 (66.7)	0.187
No	18 (23.1)	19 (33.3)	
MSH-2 expression			
Yes	65 (83.3)	48 (84.2)	0.892
No	13 (16.7)	9 (15.8)	
MLH-1/MSH-2 expression			
MLH-1 (+)/MSH-2 (+)	52 (66.7)	32 (56.1)	
MLH-1 (+)/MSH-2 (-)	8 (10.3)	6 (10.5)	0.448
MLH-1 (-)/MSH-2 (+)	13 (16.7)	16 (28.1)	
MLH-1 (-)/MSH-2 (-)	5 (6.4)	3 (5.3)	
p53 expression			
Yes	32 (41.0)	22 (38.6)	0.776
No	46 (59.0)	35 (61.4)	
BRAF mutation			
Yes	33 (42.3)	20 (35.1)	0.396
No	45 (57.7)	37 (64.9)	

Table 4. General characteristics of gastric and colorectal tumor groups

	Gastric (n = 75) n (%)	Colorectal (n = 60) n (%)	P
Gender			
Male	47 (62.7)	32 (53.3)	0.274
Female	28 (37.3)	28 (46.7)	
Age group, years			
≤ 50	24 (32.0)	11 (18.3)	0.072
> 50	51 (68.0)	49 (81.7)	
Diagnosis			
MAC	20 (26.7)	58 (96.7)	0.001
SRCC	55 (73.3)	2 (3.3)	
Tumor size, cm			
≤ 5	11 (14.7)	14 (23.3)	0.198
> 5	64 (85.3)	46 (76.7)	
Macroscopic type			
Early	3 (4.0)	0 (0.0)	
Fungating	4 (5.3)	17 (28.3)	0.001
Infiltrative	19 (25.3)	8 (13.3)	
Ulceroinfiltrative	49 (65.3)	35 (58.3)	

found the mean age to be higher in MACs. There are studies reporting no age difference between MAC and SRCC patients [19, 20].

The differences between MAC and SRCC have also been investigated in terms of tumor characteristics. In the present study, tumor size was similar in MACs and SRCCs. Bozkaya et al. [19] reported that tumor size was greater for MACs when compared with that of SRCCs. In the present study, depth of tumor invasion was greater but lymph node involvement was less common in the MAC group than in the SRCC group. Jiang et al. [20] found deeper tumor invasion and a higher rate of lymph node involvement in the MAC group, whereas Bozkaya et al. [19] found no difference between MAC and SRCC in terms of invasion depth. In the present study, no difference was found between the MAC and SRCC groups in terms of lymphatic and vascular invasion; however, perineural invasion was more common in the SRCC group (89.5% vs. 51.3%; P = 0.001). In the study of Bozkaya et al. [19] both lymphovascular invasion and perineural invasion were found to be more common in the SRCC group.

MLH-1 and MSH-2 expression has been investigated as a prognostic factor in gastrointestinal cancers [26, 27]. In the literature, MLH-1 and MSH-2 expression have been mainly investigated in colorectal cancers [18, 28-32]. Ogino et al. [18] found the loss of MLH-1 expression to be 22% in colorectal MACs and 30% in the SRCCs. In the present study, likewise, loss of MLH-1 expression was found to be 23.1% in MACs and 33.3% in SRCCs and there was no significant difference between the two groups. Immunohistochemical evaluation of MLH-1 and MSH-2 expression gives information about the presence of microsatellite instability (MSI) in tumors [33]. Within this context, we also considered the cases with no loss of MLH-1 and MSH-2 expression as microsatellite stable (MSS); whereas the cases with both MLH-1 and MSH-2 expres-

Table 5. Pathological prognostic features of tumors in gastric and colorectal locations

	Gastric (n = 75) n (%)	Colorectal (n = 60) n (%)	P
Depth of Tumor Invasion			
T0	0 (0.0)	0 (0.0)	
T1	5 (6.7)	1 (1.7)	0.001
T2	29 (38.7)	3 (5.0)	
T3	37 (49.3)	41 (68.3)	
T4	4 (5.3)	15 (25.0)	
Lymph Node Invasion			
N0	10 (13.3)	24 (40.0)	
N1	26 (34.7)	18 (30.0)	0.001
N2	21 (28.0)	18 (30.0)	
N3	18 (24.0)	0 (0.0)	
Growth Pattern			
Expansive	10 (13.3)	13 (21.7)	
Infiltrative	55 (73.3)	38 (63.3)	0.462
Expansive-Infiltrative	10 (13.3)	9 (15.0)	
Lymphatic Invasion			
Yes	68 (90.7)	45 (75.0)	0.014
No	7 (9.3)	15 (25.0)	
Vascular Invasion			
Yes	28 (37.3)	12 (20.0)	0.028
No	47 (62.7)	48 (80.0)	
Perineural Invasion			
Yes	65 (86.7)	26 (43.0)	0.001
No	10 (13.3)	34 (56.7)	

Table 6. Immunohistochemical and genetic features of tumors with gastric and colorectal location

	Gastric (N = 75) n (%)	Colorectal (N = 60) n (%)	P
MLH-1 expression			
Yes	52 (69.3)	46 (76.7)	0.343
No	23 (30.7)	14 (23.3)	
MSH-2 expression			
Yes	61 (81.3)	52 (86.7)	0.404
No	14 (18.7)	8 (13.3)	
MLH-1/MSH-2 expression			
MLH-1 (+)/MSH-2 (+)	43 (57.3)	41 (68.3)	
MLH-1 (+)/MSH-2 (-)	9 (12.0)	5 (8.3)	0.629
MLH-1 (-)/MSH-2 (+)	18 (24.0)	11 (18.3)	
MLH-1 (-)/MSH-2 (-)	5 (6.7)	3 (5.0)	
p53 expression			
Yes	28 (37.3)	26 (43.3)	0.480
No	47 (62.7)	34 (56.7)	
BRAF mutation			
Yes	27 (36.0)	26 (43.3)	0.386
No	48 (64.0)	34 (56.7)	

sion loss were considered as MSI. In the present study, the prevalence of MSI was 6.4% among MAC cases and 5.3% among SRCC cases. Kazama et al. [29] reported the prevalence of MSI in the colorectal MACs to be 30.8%, while Ogino et al. [18] found the prevalence of MSI to be 28% in the colorectal MACs and 31% in the SRCCs. Song et al. [34] reported the prevalence of MSI to be 36% in the colorectal MACs. Kakar & Smyrk [28] determined the percentage of MSI-high (tumors with instability at > 30% of informative markers and/or loss of hMLH1 or hMSH2 expression) cases among the colorectal SRCCs as 31%. Most of these studies do not give any detail about the subgroups of the tumors. In the present study, the prevalence of MSI was lower than that reported in the literature. Thus we raise a new question about MSI status of MAC and SRCC in the colon. MSI cases may be lower in number in these tumors compared to the non-MAC and non-SRCC in general. Besides, this finding might have arisen from a geographical/ethnic difference. Genetic investigation of MSI is required to gain a clear understanding of this situation.

There are only a few reports about the MSI incidence in gastric tumors. In most of these studies, the subgroup detail has not been given. The MSI ratio in gastric carcinomas in general is similar to the ratio of MSI in gastric MAC and SRCC in our study (**Tables 3**, **6**). There is only one study comparing MSI status in mucinous and nonmucinous gastric carcinomas [35]; in that study, no difference was found between these two groups in terms of MSI status, similar to our results.

In the literature, BRAF mutation has been mainly investigated in colorectal carcinomas [18, 36-40]. The prevalence of BRAF mutation was reported to be 8-14% in colorectal

cancer [38-41]. Studies investigating BRAF mutation in gastric cancers are limited in number, and they reported no BRAF mutation in gastric cancers [40-44]. The present study is also important as it is one of the few studies investigating BRAF mutation in gastric cancer. Evaluation of the molecular and genetic dimensions of the present study in the light of this literature information revealed that the most striking finding was the presence of BRAF mutation in the gastric MACs and SRCCs, which has been reported to be absent in gastric carcinomas. Evaluation of the previous literature about this subject has revealed that most of the studies had been done with tissue lines and in the other ones, the subtype of the carcinoma had not been given. In our study, BRAF mutation was found in 36% of the gastric MACs and SRCCs, whereas it was found in 43.3% of the colorectal MACs and SRCCs. No difference was determined between the gastric and colorectal cancers in terms of BRAF mutation (P = 0.386). Ogino et al. [18] found the prevalence of BRAF mutation to be 22% in the colorectal MACs and 28% in the SRCCs. Song et al. [34] reported the prevalence of BRAF mutation to be 18% in the colorectal MACs. In the present study, BRAF mutation was present in 42.3% of the colorectal MACs and 35.1% of the SRCCs (P = 0.396). The high ratio of BRAF positivity in our results is probably due to the special tumor type we selected (MAC and SRCC).

p53 expression has been investigated as a prognostic factor in gastrointestinal cancers [27, 45]. In a large scale study in colorectal cancers, Russo et al. [45] found the prevalence of p53 mutation to be 34% for proximal colon cancers and 54% for distal colon and rectum cancers. Ogino et al. [18] reported the prevalence of p53 expression to be 37% in MACs and 50% in SRCCs. Song et al. [34] reported the prevalence of p53 inactivation to be 24-33% in the colorectal MACs. In the present study, the prevalence of p53 expression was similar in MACs and SRCCs (41% and 38.6%, respectively; P = 0.776). The prevalence of p53 expression was also similar in MACs and SRCCs in the gastric and colorectal localizations (36% and 43.3%, respectively; P = 0.386).

In conclusion, in the present study comparing the characteristics of MAC and SRCC groups, we found that MAC patients are older than the SRCC patients. MACs are usually localized in the colorectal region, whereas SRCCs are usually located in the gastric region. Macroscopically, ulceroinfiltrative type was the leading type in both groups followed by fungating type in the MACs and infiltrative type in the SRCCs. Tumor invasion stage was higher, the percentage of cases with infiltrative growth pattern and those with perineural invasion was lower, and lymph node involvement was less common in the MAC group in comparison to that of the SRCC group. No difference was determined between the MAC and SRCC groups in terms of MLH-1 and MSH-2 expressions, p53 expression, and presence of BRAF mutation. When the results were compared with the literature, it was seen that MSI cases may be lower in number in these tumors compared to the non-MAC and non-SRCC in general or this difference may be due to geogrophic difference. Finally, this study is the only study investigating and comparing the gastric/colorectal MACs and SRCCs in terms of clinical, histologic, and molecular characteristics especially in terms of defects in two common MMR genes (MSI-2, MLH-1) in a Turkish population. The results will help improve treatment modalities for this population.

Disclosure of conflict of interest

None.

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