

Clinicopathological Factors Affecting Mucin 1, Mucin 2, and Mucin 5AC Staining in Patients who Underwent Resection for Colorectal Cancer

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ABSTRACT

Objective: To investigate the clinicopathological factors affecting mucins (MUC 1, MUC 2, and MUC 5AC) staining in patients who underwent resection for colorectal cancer.

Study Design: An observational study.

Place and Duration of the Study: Department of General Surgery and Department of Pathology, Kafkas University Faculty of Medicine, Kars, Turkey, between January 2020 and January 2021.

Methodology: Patients operated on for colorectal adenocarcinoma were included in the study. Patients who underwent colorectal surgery for benign diseases or had a pathological diagnosis other than adenocarcinoma were excluded from the study. Clinicopathological factors affecting MUC1, MUC2, and MUC5AC staining were evaluated with appropriate statistical tests, assuming a significant p-value of less than 0.05.

Results: Of the 30 patients who met all study criteria, 18 (60%) were males. The mean age of all patients was 62.83±16.79 (21-88). MUC1 strongly positive staining was observed in 18 (60%) cases, and high expression was detected in pT₄ and pT₃ cases (p=0.005). In addition, increased expression was also noted in cases with lymph node involvement (p=0.045). MUC2 expression was more than 60% (strongly positive) in 20 (66.7%). The MUC2 expression was increased in moderately differentiated cases (p=0.032). There was no staining (negativity) in 22 (73.3%) cases with MUC5AC, and more than 60% staining (strongly positive) was observed in 3 (10%) cases. In addition, strong expression was noted in rectosigmoid tumours (p=0.001), female patients (p=0.046), and patients with pT₃ and pT₄ tumours (p=0.05).

Conclusion: High MUC1 and high MUC5AC staining were observed in advanced colorectal cancer, whereas high MUC2 staining was observed in patients with moderate tumour differentiation.

Key Words: Colorectal cancers, Gene expressions, Mucin.

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INTRODUCTION

One of the top five expected numbers of new cases and deaths, Colon cancer, is an important health problem worldwide.¹ Symptoms vary according to the localisation of the tumour, its macroscopic features, stage, and complications. The first and most common finding is a change in bowel habits, and this change is more common in left colon tumours.²

Iron deficiency anaemia is common in tumours located in the right colon, while haematochezia and tenesmus are common in tumours in the rectosigmoid region. Generalised peritonitis can be seen at hospital admission due to obstruction, peritoneal invasion or intestinal perforation.³

A stool occult blood test colonoscopy and radiological imaging tools are helpful in the diagnosis and staging of colorectal carcinomas. Radiographs with barium, computed tomography, magnetic resonance imaging, and transrectal ultrasonography provide information about tumour depth and regional and distant metastases. However, it should be noted that pathological examination is required for the definitive staging of colorectal carcinomas.⁴

As in all types of cancer, basic therapeutic decisions are made in colorectal cancer by staging the tumour with TNM (tumour, node, metastasis) classification. Even if the patients have the

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same pathological diagnosis, the final treatment decision of the patients is made with additional immunohistochemical and genetic tests.⁵ The identification of these additional prognostic markers/tests have greatly contributed to the treatment processes of patients. In addition, the relationship with prognosis can be determined by looking at the expression levels of various materials with immunohistochemical evaluation.

In cancer-related deaths, one of the most important factors affecting the mortality rate and disease prognosis is cancer progression within the tissue and metastasis to the other tissues. In regional tumour spread, proteins need to be rearranged by proteolytic activity and act as a barrier to prevent cancer cells from spreading.⁶ Mucins are one of the important proteins that act as a chemical barrier, have an inhibitory effect, and are effective in the biochemical and immune events.⁷ Although some mucins are membrane-bound due to a hydrophobic membrane-spanning domain favours retention in the plasma membrane, most mucous membranes are secreted as principal components of mucus by mucous membranes or are secreted to become a component of saliva.⁸

Mucins (MUCs) are high-molecular-weight glycosylated proteins. Different tissue-specific genes encode them, and there are 22 different *MUC* genes.³ It is known that tissue-specific mucin genes and mucin carbohydrate antigens change in colorectal carcinoma and are responsible for the malignant behaviour of cancer cells. Although the *MUC1* gene is not significantly expressed in normal colon tissue, its expression increases in colorectal carcinoma and is higher in metastatic tumours. *MUC 2* gene expression is decreased in well-to-moderate adenocarcinomas, while mucinous carcinomas are increased. The *MUC5AC* gene is not expressed in normal colon, they show aberrant expression in colon carcinoma.⁹

This study aimed to investigate the clinicopathological factors affecting *MUC 1*, *MUC 2* and *MUC 5AC* staining in patients who underwent resection for colorectal cancer.

METHODOLOGY

This single-centre study was designed as a retrospective study after Ethical Committee approval from Kafkas University Faculty of Medicine. The study was conducted in Kafkas University Faculty of Medicine Department of General Surgery, between January 2020 and January 2021. Thirty patients who were operated on for colorectal adenocarcinoma were included in the study. Pathology specimens of these patients were evaluated in the pathology department of the hospital. Patients who underwent colorectal surgery for benign reasons and had a pathological diagnosis other than adenocarcinoma were excluded from the study.

Demographic data (age, gender), tumour locations, resection types, and specimen pathology reports were collected from the patients' files. In pathology reports, the diameter of the tumour, the structural features of the tumour, the presence of perforation, histological grade, depth of invasion (pT), and lymph node

involvement (pN) were examined. In addition, the *MUC1*, *MUC2* and *MUC5AC* staining status of tumours were investigated.

Sections were taken from tumour blocks selected from the cases, Haematoxylin & Eosin staining was performed, and re-evaluation was performed. According to the evaluation, the diagnosis of adenocarcinoma was confirmed in all of them. Haematoxylin & Eosin-stained slides of the cases were examined under a microscope, and paraffin blocks suitable for research were selected. A section was taken from the determined paraffin blocks by writing *MUC1*, *MUC2*, and *MUC5AC* on 3 separate adhesive slides with a thickness of 3-4 microns. Sections were kept in an oven at 56 degrees overnight. The sections were kept in three separate xylenes for 5 minutes the next day. Then, they were kept in graded alcohols for 5 minutes and washed in distilled water for 1 minute. They were boiled in 10% citrate buffer (Sigma-Aldrich, ph. 6.0) solution for 10 minutes.

The container's lid containing the materials was opened and kept at room temperature for 20 minutes. After 20 minutes, the sections were passed through distilled water, kept in 10% hydrogen peroxide solution for 10 minutes, washed again in distilled water, and kept in W block (Thermo) for 5 minutes. At the end of the period, the primary antibodies were dripped by shaking the W block on the sections without washing. Antibodies were incubated for 60 minutes. After incubation, washing was done in distilled water for 10 minutes. Then, it was passed to the secondary antibody stage and then kept in Biotin (Thermo) solution for 20 minutes, washed in distilled water for 5 minutes and then kept in Streptavidin (Thermo) solution for 20 minutes. After washing in distilled water for 5 minutes, it was incubated in DAP chromogen (Thermo) for 7 minutes and washed. Finally, after 5 minutes of staining in Mayer's Haematoxylin (Bio-Optica), it was passed through alcohol and xylene and closed with the amount.

Two pathologists evaluated the stainings. Significant membranous and/or granular cytoplasmic staining was considered positive. Staining evaluation was made by categorising 0 (no staining), 1 (staining in 30% cells), 2 (staining in 30-60% cells), 3 (staining in cells above 60%) and giving a percentage of staining.

Statistical analyses were performed using the IBM Statistical Analyses for Social Sciences (SPSS) ver. 26.0 for Windows. Quantitative variables were expressed as mean \pm standard deviation (SD), median, range (minimum-maximum), and interval. Qualitative variables were reported as numbers and percentages. A Shapiro Wilk test was used to evaluate the normality distribution. Due to normality test results, the Kruskal Wallis test was used to compare groups. A Likelihood ratio test was used to compare qualitative variables. A p-value below 0.05 was considered statistically significant.

RESULTS

Of the 30 patients who met all study criteria, 18 (60%) were males, and 12 (40%) were female. The mean age of all patients was 62.83 ± 16.79 (21-88) years. The most common tumour location was caecum with 40%, while the most common surgery type was right hemicolectomy with ileal resection (33.3%). Table I shows the demographic and operative data of patients.

Table I: Demographic and operative data of patients.

Patient No.	Gender	Age	Tumour location	Specimen
1	M	83	TC (HF)	Right hemicolectomy+Transvers colectomy
2	F	53	SC	Sigmoid colectomy
3	M	42	RC	Right hemicolectomy
4	F	64	RC	Right hemicolectomy
5	M	65	Caecum	Right hemicolectomy+Ileal resection
6	F	79	TC	Right hemicolectomy+Transvers colectomy
7	M	76	LC	Left hemicolectomy
8	M	60	Caecum	Right hemicolectomy+Ileal resection
9	F	70	Caecum+Appendix	Right hemicolectomy+Ileal resection
10	M	85	RC	Right hemicolectomy
11	M	37	Caecum	Right hemicolectomy+Ileal resection
12	F	64	Caecum+Appendix	Right hemicolectomy+Ileal resection
13	F	88	LC	Left hemicolectomy
14	F	78	Caecum	Right hemicolectomy+Ileal resection
15	M	76	LC	Left hemicolectomy
16	F	72	Caecum	Right hemicolectomy
17	M	60	Caecum	Right hemicolectomy
18	M	63	SC	Sigmoid colectomy
19	M	53	LC	Left hemicolectomy
20	M	54	Caecum+Appendix	Right hemicolectomy+Ileal resection
21	M	51	TC (HF)	Right hemicolectomy+Transvers colectomy
22	M	60	Caecum	Right hemicolectomy+Ileal resection
23	M	77	RC	Right hemicolectomy
24	F	68	SC	Sigmoid colectomy
25	F	83	SC	Sigmoid colectomy
26	M	22	Caecum	Right hemicolectomy+Ileal resection
27	F	21	LC	Left hemicolectomy
28	F	64	Upper rectum	LAR
29	M	54	Caecum	Right hemicolectomy+Ileal resection
30	M	63	SC	Sigmoid colectomy

TC: Transverse colon, HF: Hepatic flexure, SC: Sigmoid colon, RC: Right colon, LC: Left colon, LAR: Low anterior resection.

Table II: The relationship of MUC1 and MUC2 staining with preoperative, operative factors and pathological factors.

	MUC 1 (-) (n=2)	MUC 1 (+) (n=3)	MUC 1 (++) (n=7)	MUC 1 (+++) (n=18)	P	MUC 2 (-) (n=1)	MUC 2 (+) (n=3)	MUC 2 (++) (n=6)	MUC 2 (+++) (n=20)	P
Age (median, IQR)	NR	54 (IQR=NR)	76 (IQR=36)	64 (IQR=15)	0.875*	NR	70 (IQR=NR)	61.50 (IQR=19)	64.50 (IQR=24)	0.779*
Gender n (%)					0.064**					0.572**
Female	0 (0)	0 (0)	2 (16.7)	10 (83.3)		0 (0)	2 (16.7)	8 (66.7)	8 (66.7)	
Male	2 (11.1)	3 (16.7)	5 (27.8)	8 (44.4)		1 (5.6)	1 (5.6)	4 (22.2)	12 (66.7)	
Tumour location n (%)					0.231**					0.187**
Right	2 (10.5)	3 (15.8)	3 (15.8)	11 (57.9)		1 (5.3)	3 (15.8)	3 (15.8)	12 (63.2)	
Left	0 (0)	0 (0)	3 (60)	2 (40)		0 (0)	0 (0)	0 (0)	5 (100)	
Rectosigmoid	0 (0)	0 (0)	1 (16.7)	5 (83.3)		0 (0)	0 (0)	3 (50)	3 (50)	
Tumour diameter (Median, IQR)	NR	65 (IQR=NR)	50 (IQR=30)	61 (IQR=28)	0.225*	NR	80 (IQR=NR)	53.50 (IQR=29)	57.50 (IQR=24)	0.139*
Tumour structure										
Annular n (%)					0.660**					0.315**
Yes	0 (0)	0 (0)	1 (25)	3 (75)		0 (0)	0 (0)	0 (0)	4 (100)	
No	2 (7.7)	3 (11.5)	6 (23.1)	15 (57.7)		1 (3.8)	3 (11.5)	6 (23.1)	16 (61.5)	
Ulcerovegetan n (%)					0.752**					0.458**
Yes	2 (7.4)	3 (11.1)	6 (22.2)	16 (59.3)		1 (3.7)	3 (11.1)	6 (22.2)	17 (63)	
No	0 (0)	0 (0)	1 (33.3)	2 (66.7)		0 (0)	0 (0)	0 (0)	3 (100)	
Exophytic n (%)					0.061**					0.081**
Yes	2 (20)	2 (20)	1 (10)	5 (50)		1 (10)	1 (10)	4 (40)	4 (40)	
No	0 (0)	1 (5)	6 (30)	13 (65)		0 (0)	2 (10)	2 (10)	16 (80)	
Tumour perforation n (%)					0.660**					0.315**
Yes	0 (0)	0 (0)	1 (25)	3 (75)		0 (0)	0 (0)	0 (0)	4 (100)	
No	2 (7.7)	3 (11.5)	6 (23.1)	15 (57.7)		1 (3.8)	3 (11.5)	6 (23.1)	16 (61.5)	
Histological Grade n (%)					0.892**					0.032**
Well	0 (0)	0 (0)	0 (0)	1 (100)		0 (0)	1 (100)	0 (0)	0 (0)	
Moderate	2 (7.7)	3 (11.5)	6 (23.1)	15 (57.7)		1 (3.8)	1 (3.8)	4 (15.4)	20 (76.9)	
Poor	0 (0)	0 (0)	1 (33.3)	2 (66.7)		0 (0)	1 (33.3)	2 (66.7)	0 (0)	
pT n (%)					0.005**					0.192**
T ₂	2 (66.7)	1 (33.3)	0 (0)	0 (0)		1 (33.3)	1 (33.3)	0 (0)	1 (33.3)	
T ₃	0 (0)	2 (10.5)	6 (31.6)	11 (57.9)		0 (0)	1 (5.3)	5 (26.3)	13 (68.4)	
T ₄	0 (0)	0 (0)	1 (12.5)	7 (87.5)		0 (0)	1 (12.5)	1 (12.5)	6 (75)	
pN n (%)					0.045**					0.509**
N ₀	0 (0)	3 (23.1)	4 (30.8)	6 (46.2)		0 (0)	1 (7.7)	2 (23.1)	9 (69.2)	
N ₁	2 (16.7)	0 (0)	3 (25)	7 (58.3)		1 (8.3)	2 (16.7)	1 (8.3)	8 (66.7)	
N ₂	0 (0)	0 (0)	0 (0)	5 (100)		0 (0)	0 (0)	2 (40)	3 (60)	

NR: Not reported, IQR: Interquartile range. * Kruskal Wallis test, ** Likelihood ratio test.

According to the pathological evaluation, the most common tumour structure was the ulcerovegetan type with 56.7%, and the mean tumour diameter was 62.67 (28-90) mm. There were significant perforation in 3 (10%) cases and suspicious perforation in 1 (3.3%) case in the pathological specimen. There were moderately differentiated tumours in 26 (86.7%) cases, poorly differentiated tumours in 3 (10%) cases and well-differentiated tumours in 1 (3.3%) case. The most common pathological T stage was T₃, pathological N stage N₀, and the percentages were 60% and 50%, respectively.

In MUC1 immunohistochemical evaluation, more than 60% staining (strongly positivity) was observed in 18 (60%) cases. High expression was detected in pT₄ and pT₃ cases (p=0.005). In addition, high expression was also noted in cases with lymph node involvement (p=0.045). MUC1 negative staining was only observed in patients with tumour invasion depth pT₂. MUC2 expression was more than 60% (strongly positivity) in 20 (66.7%). The MUC2 expression was increased in moderately differentiated cases (p=0.032), but pT staging was not significantly associated with staining (p=0.192).

Table III: The relationship of MUC5AC staining with preoperative, operative factors and pathological factors.

	MUC 5AC (-) (n=22)	MUC 5AC (+) (n=3)	MUC 5AC (++) (n=2)	MUC 5AC (+++) (n=3)	P
Age (median, IQR)	61.50 (IQR=25)	70 (IQR=NR)	58 (IQR=NR)	68 (IQR=NR)	0.444*
Gender n (%)					0.046**
Female	6 (50)	2 (16.7)	1 (8.3)	3 (25)	
Male	16 (88.9)	1 (5.6)	1 (5.6)	0 (0)	
Tumour location n (%)					0.001**
Right	17 (89.5)	2 (10.5)	0 (0)	0 (0)	
Left	4 (80)	1 (20)	0 (0)	0 (0)	
Rectosigmoid	1 (16.7)	0 (0)	2 (33.3)	3 (50)	
Tumour diameter (Median, IQR)	67.50 (IQR=30)	60 (IQR=NR)	50 (IQR=NR)	40 (IQR=NR)	0.074*
Tumour structure					0.353**
Annular n (%)					
Yes	3 (75)	0 (0)	1 (25)	0 (0)	
No	19 (73.1)	3 (11.5)	2 (3.8)	3 (11.5)	
Ulcerovegetan n (%)					0.344**
Yes	20 (74.1)	3 (11.1)	1 (3.7)	3 (11.1)	
No	2 (66.7)	0 (0)	1 (33.3)	0 (0)	
Exophytic n (%)					0.430**
Yes	8 (80)	0 (0)	1 (10)	1 (10)	
No	14 (70)	3 (15)	1 (5)	2 (10)	
Tumour perforation n (%)					0.529**
Yes	3 (75)	1 (25)	0 (0)	0 (0)	
No	19 (73.1)	2 (7.7)	2 (7.7)	3 (11.5)	
Histological Grade n (%)					0.348**
Well	0 (0)	1 (100)	0 (0)	0 (0)	
Moderate	19 (73.1)	2 (7.7)	2 (7.7)	3 (11.5)	
Poor	3 (100)	0 (0)	0 (0)	0 (0)	
pT n (%)					0.05**
T ₂	3 (100)	0 (0)	0 (0)	0 (0)	
T ₃	15 (78.9)	0 (0)	1 (5.3)	3 (15.8)	
T ₄	4 (50)	3 (37.5)	1 (12.5)	0 (0)	
pN n (%)					0.464**
N ₀	10 (76.9)	1 (7.7)	0 (0)	2 (15.4)	
N ₁	8 (66.7)	2 (16.7)	1 (8.3)	1 (8.3)	
N ₂	4 (80)	0 (0)	1 (20)	0 (0)	

NR: Not reported, IQR: Interquartile range. * Kruskal Wallis test, ** Likelihood ratio test.

It was observed that *MUC1* and *MUC2* stainings were intensely expressed in exophytic tumour types (without statistical significance). Again, no significant relationship was found between tumour diameter and perforation status. The relationship of *MUC1* and *MUC2* staining with preoperative, operative factors and pathological factors are shown in Table II. There was no staining (negativity) in 22 (73.3%) cases with *MUC5AC*, and more than 60% staining (strongly positivity) was observed in 3 (10%) cases. It was observed that the staining rate of *MUC5AC* was lower than the other two antibodies. In addition, strongly expression was noted in rectosigmoid tumours ($p=0.001$), female patients ($p=0.046$), and patients with pT₃ and pT₄ tumours ($p=0.05$). The relationship of *MUC5AC* staining with preoperative, operative factors and pathological factors are shown in Table III.

DISCUSSION

This is the first study to examine the relationship of *MUC1*, *MUC2* and *MUC5AC* with clinicopathological factors. According to the results of this study, while high *MUC1* expression was detected in pT₄ and pT₃ cases and the cases with lymph node involvement, *MUC2* expression was higher

in moderately differentiated cases. In addition, it was observed that *MUC1* and *MUC2* stainings were intensely expressed in exophytic tumour types (not statistically significant). The *MUC5AC* staining rate was lower than for the other two antibodies, and intense expression was noted in rectosigmoid tumours, female patients, and patients with pT₃ and pT₄ tumours.

In gastrointestinal cancers, mucin glycoproteins are altered in two ways. The first change occurs by incorrect glycosylation. At this stage, incomplete glycosylation and de-O-acetylation of O-acetyl sialic acid cause peripheral false glycosylation. The second is that the epitopes in the mucin polypeptide are inappropriately expressed due to sparse and/or insufficient glycosylation, alteration of transcription, or dysregulation of the mucin gene.¹⁰

The *MUC1* antibody is strongly expressed in mucus-forming cells in the gastric surface and neck epithelium. At the same time, it also shows perinuclear expression in crypt cells and submucous glands in the duodenum, jejunum, or ileum.¹¹ *MUC1* expression is also found in the apical membranes of ductal epithelial tissues such as the gallbladder, oesoph-

agus, breast tissue, prostate, endometrium, and endocervix. In addition, *MUC1* can be found at very low levels in normal colon tissue.¹² While *MUC1* is expressed at a very low level in normal colon, it is expressed at 70-85% in colon cancer. On the other hand, *MUC1* expression is significantly increased in severe dysplastic adenomas and tubular differentiated tumours. Nakamori *et al.* found that *MUC1* expression in primary colon and metastatic tumours in Dukes C and D stages showed higher expression than in tumours without metastasis.¹³ However, in another study, no correlation was found between *MUC1* expression and tumour stage, tumour localisation, and differentiation in patients with Dukes B and D tumours. In the light of these studies, immunotherapy and vaccine against *MUC1* have been developed.¹⁴ In this study, *MUC1* negativity was found with a rate of 6.7%, and it was shown that high *MUC1* expression was detected in pT₄ and pT₃ cases and the cases with lymph node involvement.

Expression of the *MUC2* gene in normal tissues was limited in the intestinal epithelium.¹⁵ Both immunohistochemical studies and *in situ* hybridisation studies have shown that *MUC2* apomucin is located supranuclear and perinuclearly in small intestine cells, normal colon cells and cells with colon cancer, and goblet cells. They act as lubrication and protection by being released after the warning mechanisms.¹⁶ In a study by Blank *et al.*, the *MUC2* protein epitope was 21% strongly positive in normal colon tissue, 40% strongly positive in villous adenomas, and 48% strongly positive in tubular adenomas.¹⁷ In another study by Matsuda *et al.*, it was observed that *MUC2* expression decreased as the dysplasia type progressed towards severe dysplasia in both flat and polypoid type adenomas. While *MUC2* expression was observed in 92% in low-grade dysplasia, this rate decreased to 60% in high-grade dysplasia.¹⁸ In this study, the rate of *MUC2* strongly positive expression was 66.7%. However, the expression was increased in moderately differentiated cases; pT and pN staging were not significantly associated with staining. In addition, *MUC2* stainings appeared to be prone to intense expression in exophytic tumour types.

The *MUC5AC* gene encodes secretory mucin and is strongly expressed in gastric superficial and neck mucus cells. The *MUC5AC* gene shows expression in the glandular epithelium of the respiratory system, in the gallbladder epithelium, and the endocervix. However, the *MUC5AC* gene is not expressed in normal colon tissue but non-neoplastic mucosa close to cancer tissue. In some studies, it has been shown that there is an expression at an intensity of 5-20% in the transitional mucosa.⁵ The precancerous field effect has explained the expression of distant normal colon tissue in cancer tissue, albeit very little. In another study, expression was observed in the normal colon at 14%. *MUC5AC* and *MUC6* protein and mRNA levels were minimal or absent in normal colon tissue, while *de novo* expressions were demonstrated in colonic polyps.¹⁹ In a study by Buisine *et al.*, *MUC5AC* was aberrantly

expressed in all cases with rectosigmoid villous adenoma. *MUC5AC* expression was stronger in 91% of patients with low-grade dysplasia, while weak expression was observed in 9%. This rate decreased in patients with high-grade dysplasia (25% strong, 75% weak). While staining was detected in 33% of high-grade dysplasia positive invasive adenocarcinoma cases, no expression was observed in tissues with adenocarcinoma.²⁰ In the study of Bartman *et al.*, low-intensity *MUC5AC* staining was rarely observed in normal colon and hyperplastic polyps. High staining was observed in cases of moderate dysplasia with moderate villous structures in large adenomas.²¹ However, it has been reported that the *MUC5AC* gene is expressed in pancreatic adenocarcinomas and mucinous carcinomas; there are not many studies indicating the percentage of expression in colon carcinoma.²² Kocer *et al.* showed that *MUC5AC* was expressed in 34.1% of tumour samples, 24.4% of normal colonic mucosa samples and 19% of lymph node metastases. *MUC5AC* showed ectopic expression in colorectal carcinoma and strongly expressed in mucinous carcinoma (60%). The number of *MUC5AC*-expressing tumours was lower in patients older than 60 years, tumours located in the rectum, and patients with evidence of recurrence and metastasis postoperatively. Patients with *MUC5AC*-negative tumours had a lower incidence of disease-freeness and overall survival.²³ In this study, *MUC5AC* negative staining was observed in 73.3%. However, strong positivity was observed in 10% of all cases. In addition, intense expression was noted in rectosigmoid tumours, female gender, and patients with pT₃ and pT₄ tumours.

The present study has several limitations. First, it involved a single centre and included a small number of patients. Second, a retrospective study performed using electronic medical records might have introduced the potential for information and statistical bias. Therefore, a multicentre, prospective, and randomised controlled study is necessary to identify the clinicopathological factors affecting *Mucin 1*, *Mucin 2* and *Mucin 5AC* staining in patients who underwent resection for colorectal cancer.

CONCLUSION

High *MUC1* expression was mostly noted in pT₄ and pT₃ cases and cases with lymph node involvement, high *MUC2* expression in moderately differentiated cases, and high *MUC5AC* expression in rectosigmoid tumours, female gender, and patients with pT₃ and pT₄ tumours.

ETHICAL APPROVAL:

Ethical approval was obtained from Kafkas University Non-invasive Ethical Committee (Decision No. 172 / Date: 01.07.2021 (07)).

PATIENTS' CONSENT:

Since it was a retrospective study, no patient consent was required.

COMPETING INTEREST:

The authors declared no competing interest.

AUTHORS' CONTRIBUTION:

TA, HB, YP, OO, TK: Study concept and design.

TA, HB: Data acquisition.

TA, HB, YP: Data analysis and interpretation.

TA, TK: Drafting of the manuscript.

TA, HB, YP, OO, TK: Critical revision and final approval of the manuscript.

All the authors have approved the final version of the manuscript to be published.

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