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Analysis of Demographic, Clinicopathologic and Oncologic Outcomes of Patients with Operated Colorectal Signet Ring Cell Carcinoma

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ABSTRACT

Introduction: Colorectal signet ring cell carcinoma is a rarely encountered histopathological subtype of colorectal adenocarcinoma and displays unique clinicopathological and prognostic characteristics.
Objective: In order to extend the expected survival time and improve the quality of life, this study aimed to determine the factors affecting survival.
Method: This retrospective study was directed towards examining the clinicopathological features of colorectal signet ring cell carcinoma and to investigate prognostic factors affecting survival of the patients. Of 2622 patients having colorectal surgery in a state hospital between 1 January 2015 and 1 June 2025, 30 were found to have colorectal signet ring cell carcinoma and included in the study. Kaplan-Meier method was adopted to calculate the rates of cumulative events and Log-rank test was utilized for comparison of survival in terms of prognostic factors.
Results: Curative surgery created a significant difference in survival. In fact, the patients undergoing curative surgery had longer survival. However, female gender, T4 invasion depth, N2-N3 lymph node involvement, presence of metastasis and a stage 3C or higher stage of disease were found to be the factors of poor prognosis.
Conclusion: This study has a retrospective design. Therefore, randomized studies are needed to understand the biological behavior of signet ring cell adenocarcinoma and to determine prognostic factors that can affect survival.

Keywords: Signet Ring Cell Carcinoma, Mucinous Carcinoma, Colorectal Oncological Surgery.

INTRODUCTION

Colorectal signet ring cell carcinoma is a rare histopathological subtype accounting for about 0,6-2,7% of all colorectal adenocarcinoma cases and has unique clinicopathological and prognostic features (1). Mucin production is a histological feature frequently observed in colorectal cancers. When the rate of mucin production is over 50% in colorectal tumors, they are regarded as a different clinical and pathological entity and called mucinous carcinoma. Signet ring cell mucinous carcinoma is a subtype of mucinous carcinoma and has intracellular mucin production. When more than 50% of neoplastic cells look like signet ring cells, the condition is called signet ring cell carcinoma (2). The primary tumor is more frequently located in the right colon (3). It has a highly poor prognosis (4). Most of the patients have an advanced disease stage at the time of diagnosis (5). It typically appears in young adults (6). Its incidence is higher in the female gender (7). The aim of the present study was to examine the clinicopathological features of colorectal signet ring cell carcinoma, a rare condition with an aggressive course, and to investigate the prognostic factors effective in survival of the patients.

METHOD

Data about 2622 colorectal surgeries performed in Adana City Training and Research Hospital between 1 January 2015 and 1 June 2025 were retrospectively reviewed. Of 2622 cases, 30 had signet ring cell adenocarcinoma. Data about their demographic, clinicopathologic and oncologic features were analyzed by using Statistical Package for the Social Sciences 23.0 (SPSS 23.0, Chicago, Illinois). Effects of 13 factors on survival including age (age of lower than 50 years and age of 50 years and higher), gender, location of the primary tumor, endoscopic appearance, macroscopic appearance, tumor diameter, intestinal obstruction, the number of primary tumor foci, surgical treatment, depth of tumor invasion, (T), lymph node involvement (N), distant metastasis (M), stage of primary tumor (TNM) were investigated. Tumor stages were determined by using American Joint Committee on Cancer (2025)

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TNM staging version 9. General survival was considered as time elapsing from histological diagnosis to death or the last follow-up. Disease-free survival was regarded as time from histological diagnosis to tumor progression or distant organ metastasis. The mean values of general survival and disease-free survival were considered as constant variables. The rates of cumulative events were calculated by utilizing Kaplan-Meier method and data about survival were compared by utilizing Log-rank (Mantel-Cox) test. Adjusted hazard rates and 95% confidence interval were used for predictions. The statistical significance level was set at $p < 0,05$ for all the tests.

Ethical approval was obtained from the ethical committee of Adana City Training and Research Hospital (Ethical approval number: 550 and ethical approval date: 4 June 2025).

RESULTS

A total of 2622 patients undergoing surgery for colorectal adenocarcinoma between 2015 and 2025 were retrospectively reviewed and 30 patients were found to have signet ring cell adenocarcinoma, 50% of which had a signet ring cell appearance confirmed on histological examinations. The rate of signet ring cell adenocarcinoma was found to be 1,1% in the present study.

Table 1. Analysis of the Factors Affecting Mortality and Life Expectancy
(Result of univariate and multivariate analyses)

	Total n	Ex	Censored	Mean ± SD	95% CI	Log rank p
Total	30	15 (50%)	15 (50%)	38,94 ± 6,93	25,35 - 52,53	
Age						
Younger than 50 years old	7	3 (43%)	4 (57%)	30,1 ± 4,23	21,81 - 38,39	0,825 ($\chi2$=0,049)
50 years old or older	23	12 (52%)	11 (48%)	38,9 ± 7,97	23,29 - 54,52	
Gender						
Male	19	6 (32%)	13 (68%)	47,02 ± 8,42	30,52 - 63,53	0,045* ($\chi2$=4,018)
Female	11	9 (82%)	2 (18%)	24,73 ± 7,79	9,45 – 40	
Location of primary tumor						
Right colon	16	7 (44%)	9 (56%)	41,26 ± 8,71	24,18 - 58,34	
Left colon	10	7 (70%)	3 (30%)	30,64 ± 8,76	13,48 - 47,81	0,62 ($\chi2$=0,956)
Rectum	4	1 (25%)	3 (75%)	23,33 ± 5,44	12,66 – 34	
Endoscopic appearance						
Annular	13	7 (54%)	6 (46%)	20,19 ± 4,92	10,53 - 29,84	0,106 ($\chi2$=2,619)
Exophytic	17	8 (47%)	9 (53%)	45,44 ± 8,26	29,26 - 61,63	
Macroscopic appearance						
Ulcerative	15	7 (47%)	8 (53%)	47,82 ± 8,78	30,61 - 65,03	0,06 ($\chi2$=3,546)
Infiltrative	15	8 (53%)	7 (47%)	19 ± 4,22	10,72 - 27,28	
Primary tumor diameter						
Shorter than 5cm	6	3 (50%)	3 (50%)	49,8 ± 11,71	26,85 - 72,75	0,322 ($\chi2$=0,979)
5 cm or longer	24	12 (50%)	12 (50%)	34,51 ± 7,17	20,47 - 48,56	
Intestinal obstruction						
Yes	10	5 (50%)	5 (50%)	38,03 ± 13,34	11,89 - 64,18	0,398 ($\chi2$=0,714)
No	20	10 (50%)	10 (50%)	38,58 ± 6,86	25,13 - 52,04	
Surgical treatment						
Curative	16	6 (37%)	10 (63%)	52,97 ± 8,98	35,36 - 70,57	0,006* ($\chi2$=7,677)
Palliative	14	9 (64%)	5 (36%)	20,87 ± 6,14	8,84 - 32,89	
Tumor invasion depth						
T3	16	6 (37%)	10 (63%)	49,51 ± 9,33	31,23 - 67,8	0,035* ($\chi2$=4,455)
T4	14	9 (64%)	5 (36%)	20,57 ± 5,47	9,85 - 31,29	
Lymph node involvement						
N0-N1	13	5 (38%)	8 (62%)	53,46 ± 9,6	34,64 - 72,27	0,022* ($\chi2$=5,244)
N2-N3	17	10 (59%)	7 (41%)	20,15 ± 3,82	12,67 - 27,64	
Metastasis						
No	14	2 (14%)	12 (86%)	72 ± 7,86	56,6 - 87,4	0,002* ($\chi2$=9,504)
Yes	16	13 (81%)	3 (19%)	21,66 ± 3,96	13,89 - 29,43	
Tumor stage						
Stage 3B and lower	13	5 (38%)	8 (62%)	53,46 ± 9,6	34,64 - 72,27	0,022* ($\chi2$=5,244)
Stage 3C and higher	17	10 (59%)	7 (41%)	20,15 ± 3,82	12,67 - 27,64	

*Statistical Significance: $p<0,05$; SD: Standard Deviation; 95% CI: 95% Confidence Interval; $\chi2$: Chi-Square Test.

The mean age of the patients was 63,23±18,4 years (range: 18-92 years). Of all the patients included in the study (n=30), 23.3% was younger than 50 years and 76.6% was 50 years old or older. There was no significant difference between the patients younger than 50 years old and those aged 50 or older in terms of survival (p<0,825). (Table 1)

Out of all the patients (n=30), 63,3% was male (n=19) and 36,7% was female (n=11). The mean survival was 47,02 ± 8,42 months in the former group (95% CI; 30,52-63,53 months) and 24,73 ± 7,79 months in the latter group (95%CI; 9,45-40 months). Gender created a significant effect on survival (p<0,045).

Medical history and family history of the patients showed no malignancy. Only one patient had synchronous gastric signet cell carcinoma (pT3N3M0 stage 3C). The patient had stage 3C colorectal signet cell adenocarcinoma (pT3N2M0) and died in the early postoperative period. Inclusion of this patient in the sample was considered to have no effect on survival rates since the study sample included other patients having the same disease stage and dying in the postoperative period.

In this study, eight patients (26,7%) underwent emergency surgery for intestinal obstruction and two patients (6,7%) underwent emergency surgery for perforation. Only one patient (25%) had diverting colostomy and received neoadjuvant therapy because the tumor was located in the distal part of the rectum. Three patients with a tumor located in the rectum (75%) underwent surgery following neoadjuvant therapy. Five out of 26 patients with a tumor located in the colon (19,2%) could not receive adjuvant therapy since they died in the postoperative period. Besides, 6,6% of the patients had distant metastasis-peritoneal involvement at diagnosis (M1) n= 2). The disease stage was pT4N2M1 stage 4A in the male patient aged 67 years and pT4N2M1 stage 4A in the male patient aged 74 years. Both died in the first postoperative month.

The primary tumor was located in the right colon in 53,3% of the patients (n= 16), in the left colon in 33,3% of the patients (n=10) and in the rectum in 13,3% of the patients (n=4). The mean survival was 41,26 ± 8,71 months in the patients with a tumor in the right colon (95% CI; 24,18-58,34 months), 30,64 ± 8,76 months in the patients with a tumor in the left colon (95% CI; 13,48-47,81 months) and 23,33±5,44 months in the patients with a tumor in the rectum (95% CI; 12,66-34 months). However, survival did not significantly differ with respect to the location of the primary tumor (p<0,62).

Regarding endoscopic appearance, 56,7% of the carcinomas was exophytic (n=17) and 43,3% of the carcinomas was annular (n=13). The mean survival was 45,44±8,26 months in the former group (95% CI; 29, 26 - 61, 63 months) and 20,19 ± 4,92 months in the latter group (95% CI; 10,53-29,85 months). The mean survival was not significantly different in terms of appearance on endoscopy (p<0,106).

On macroscopy, 50% of the tumors was infiltrative (n=15) and 50% was ulcerative (n=15) and the mean survival was 19 ±4,22 months in the former group (95% CI; 10, 72-27,28 months) and 47,82 ±8,78 months in the latter group (95% CI; 30,61-65,03 months). Macroscopic appearance caused a notable difference in the mean survival (p < 0,06), though it was not significant.

The mean primary tumor diameter was 6,3 cm ±2,11 cm (minimum 3 cm and maximum 11 cm). It was shorter than 5 cm in 20% of the patients (n=6) and 5 cm or longer in 80% of the patients (n=24). The mean survival was 49,8 ±11,71 months (95% CI; 26,85-72,75 months) in the former group and 34,51±7,17 months (95% CI; 20,47-48,56 months) in the latter group. Tumor diameter was not significantly different in terms of survival (p<0,322).

There were multiple primary foci of tumor in 6,6% of the patients (n=2) and a single primary focus of the tumor in 93,4% of the patients (n=28). Two primary foci were found in two patients with the disease located in the right colon. One of them was male and 40 years old, had a tumor stage of pT4N2M0 and one primary focus 7cm in diameter and another primary focus 3 cm in the ascending colon. The other patient was male and 44 years old, had a tumor stage of pT4N3M0 and one primary focus 11 cm in diameter in the cecum and another primary focus 7 cm in diameter in the ascending colon. The number of primary foci did not cause a significant difference in the mean survival (p<0,173).

Of all the patients (n=30), 53,3% (n=16) underwent curative resection and the remaining 46,7% (n=14) underwent palliative surgery. The mean survival was 52,97 ± 8,98 months (95% CI; 35,36 – 70,57 month) in the patients undergoing curative resection and 20,87 ± 6,14 months (95% CI; 8,84 – 32,89 months) in the patients undergoing palliative resection. There was a significant difference in the mean survival between curative surgery and palliative surgery (p<0,006).

Of all the patients (n=30), 53,3% had T3 tumor (n=16) and 46,7% had T4 tumor (n=14). The mean survival was 49,51±9,33 months in the former group (95% CI; 31,23–67,8 months) and 20,57 ± 5,47 months in the latter group (95% CI; 9,85–31,29 months). Tumor invasion depth was significantly different between the patients with T3 tumor and those with T4 tumor (p < 0,035).

There was lymph node involvement in 27 patients (90%). Lymph node involvement status was N0 in three patients (10%), N1 in 10 patients (33,3%), N2 in 16 patients (53,3%) and N3 in one patient (3,4%). The rate of N0-N1 lymph node involvement (involvement of 0-3 lymph nodes) was 43,3% (n=13) and the rate of N2-N3 lymph node involvement (involvement of 4 or more lymph nodes) was 56,7% (n=17). The mean survival was 53,46 ± 9,6 months in the presence of N0-N1 involvement (95% CI; 34,64–72,27 months) and 20,15±3,82 months in the presence of N2-N3 lymph node involvement (95% CI; 12,67–27,64 months). The mean survival significantly differed between N0-N1 and N2-N3 (p< 0,022).

Distant metastasis developed in 53.3% of the patients (n=16). Of all the patients with distant metastasis, 36.6% had peritoneal metastasis (n=11), 6.6% had liver metastasis (n=2), 3.3% had brain metastasis (n=1) and 3.3% had ovarian metastasis (n=1). The mean survival was 21,66 ± 3,96 months in the presence of metastasis (95% CI; 13,89–29,43 months) and 72 ± 7,86 months in the absence of metastasis (95% CI; 56,6–87,4 months). The mean survival was significantly different between the patients with metastasis (M1) and those without metastasis (M0) (p<0,002).

On admission, 10% of the patients had stage 2A disease, 33,4% had stage 3B disease, 50% had stage 3C disease and 6,6% had stage 4A disease. The mean survival was 53,46 ±9,6 months (95% CI; 34,64–72,27 months) in the patients with stage 2A and 3B (n=13; 43,3%) and 20,15±3,82 months (95% CI and 12,67–27,64 months) in the patients with stages 3C and 4A (n=17; 56,6%). There was a significant difference in the mean survival between the two groups of the patients (p < 0,022).

However, no significant difference was found in the mean survival with regard to age (younger than 50 years old versus 50 years old and older), gender, primary tumor location, endoscopic appearance, macroscopic appearance, tumor diameter, number of foci and intestinal obstruction (p> 0,05) (Table1).

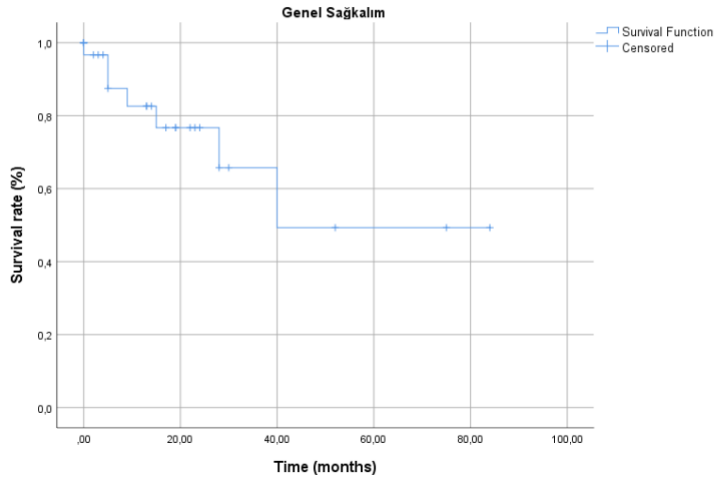

Figure 1. Kaplan Meier Survival Curve

As shown in Kaplan Meier survival graph, 50% of the patients were alive when the present article was written (Figure 1).

The mean survival of the all study sample was 38,03 ± 7,55 months (95% CI; 23–52,83 months).

DISCUSSION

Colorectal signet ring cell carcinoma is a scarcely encountered histological subtype responsible for 0,6%-2,7% of all colorectal carcinoma cases and has distinctive clinicopathological and prognostic features (8). In the present study, the incidence of signet ring cell adenocarcinoma was found to be 1,1%, which is congruent with the literature.

In a study by Bittorf et al., the patients with signet ring cell adenocarcinoma were shown to be young adults with a median age of 40 years and the disease was found to be more frequent in the male gender (68,2%) (9). Compatible with the literature, the present study demonstrated that the male gender was more frequently affected (63,3%). The mean age of the patients in this study was 63,23 ± 18,4 years (minimum 18 years and maximum 92 years). The reason why the patients were older can be attributed to the fact that life expectancy has increased at present. In the current study, age was found to have no effect on survival.

In the present study, however, the female gender was found to be a negative prognostic factor of survival. There has been one more study in the literature to suggest that the female gender had a worse effect on survival (10).

It has been reported in the literature that primary signet ring cell adenocarcinoma is more frequently located in the right colon and that the right colon tumor more adversely affects survival than the one located in the left colon and rectum (10). In the current study, the most frequent location of the tumor was found to be the right colon at the rate of 53,3%. Nevertheless, tumor location did not create a significant effect on survival.

Although there have been various studies supplying information about the size of primary signet ring cell adenocarcinoma, the tumor diameter is reported to be 5,5-6,2 cm at the time of the diagnosis (11). In the current study, the mean primary tumor diameter was found to be 6,3 cm±2,11 cm (minimum 3 cm and maximum 11 cm). This longer diameter can be ascribed with the annular involvement and the presence of mucin. Consistent with the literature, tumor diameter did not create a significant effect on survival in the current study.

It is stated in the literature that mucinous adenocarcinoma develops on the basis of hereditary nonpolyposis coli, which is a comorbidity, and that patients with ulcerative colitis more frequently develop signet ring cell carcinoma compared to the normal population (12). However, none of the patients in the present study had hereditary nonpolyposis coli or ulcerative colitis.

Messerini et al. noted that 70,6% and 29,4% of signet ring cell carcinomas had an infiltrative pattern and exophytic growth respectively, which caused difficulty in its diagnosis (13). In the present study, 50% of the patients had infiltrative tumor on macroscopy and 56,7% of the patients had exophytic tumor on endoscopy. Although as a result of its biological behavior, signet ring cell carcinoma are observed to be annular infiltrative and ulcerative exophytic, an annular wall involvement and infiltrative growth pattern were shown to have no significant effect on survival in the current study.

Belli et al. discovered that curative surgery brought about favorable prognosis and had a positive effect on survival (13). Consistent with their finding, the present study showed a significant difference in survival between curative surgery and palliative surgery. Curative surgery lengthens the expected duration of life.

Surgical therapies performed for intestinal obstruction have been reported to have a significant negative impact on survival (13). However, the present study did not show a significant effect of these therapies.

It has been stated in the literature that 98,2% of the patients with colorectal signet ring cell adenocarcinoma had a stage 3 and 4 disease at the time of the diagnosis and that the TNM had a

significant effect on survival (14). Congruent with the literature, the present study revealed a significant difference in tumor invasion depth between T3 and T4 tumors. The patients with T4 invasion depth have a shorter survival.

In a study with a large case series, involvement of four or more lymph nodes was shown to be a poor prognostic factor (14). In the current study, survival was significantly different between involvement of fewer than four lymph nodes (N0-N1) and involvement of four or more lymph nodes (N1-N2). In the presence of involvement of three or fewer lymph nodes, life expectancy is longer.

The presence of a distant metastasis has been reported to be a poor prognostic factor in the literature (14). In the present study, 53,3% of the patients were shown to develop distant metastasis, which had a significant effect on survival. Life expectancy is shortened in the presence of distant metastasis.

Stage 3 and 4 disease has been reported to be an independent poor prognostic factor (14). The present study showed a significant difference in survival between the patients with a stage 3B or lower stage of disease and those with a stage 3C or higher stage of disease. This suggests that the TNM stage of primary tumor affects survival. In fact, life expectancy is shorter in patients with a stage 3C or lower stage of disease.

CONCLUSION

In light of the results of this study, while curative surgery is a favorable prognostic factor, female gender, T4 invasion depth, N2-N3 lymph node involvement, presence of metastasis and a stage 3C or higher stage of disease are poor prognostic factors.

This study has a retrospective design. Therefore, randomized studies are needed to understand the biological behavior of signet ring cell adenocarcinoma and to determine prognostic factors that can affect survival.

DESCRIPTIONS

No financial support.

No conflict of interest.

AI Statement: Not used

Data Availability: The data supporting this study's findings are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Ethical Declaration: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Our institution has granted ethics committee approval. As this was retrospective research, no informed consent was obtained from participants.

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REFERENCES

1. Thota R, Fang X, Subbiah S. Clinicopathological features and survival outcomes of primary signet ring cell and mucinous adenocarcinoma of colon: retrospective analysis of VACCR database. Journal of gastrointestinal oncology. 2014;5(1):18-24. doi: 10.3978/j.issn.2078-6891.2013.051

2. Öngörü Ö, Sentürk T, Karslıoğlu Y et al. Müsinöz kolorektal karsinomalarda taşlı yüzük hücreli komponentin prognostik önemi. Gülhane Tıp Dergisi. 2015;57: 118-120. doi: 10.5455/gulhane.28063

3. Lee W S, Chun H K, Lee W Y et al. Treatment outcomes in patients with signet ring cell carcinoma of the colorectum. Am J Surg. 2007 Sep;194(3):294-298. doi: 10.1016/j.amjsurg.2006.12.041

4. Min B S, Kim N K, Ko Y T, et al. Clinicopathological features of signet-ring cell carcinoma of the colon and rectum: a case-matched study. Hepato-Gastroenterology journal 2009;56(93):984–988.

5. Casavilca Z S, Sanchez L J, Zavaleta A. Colon and rectum signet-ring cell carcinoma in the National Institute of Neoplastic Diseases. Rev Gastroenterol Peru. 2004;24(3):234-237.

6. Kaw L L Jr, Punzalan C K, Crisostomo A C, Bowyer M W, Wherry D C. Surgical pathology of colorectal cancer in Filipinos: implications for clinical practice. J Am Coll Surg. 2002;195(2):188–195. doi: 10.1016/s1072-7515(02)01186-9

7. She K M, Wang H M, Chen J B, et al. Colorectal cancer in younger than 30 years old group is not associated with poor prognosis. J Soc Colon Rectal Surgeon. 2011;22:93-98. www.crs.org.tw/data/journal/file/1737515414DO20N.pdf

8. Weng M T, Chao K H, Tung C C et al. Characteristics of primary signet ring cell carcinoma of colon and rectum: a case control study, BMC Gastroenterology. 2022;22(1):173. doi.org/10.1186/s12876-022-02258-1

9. Bittorf B, Merkel S, Matzel KE, Wein A, Dimmler A, Hohenberger W. Primary signet-ring cell carcinoma of the colorectum. Langenbecks Arch Surg. 2004;389(3):178–183. doi: 10.1007/s00423-004-0474-y

10. Ishihara S, Watanabe T, Akahane T, et al. Tumor location is a prognostic factor in poorly differentiated adenocarcinoma, mucinous adenocarcinoma, and signet-ring cell carcinoma of the colon. Int J Colorectal Dis. 2012;27(3):371–379. doi: 10.1007/s00384-011-1343-0.

11. Chen J S, Hsieh P S, Hung S Y, et al. Clinical significance of signet ring cell rectal carcinoma. Int J Colorectal Dis. 2004;19(2):102–107. doi: 10.1007/s00384-003-0515-y

12. Psathakis D, Schiedeck T H, Krug F, Oevermann E, Kujath P, Bruch H P. Ordinary colorectal adenocarcinoma vs. primary colorectal signet-ring cell carcinoma: study matched for age, gender, grade, and stage. Dis Colon Rectum. 1999;42(12):1618-1625. doi: 10.1007/BF02236218

13. Belli S, Aytac H O, Karagulle E, Yabanoglu H, Kayaselcuk F, Yildirim S. Outcomes of surgical treatment of primary signet ring cell carcinoma of the colon and rectum: 22 cases reviewed with literature. Int Surg. 2014;99(6):691-698. doi: 10.9738/INTSURG-D-14-00067.1

14. Yang L L, Wang M, He P. Clinicopathological characteristics and survival in colorectal signet ring cell carcinoma: a population-based study. Sci Rep. 2020;10(1):10460. doi: 10.1038/s41598-020-67388-6