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Original Article

Signet ring cell component predicts the response to neoadjuvant chemoradiotherapy in rectal cancer. Long interim results of a single institution experience

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Abstract: There are limited studies evaluating the correlation between the presence of signet ring carcinoma and tumor response to neoadjuvant therapy in the rectum. Hereby, we aimed to report for the first time our experience from Upper Egypt through assessing the predictive role of signet ring cell component (SRCC) in the response to preoperative chemoradiotherapy (PCRT) and the impact of histological types (SRCC versus other types) on survival. This retrospective study analysed the medical records of 195 patients with locally advanced rectal cancer treated from 2011, to 2018. Patients were divided into two groups according to histological types: SRCC group and non SRCC group. All patients received PCRT followed by surgery. SRCC group was associated with significant higher rate of complete clinical response (cCR) and pathologic complete response (pCR) (83.3% and 88.9% respectively) as compared to non SRCC group (9.0% and 10.2% respectively); $P<0.0001$. Fifteen cases (93.8%) who were diagnosed by magnetic resonance tumor regression grade (mrTRG) and diffusion weighted imaging (DWI) as cCR after PCRT, also achieved pCR, in contrast to 88.9% of cases without SRCC. Signet ring histology was the only predictor of pCR in multivariate analysis ($P=0.027$). There was no statistically significant difference between both histological groups as regard to survival. SRCC is an important predictor of pCR and assessing their response to PCRT using mrTRG and DWI showed high sensitivity for the detection of cCR, making them good candidates for watch-and-wait approach. Histological types did not significantly affect the survival outcome.

Keywords: Rectal cancer, signet ring cell component, preoperative chemoradiotherapy, prediction, magnetic resonance imaging

Introduction

Preoperative chemoradiotherapy (PCRT) is the treatment of choice for locally advanced rectal cancer as it achieves sphincter preservation [1] and local control [2, 3]. This is followed by total mesorectal excision (TME) which reduced the local recurrence rate to <10% compared to 20-45% local failure rate with conventional surgery [4, 5].

Magnetic resonance imaging (MRI) is currently considered the standard for assessing the involvement of mesorectal fascia by tumor as these patients have high risk of positive circumferential margin (CRM) which necessitate downstaging before surgery. Furthermore, MRI plays a crucial role in restaging of rectal cancer after neoadjuvant therapy through determining the degree of response to therapy [6].

Patients with pathologic complete response (pCR) to PCRT, had better outcome compared to those with partial response to PCRT [7-9]. With the advancement in preoperative therapies, patients can achieve complete response

(CR) to PCRT and this encouraged the clinicians to investigate the wait and watch policy in these cohorts based on clinical and radiologic assessment to avoid morbidity to surgery [10]. However, this requires proper selection of patients with CR potential at the time of initiation of treatment. Several studies have evaluated the predictive factors for pCR and these included clinical stage at presentation, tumor size, histological types, negative circumferential margin, interval between preoperative chemoradiation and surgery and adjuvant regimens [11-14].

There are limited studies evaluating the correlation between histological types and the tumor response to neoadjuvant therapy in the rectum [12, 15]. These studies demonstrated that signet ring carcinoma (SRC) might have a predictive pCR to PCRT in rectum. Hence, the aim of our study was to assess whether the presence of signet ring cell component (SRCC) in pretreatment biopsy had a predictive role as regard to the response to PCRT and the impact of histological types (SRCC versus other types) on survival outcome.

Patients and methods

Selection criteria for the study

Our Institutional database was collected for all patients with rectal cancer who received PCRT followed by surgery, from January 1, 2011 to January 1, 2018. Inclusion criteria comprised, different subtypes of histologically proven rectal cancer, age ≥18 years and radiologically proven T3/4, or N^+ disease before CRT. Patients with concurrent malignancy, past history of previous malignancy, previous treatment at other centers or stage IV disease were excluded.

Data collection and extraction

The medical records of 195 patients met the inclusion criteria and retrospectively reviewed to extract the study relevant data. Data that were collected included: patients' age; gender; primary tumor site; extent of surgical resection; radiologically assessed response to combined therapy; local and systemic recurrences and survival outcome.

The study population was grouped according to histopathological types into.

-signet ring cell component (SRCC) group which included signet ring carcinoma (presence of signet ring cells in more than 50% of tumor) [16], and adenocarcinoma with signet ring cell component (presence of less than 50% signet ring cells).

-non SRCC group.

These two groups were retrospectively compared with respect to the clinicopathologic characteristics, the response to PCRT, and survival outcome.

This study was approved by the Committee of Medical Ethics of South Egypt Cancer Institute with IRB no: IORG0006563-533 and deemed not to require patient consent.

All patients' files were retrospectively reviewed as regard to initial diagnosis, treatment strategy and follow up, histopathologic data, radiologic response evaluation and treatment outcome.

Initial diagnosis

Pre-treatment evaluation of all patients was based on complete history including age, family history, Eastern Cooperation Oncology Group Performance Status (ECOG PS) [17], physical examination, digital rectal examination, serum carcinoembryonic antigen (CEA).

The initial computed tomographic (CT) scans of the chest and abdomen, as well as the MRI scans of the pelvis, were reviewed by two independent radiologists with 13 and 16 years of experience for determination of pre-treatment tumor and nodal stages. The report included the tumor location and morphology, its T and N categories, the presence of extramural vascular invasion, and its relationship with the surrounding structures including the sphincter complex and the circumferential resection margin (CRM). All patients underwent pelvic MRI 1.5T scanner (Achieva, Philips Healthcare; Amsterdam, Netherlands) with a phased-array surface coil for the primary staging and for the restaging after chemoradiation with the same parameters. Patients were imaged in the supine position. The examination included a wide field of view (FOV) turbo spin-echo (TSE) T2-weighted sequence in the axial (from the aortic bifurcation to the anal sphincter) and the sagittal planes. A small FOV two-dimensional TSE T2-weighted sequence was also done, with a

section thickness of 3 mm for better resolution. Images were obtained in the (a) axial oblique plane (perpendicular to the tumor), (b) sagittal plane, drawn along the longitudinal tumor axis; and (c) oblique coronal plane (parallel to the anal canal), to better evaluate the relationship with the anal sphincter. Diffusion-weighted image (DWI) was also obtained at multiple b values (b=50, 600, 1000 sec/mm^2) in oblique axial plane.

Pre-treatment rectoscope with biopsy which was available for review by pathologists to determine the histological types and grading (poorly differentiated, moderately differentiated and well differentiated) based on the World Health Organization criteria [16]. Pre-treatment clinical staging was performed according to AJCC classification, 7th edition [18].

Treatment strategy and follow up

Preoperative concurrent CRT

All patients received concurrent CRT.

Radiotherapy: CT scan was performed in the treatment position for three dimentional conformal radiotherapy planning. A slice thickness of 5-mm and 5-mm spacing between images were used throughout the scan.

Target volume: Gross tumor volume (GTV), included the tumor and any involved pelvic lymph nodes. Clinical target volume (CTV), included the entire rectum, mesorectum and the regional lymph nodes (presacral lymph nodes, pararectal and hypogastric). The planning target volume (PTV), was constructed by adding 10 mm margin around the CTV.

Dose and energy: Patients were treated by a photon beam of either 6 or 15 MeV using three fields technique (one posterior and two opposing wedged lateral fields). The total dose of 50.4 Gy (pelvis dose of 45 Gy/25 fractions and a boost dose of 5.4 Gy/3 fractions to the GTV plus 2 cm margin) was prescribed at the isocenter of the plan according to ICRU report No. 50.

Concurrent chemotherapy: Concurrent chemotherapy consisted of Capecitabine, 825 mg/m^2, twice daily for 5 days/weeks and it was initiated on the first day of pelvic radiotherapy.

Surgery

All patients underwent TME (R0 resection) which was performed 6-8 weeks after completion of CRT. TME involves en-bloc resection of the rectum, perirectal fat and lymphoid tissue.

Adjuvant chemotherapy

Adjuvant chemotherapy was administered as soon as the patient was medically able, and the wound was completely healed. Chemotherapy regimens included, CapeOX: oxaliplatin 130 mg/m^2 on day 1, capecitabine 1000 mg/m^2 twice daily days 1-14 every 3 weeks; mFOLFOX6: Oxaliplatin 85 mg/m^2 IV, day 1, leucovorin 400 mg/m^2 IV day 1, 5-FU 400 mg/m^2 IV bolus on day 1, then 1200 mg/m^2/day ×2 days (total 2400 mg/m^2 over 46-48 hours) continuous infusion to be repeated every two weeks or Capecitabine: 1000-1250 mg/m^2 PO twice daily days 1-14 every 3 weeks. The adjuvant therapy was given for 4 months (total of 6 months perioperative therapy). The choice between regimens was determined by the availability of the drugs.

Histopathology

Two experienced pathologists reviewed both pre-treatment and post-treatment H&E slides tissue sections from all rectal carcinoma cases and this was done blindly to clinical outcome. The pre-treatment rectal slides were assessed for tumor type and grade (poorly differentiated, moderately differentiated and well differentiated) based on the World Health Organization criteria [16]. SRC was recognized as the presence of signet ring cells in more than 50% of tumor [16], while the presence of less than 50% signet ring cells considered adenocarcinoma with signet ring cell component (**Figure 1**) and we combined both histology for subsequent analysis; (SRCC) group. According to International tumour Budding Consensus Conference (ITBCC) criteria, tumor budding is defined as, the presence of one signet ring cell dissociated from the main tumor at the invasive front or group of less than five cells separated from tumor nests within peritumoral stroma, considered low intratumoral budding. The presence of group of cells; 5-9 and more than 10, considered moderate and high intratumoral budding, respectively [19, 20] (**Figure 1**). Poorly differentiated clusters (PDCs), defined as the presence of

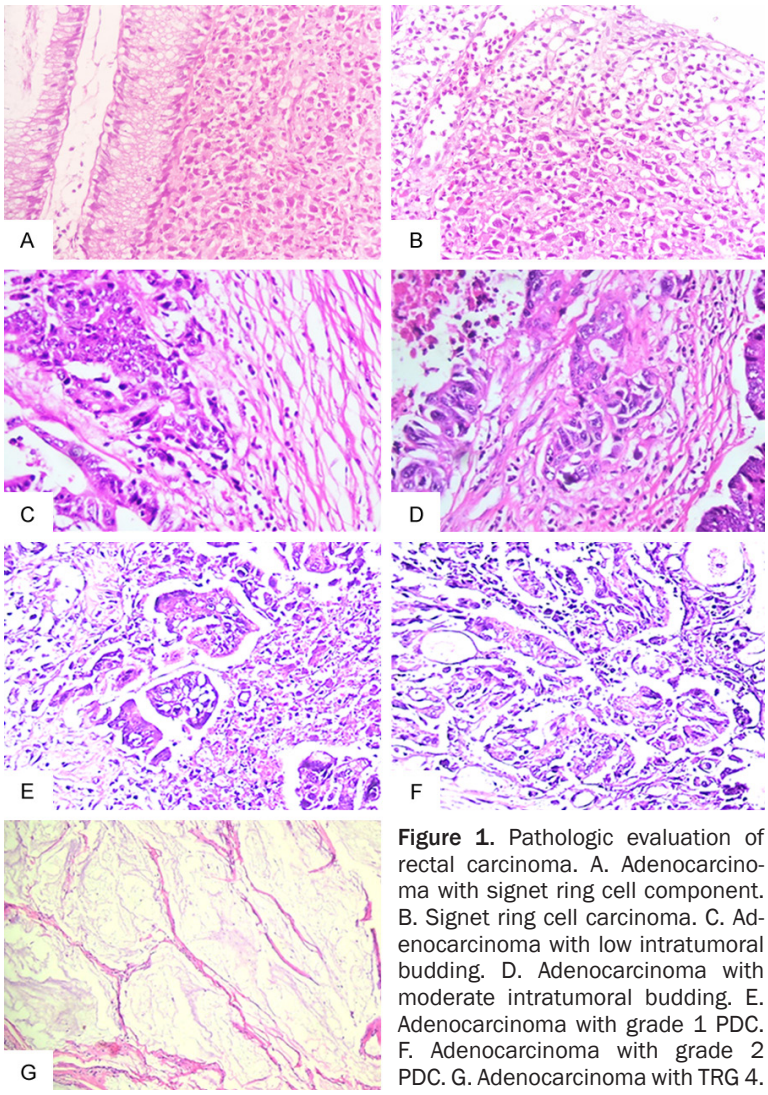

Figure 1. Pathologic evaluation of rectal carcinoma. A. Adenocarcinoma with signet ring cell component. B. Signet ring cell carcinoma. C. Adenocarcinoma with low intratumoral budding. D. Adenocarcinoma with moderate intratumoral budding. E. Adenocarcinoma with grade 1 PDC. F. Adenocarcinoma with grade 2 PDC. G. Adenocarcinoma with TRG 4.

group formed of at least 5 cells lacking glandular structure by the use of 20× power field. PDCS graded as GRADE 1, 2, 3, which corresponds to absence of PDCs, 1-2 PDCS and >2 PDCs, respectively [21] (**Figure 1**).

Radiologic response evaluation

Clinical response was evaluated using MRI scans of the pelvis which were performed four weeks after finishing PCRT to assess primary tumor and nodal response. The pre- and post-treatment MRI scans were reviewed independently by the radiologists and the diagnosis was made in consensus (**Figure 2**). The local radiological T and N stage were made according to previously published criteria [22]. MRI tumor regression grade (mrTRG) as defined by Taylor et al. [22], determines the degree of tumor replacement by fibrotic stroma. Thus, mrTRG 1, 2 and 3 were used to diagnose clinical complete response in addition to diffusion weighted imaging (DWI) to detect any residual areas of tumor restriction adjacent to the fibrosis. Additionally, evaluation of downstaging of the tumor and lymph nodes was done by the pathologic tumor regression grade (TRG) which was quantified according to a five-point scale of Dworak et al. [23]: TRG 0: no regression; TRG 1: dominant tumor mass with obvious fibrosis and/or vasculopathy; TRG 2: dominantly fibrotic changes with few tumor cells or groups (easy to find); TRG 3: very few (difficult to find microscopically) tumor cells in fibrotic tissue with or without mucous substance; TRG 4: no tumor cells, only a fibrotic mass (total regression or response). pCR defined as the absence of any tumor cells in the surgical specimen (ypT0pN0).

Study end point

The study population were followed up until the first of January, 2021, for assessment of the predictive role of the presence of signet ring cell component (SRCC) in pre-treatment biopsy regarding to the response to PCRT and the impact of histological types (SRCC versus other types) on the local recurrence (LR) rate, the distant metastasis (DM) rate and the survival outcome {overall survival (OS) Local recurrence-free survival (LRFS) and distant metastasis-free survival (DMFS).

Statistical analysis

Comparison between categorical variables was done using the χ^2 test or Fisher's exact test, while t-test was used for comparison between continuous variables. LRFS time and DMFS time were defined as the date of surgery to the date of recurrence or date of metastasis

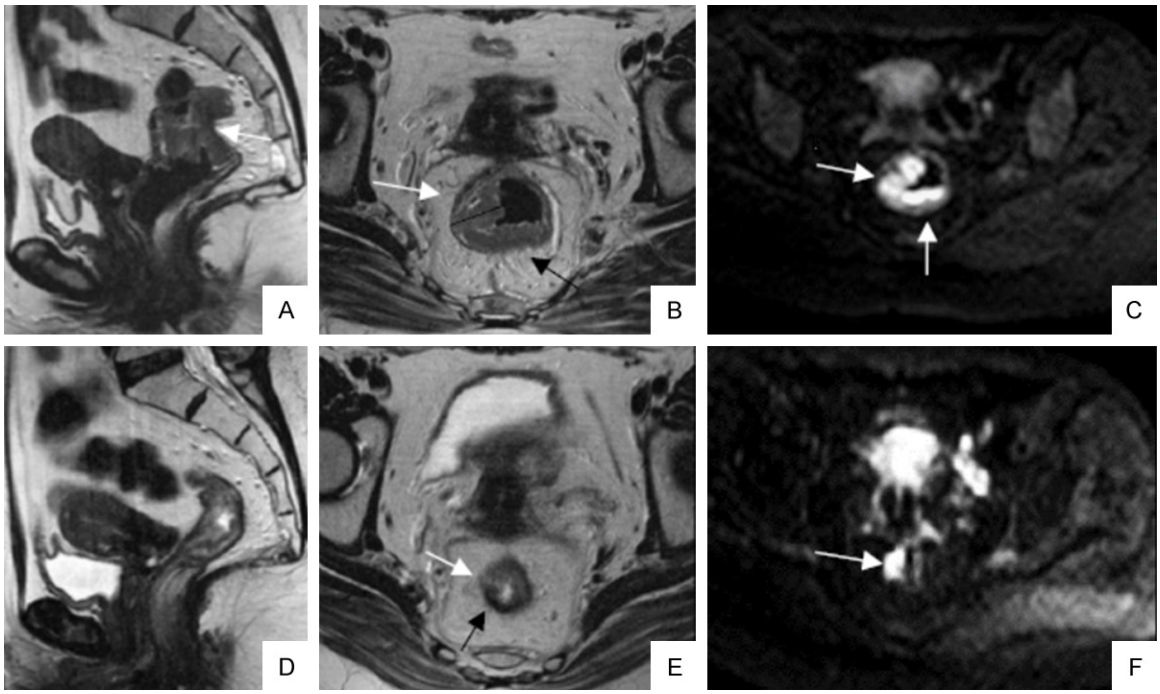

Figure 2. A 20 year-old man presented with a T3b rectal mass. MRI sagittal and axial T2WI and DWI (A-C) pre-chemotherapy showing circumferential rectal wall thickening (13 mm) (white arrow) in the upper rectum of immediate signal intensity infiltrating the muscularis propria and extending for 3 mm in the posterior MRF (black arrow) with restricted diffusion. (D, E) Post-chemoradiotherapy sagittal and axial T2WI shows that the residual mas has intermediate signal (white arrow) and has regressed by ~50% measuring 7 mm with a posterior arc of low signal intensity fibrosis (black arrow), indicating mrTRG 3. (F) DWI shows restricted diffusion of the residual mass with facilitated diffusion of the posterior arc of fibrosis.

respectively. OS time was defined as the date of surgery to the date of death from any cause. Patients were censored at the date of last contact (January 1, 2021), if they did not experience LR, DM, or death at the time of the analysis. LRFS, DMFS and OS were done using Kaplan and Meier analysis [24], and comparison between survival was done using log rank test. Univariate and multivariate logistic regression analysis was performed to identify the potential predictors of pCR. A *P*-value of ≤0.05 is considered to be the level of significance. SPSS version 23 was used for statistical analysis (IBM Corp, 1987, Chicago, USA).

Data availability

The data generated in this study are available upon request from the corresponding author.

Results

Clinicopathologic characteristics

Among reviewed 195 patients, 18 cases (9.2%) had signet ring cell component (SSRC group) and 177 patients (90.8%) had other morphological types of rectal cancer (non SSRC group) in biopsy specimens. The detailed clinical and pathological data of the eligible patients are presented in **Table 1**. Patients' clinical and pathologic characteristics of both groups (SRCC group and non SRCC group), were matched. There were 143 males (73.3%) and 52 females (26.7%). The median age was 54 years (range: 32-68 years). The majority (n=113, 57.9%) of the patients had their tumors located within 5 cm to 10 cm from the anal verge. One hundred twenty-nine patients (66.2%) were cT3 and in 158 patients (81.0%) there was clinical lymph node involvement on diagnosis. Forty-four patients (22.6%) presented with T4N+ve disease where inoperable due to infiltration of bladder neck (23 patients), sacrum (6 patients) and vagina (15 patients).

Treatment

Surgical exploration was carried out after six to eight weeks of completion of CRT and the circumferential resection margin was not involved

Table 1. Clinicopathological characteristics of 195 patients with rectal cancer according to histological groups

Variables	SRCC group 18 (100%)	Non SRCC group 177 (100%)	Total 195 (100%)	*P*-value*
Sex				0.502
Male	13 (72.2)	130 (73.4)	143 (73.3)	
Female	5 (27.8)	47 (26.6)	52 (26.7)	
Age				0.121
Median (range)	51 (34-63)	55 (40-68)	55 (34-68)	
PS				0.315
0	7 (38.9)	42 (23.7)	49 (25.1)	
1	9 (50.0)	99 (55.9)	108 (55.4)	
2	2 (11.1)	36 (20.3)	38 (19.5)	
Pre-treatment CEA level (μg/L)				0.455
≤5	8 (44.4)	95 (53.7)	103 (52.8)	
>5	10 (55.6)	82 (46.3)	92 (47.2)	
Tumor location				0.264
<5 cm	8 (44.4)	56 (31.6)	64 (32.8)	
5-10 cm	10 (55.6)	103 (58.2)	113 (57.9)	
10-15 cm	0	18 (10.2)	18 (9.2)	
Clinical tumor stage				0.274
cT3	14 (77.8)	115 (65.0)	129 (66.2)	
cT4	4 (22.2)	62 (35.0)	66 (33.8)	
Clinical nodal stage				0.926
N0	4 (22.2)	33 (18.6)	37 (19.0)	
N1	6 (33.3)	59 (33.3)	65 (33.3)	
N2	8 (44.4)	85 (48.0)	93 (47.7)	
Stage				0.753
II	4 (22.2)	33 (18.6)	37 (19.0)	
III	14 (77.8)	144 (81.4)	158 (81.0)	
Histologic grade				0.347
Well	2 (11.1)	21 (11.9)	23 (11.8)	
Moderate	8 (44.4)	106 (59.9)	114 (58.5)	
Poor	8 (44.4)	50 (28.2)	58 (29.7)	
Duration from CTH to Surgery				0.982
Median	39 days	37 days	38 days	
Range	(33-51)	(27-56)	(27-56)	
Type of surgery				0.364
LAR	17 (94.4)	144 (81.4)	161 (82.6)	
APR	1 (5.6)	27 (15.3)	28 (14.4)	
Inoperable	0	6 (3.4)	6 (3.1)	

Abbreviations: CEA: Carcinoembryonic antigen, ECOG PS: Eastern Cooperation Oncology Group Performance Status, CTH: Chemotherapy, LAR: Low anterior resection, APR: Abdominoperineal resection. *Chi-square test and Fisher Exact tests were used for all comparisons except age and duration of preoperative chemotherapy (Mann-Whitney U test).

(margin of >1 mm) in all patients. One hundred sixty-one patients (82.6%) underwent low anterior resection (handsewn technique was performed in 106 patients and staplers in 55 patients) and 28 patients (14.4%) underwent abdominoperineal resection. Six patients (3.1%) remained inoperable due to sacral infiltration. Palliative colostomy was done in those patients with unresectable rectal cancer and biopsies from primary tumor and perirectal

Table 2. Comparison between initial MRI staging and post-chemoradiotherapy (yp)

Initial MRI staging	Post-chemoradiotherapy (yp) pathologic staging													
	Yp T0N0	Yp T1N0	yp T1N1	Yp T1N2	yp T2N0	Yp T2N1	yp T2N2	yp T3N0	yp T3N1	Yp T3N2	Yp T4N0	Yp T4N1	yp T4N2	Total (%)
T3N0	19	0	0	0	0	0	0	0	0	0	0	0	0	19
T3N1	12	5	3	2	8	7	3	4	2	0	0	0	0	46
T3N2	0	9	7	6	10	9	7	5	5	6	0	0	0	64
T4N0	2	2	0	0	0	0	0	5	0	0	9	0	0	18
T4N1	1	0	0	0	0	3	0	9	4	0	0	2	0	19
T4N2	0	0	0	0	5	4	0	0	5	0	0	5	10	29
Total (%)	34 (17.4)	16 (8.2)	10 (5.1)	8 (4.1)	23 (11.8)	23 (11.8)	10 (5.1)	23 (11.8)	16 (8.2)	6 (3.1)	9 (4.6)	7 (3.6)	10 (5.1)	195 (100)

Table 3. Comparison between SRCC group and non SRCC group with regard to clinical and pathological response after preoperative chemoradiotherapy

Variables	SRCC group 18 (100%)	Non SRCC group 177 (100%)	Total 195 (100%)	*P*-value*
cCR	15 (83.3)	16 (9.0)	31 (15.9)	<0.0001
non cCR	3 (16.7)	161 (91.0)	163 (83.6)	
Post CRT CEA (ng/ml)				0.187
≤5	10 (55.6)	125 (70.6)	135 (69.2)	
>5	8 (44.4)	52 (29.4)	60 (30.8)	
yp T stage				<0.0001
0	16 (88.9)	18 (10.2)	34 (17.4)	
1	1 (5.6)	33 (18.6)	34 (17.4)	
2	1 (5.6)	55 (31.1)	56 (28.7)	
3	0	45 (25.4)	45 (23.1)	
4	0	26 (14.7)	26 (13.3)	
yp N stage				<0.001
0	17 (94.4)	88 (49.7)	105 (53.8)	
1	1 (5.6)	55 (31.1)	56 (28.7)	
2	0	34 (19.2)	34 (17.4)	
UICC TNM stage				<0.0001
No tumor	16 (88.9)	18 (10.2)	34 (17.4)	
I	1 (5.6)	38 (21.5)	39 (20.0)	
II	0	32 (18.1)	32 (16.4)	
III	1 (5.6)	89 (50.3)	90 (46.2)	
TRG				<0.0001
1	0	29 (16.4)	29 (14.9)	
2	0	42 (23.7)	42 (21.5)	
3	2 (11.1)	88 (49.7)	90 (46.2)	
4	16 (88.9)	18 (10.2)	34 (17.4)	
pCR	16 (88.9)	18 (10.2)	34 (17.4)	<0.0001
Non pCR	2 (11.1)	159 (89.8)	161 (82.6)	

Abbreviations: cCR: complete clinical response, CEA: Carcinoembryonic antigen, yp T: post-chemoradiotherapy pathologic tumor stage, yp N: post-chemoradiotherapy pathologic node stage, TRG: tumor regression grade, pCR: pathological complete response. *Chi-square test was used for all comparisons.

Treatment outcome

The overall downstaging rate was achieved in 85.1% of the 195 treated patients. Tumor progression has not been observed (**Table 2**).

The cCR was detected in 31 (15.9%) cases {15/18 cases (83.3%) with SRCC and 16/177 cases (9.0%) without SRCC, *P*<0.0001}. A total of 34 patients (17.4%) achieved pCR, divided as 16 (88.9%) out of 18 cases with SRCC and 18 (10.2%) out of 177 cases without SRCC; *P*<0.0001. Thus, the sensitivity of mrTRG combined DWI in the diagnosis of cCR was 91% (95% CI: 0.71-0.93). However, the sensitivity was higher (93.8%) in case of SRCC group as compared to the non SRCC group (88.9%). **Table 3** showed the comparison between SRCC group and non SRCC group with regard to clinical and pathologic response after PCRT. Of the 195 tumors examined for TRG, 17.4% showed no viable tumor cells in the rectal wall (TRG 4), whereas 14.9%, 21.5 and 46.2% demonstrated TRG 1, TRG 2, TRG 3 respectively (**Table 3**).

lymph nodes were taken; these patients were excluded from pattern of failure and survival analysis.

Signet ring histology was found to be the only predictor of pCR in univariate analysis, as we found that patients with SRCC were about 71

Table 4. Predictors of pathologic complete response after neoadjuvant chemoradiotherapy in patients with locally advanced rectal cancer by univariate and multivariate analyses using logistic regression

Variables	n	Univariate analysis*			Multivariate analysis*		
		OR	*P*-value**	95% CI	OR	*P*-value**	95% CI
Age (years)					Not included in the model		
≤60	140	ref					
≥60	55	1.497	0.314	0.683-3.282			
Gender					Not included in the model		
Male	143	ref					
Female	52	0.668	0.380	0.272-1.643			
Pre-treatment clinical tumor stage							
3	129	ref			Not included in the model		
4	66	0.546	0.166	0.232-1.285			
Pre-treatment clinical node stage							
N0	37	ref			ref		
N1	65	0.611	0.314	0.234-1.594	1.592	0.997	0.000-NA
N2	93	0.400	0.058	0.155-1.030	0.417	0.155	0.125-1.393
Pre-treatment clinical stage					Not included in the model		
II	37	ref					
III	158	0.484	0.092	0.208-1.127			
Histological types†							
Non SRCC	177	ref			ref		
SRCC	18	70.667	0.000**	15.019-332.503	4.065	0.027**	1.176-14.085
Histologic grade†					Not included in the model		
Well	23	ref					
Moderate	114	0.720	0.561	0.238-2.177			
Poor	58	0.750	0.639	0.225-2.496			
Tumor budding†					Not included in the model		
Low	87	ref					
Intermediate	31	0.137	0.059	0.017-1.079			
High	77	1.080	0.843	0.503-2.319			
PDC†							
Grade 1	93	ref			ref		
Grade 2	42	1.491	0.446	0.534-4.163	1.511	0.441	0.529-4.312
Grade 3	60	2.711	0.022	1.158-6.346	0.000	0.997	0.00-NA
Pre-treatment CEA (ng/ml)					Not included in the model		
≤5	103	ref					
>5	92	1.766	0.137	0.834-3.740			
Interval from completion of CRT to surgery (weeks)					Not included in the model		
≤7	80	ref					
>7	115	0.643	0.244	0.306-1.351			

Abbreviations: OR: Odds ratio, CI: Confidence interval, NA: not achieved, SRCC: Signet ring cell component, PDC: Poorly differentiated clusters, †In pre-treatment biopsies, CEA: Carcinoembryonic antigen, CRT: Chemoradiotherapy. *Univariate and multivariate logistic regression analysis was performed to identify the potential predictors of pCR. **P-values ≤0.05 were considered statistically significant.

times more likely to achieve pCR than patients with other histological types (OR=70.667, 95% CI: 15.019-332.503, P<0.0001) (**Table 4**). Multivariate analysis confirmed that Signet ring histology was the only predictor of pCR (OR=4.065, 95% CI: 1.176-14.085, P=0.027) (**Table 4**).

Pattern of failure

A total of 189 patients were analysed for pattern of failure. Fourteen cases (7.4%) developed local recurrence and 40 cases (21.2%) developed distant metastasis. There was no statistically significant difference between the two histologic groups regarding the rate of LR (P-value =0.115) and DM (P-value =0.184) (**Table 5**).

Survival

The median follow up duration of 189 patients was 79 months (range 18-111 months). There

Table 5. Pattern of treatment failure according to histological groups (SRCC versus non SRCC)

Pattern of failure	SRCC 18 (100%)	Non SRCC 171 (100%)	Total 189 (100%)	*P*-Value*
Local	3(16.7)	11 (6.4)	14 (7.4)	0.115
Isolated	0	9	9	
Mixed	3	2	5	
Systemic	6 (33.3)	34 (19.9)	40 (21.2)	0.184
Isolated	3	32	35	
Mixed	3	2	5	

*Chi-square test was used for all comparisons.

was no statistically significant difference in the median follow up duration between both groups (*P*=0.285). On the date of the analysis, 142 patients (75.1%) were free from disease and 143 patients (75.7%) were alive. According to Kaplan-Meier analysis, the OS rate at 5 years was 66.7% (95% CI: 67.056-108.944) and 76.4% (95% CI: 94.693-105.526) for SRCC group and non SRCC group respectively. There was no statistically significant difference between both histologic groups (*P*=0.180) (**Figure 3**). LRFS and DMFS was 81.3% (95% CI: 82.752-120.373) and 66.7% (95% CI: 64.653-108.458) respectively for SRCC group. For non SRCC group, LRFS was 93.6% (95% CI: 109.629-117.175) and DMFS was 80.0% (95% CI: 94.642-106.380). There was no statistically significant difference between both histological groups as regard to LRFS (*P*=0.107) (**Figure 4A**) and DMFS (*P*=0.158) (**Figure 4B**).

Discussion

Identification of clinical, radiological and histological features in patients with locally advanced rectal cancer who achieved a pCR following chemoradiotherapy is crucial particularly if a non-operative approach is to be undertaken.

Studies have shown that, SRCC histology is associated with high histological grade as well as advanced tumor and nodal stage compared with mucinous carcinoma and well/moderately/poorly differentiated adenocarcinoma [25, 26]. In this retrospective study, there was no significant difference in the distribution of sex, age, PS, CEA level, tumor location, tumor stage, nodal stage, overall stage, grade, median duration from chemoradiotherapy to surgery and type of surgical intervention among the two histological groups.

In this study, the use of mrTRG (grades 1, 2 and 3) in combination with DWI showed 91% sensitivity for the diagnosis of cCR when compared to TRG as the reference standard. The concordance of MRI complete regression (cCR) with pCR (ypT0N0M0) was found in 15 cases (93.8%) with SRCC and in 16 cases (88.9%) without SRCC. Bhoday et al. [27], also reported 94% sensitivity of mrTRG for detecting patients with a pCR. However, we found that the use of DWI was necessary to exclude residual tumor foci of restricted diffusion adjacent to the fibrosis and confirm cCR. We reported a statistically significant difference (*P*<0.0001) in the rate of pCR between both histological groups (88.9% vs 10.2%); in favour of the SRCC group. Similarly, two studies reported by Jaynand et al. [12] and chao et al. [28] showed that signet ring cell histology was predictive for pCR after neoadjuvant chemoradiotherapy. Chao et al. [28] also demonstrated that 4 out of 19 patients (100%) with SRCC who achieved cCR also achieved pCR while only (60%) cCR cases without SRCC achieved pCR.

In our study, 17.4% of the patients achieved pCR. A similar response was reported by Garcia-Aguilar et al. [29] as 17% of the patients achieved pCR after receiving CRT plus 2 cycles of chemotherapy (5-FU + leucovorin + oxaliplatin). Other studies reported a substantially higher rate of pCR than what was reported by our study. The possible explanations are the administration of an additional cycle of oxaliplatin-containing neoadjuvant chemotherapeutic regimens (Capecitabine + Oxaliplatin) after PCRT (Capecitbine + Oxaliplatin + radiation) (pCR rate of 19%) [30] and the use of radiotherapy boost for a total dose of 55 Gy plus concurrent chemotherapy consisting of raltitrexed and oxaliplatin (pCR rate of 32%) [31].

The local relapse rate (7.4%) reported by our study was higher compared to other studies [30-32]. This might be attributed to limited follow up period as in the study reported by Machiels et al. (no local recurrence and only 2 patients had distant metastasis) [32], more favourable distribution of T stage [30, 32] and higher radiotherapy dose (55 Gy/5 weeks) [30]. Additionally, diagnostic MRI in our study revealed that 70% of T3 tumors extended more

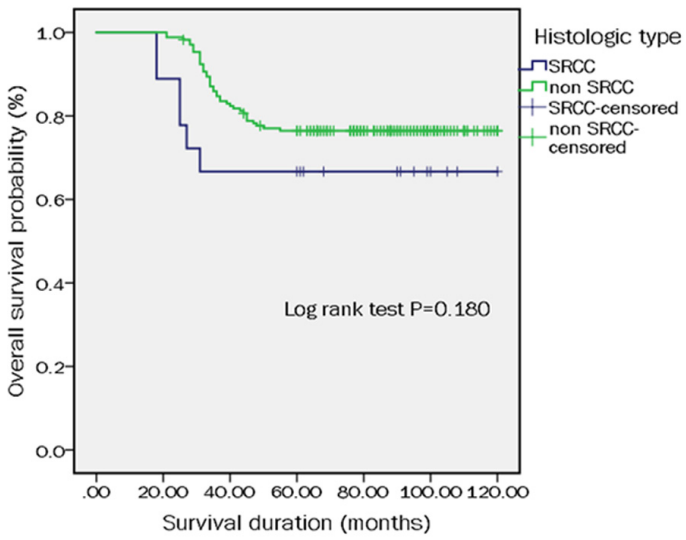

Figure 3. Kaplan-Meier curves showing OS according to histological groups. OS (*P*=0.180).

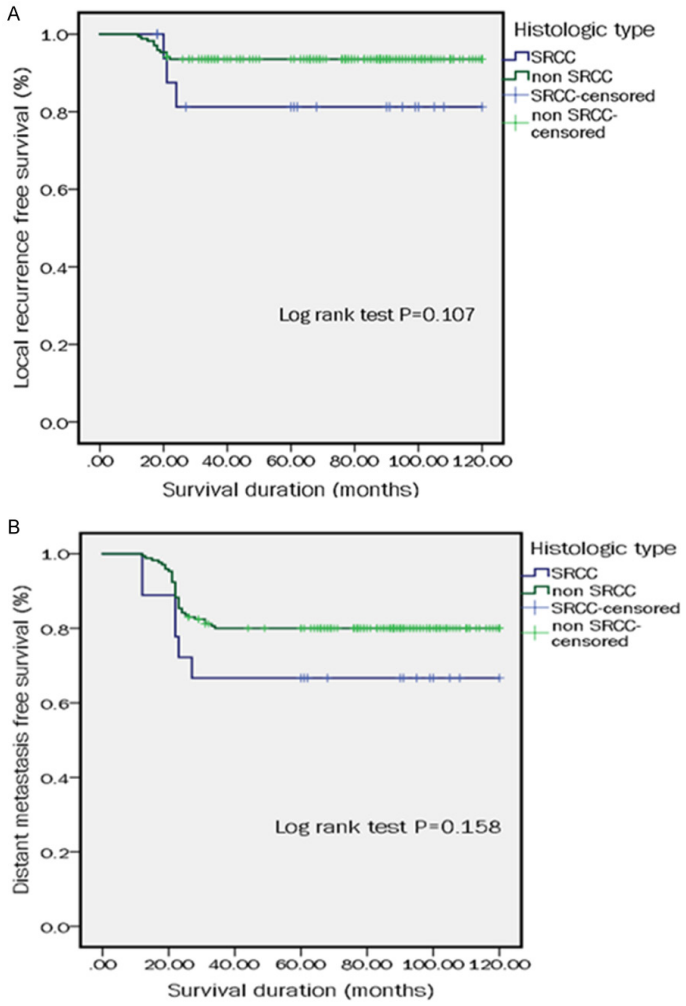

Figure 4. Kaplan-Meier curves showing (A) LRFS and (B) DMFS according to histological groups. LRFS (*P*=0.107) and DMFS (*P*=0.158).

than 5 mm beyond the muscularis propria was shown to have a significantly higher locoregional recurrence rate and poorer 5-year cancer specific survival [33]. In our study, 21.2% of the cases developed DM, which is higher than what was reported by Chau et al. [34] (10% of the patients had DM after median follow up time of 23 months in patient who received PCRT which consisted of capecitabine and oxaliplatin). This could be attributed to the longer follow up duration of our study (79 months). Additionally, Shinde et al. [35] reported a lower rate of LR (4.2%) and DM (10.9%) than what was reported by our study. This is attributed to that the pattern of failure in this study was evaluated in a cohort of patients who achieved pCR.

A study published by Shinde et al. [35], reported 3-year OS rates of 77.7%, 100%, and 92.8%, for signet, mucinous and non-signet non-mucinous histology respectively (*P*=0.20). These figures are higher than our figures as we had a longer follow up period (79 months) and they estimated survival only in patients who achieved pCR. In our study, there was no statistically significant difference between both histological groups as regard to OS (*P*=0.180), LRFS (*P*=0.107) and DMFS (*P*=0.158). Our data were further confirmed by Shinde et al. [35], who reported no significant difference in OS and DFS amongst the three groups (signet, mucinous, and non-signet non-mucinous). However, several studies [36-42] demonstrated that SRCC is an established negative prognostic factor and associated with lower survival. The lack of observed differences in overall survival or relapse rates among the histologic groups might be attributed to the small sample size of our study.

Clinical stage at presentation, tumor size, histological subtype, negative circumferential margin, interval between preoperative chemoradiation and surgery and adjuvant regimens

had been evaluated by several studies [11-14] as predictive factors for pCR. Other studies have demonstrated that tumor budding and PDCs in biopsy both predict a poor response to PCRT [20, 21]. However, our results suggest that SRCC in pre-treatment biopsy was the only predictive factor for pCR (P=0.027).

Our study has some limitations including, the retrospective nature, the small sample size and the low occurrence rate of SRC.

Conclusions

SRCC in pre-treatment biopsy is an important predictor of pCR and assessing their response to PCRT using mrTRG and DWI showed high sensitivity for the detection of complete clinical responder, making them good candidates for watch-and-wait approach. Histological types did not significantly affect the survival outcome.

Disclosure of conflict of interest

None.

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References

[1] Weiser MR, Quah HM, Shia J, Guillem JG, Paty PB, Temple LK, Goodman KA, Minsky BD and Wong WD. Sphincter preservation in low rectal cancer is facilitated by preoperative chemoradiation and intersphincteric dissection. Ann Surg 2009; 249: 236-42.

[2] Rödel C, Liersch T, Becker H, Fietkau R, Hohenberger W, Hothorn T, Graeven U, Arnold D, Lang-Welzenbach M, Raab HR, Sülberg H, Wittekind C, Potapov S, Staib L, Hess C, Weigang-Köhler K, Grabenbauer GG, Hoffmanns H, Lindemann F, Schlenska-Lange A, Folprecht G and Sauer R; German Rectal Cancer Study Group. Preoperative chemoradiotherapy and postoperative chemotherapy with fluorouracil and oxaliplatin versus fluorouracil alone in locally advanced rectal cancer: initial results of the German CAO/ARO/AIO-04 randomised phase 3 trial. Lancet Oncol 2012; 13: 679-87.

[3] Sauer R, Liersch T, Merkel S, Fietkau R, Hohenberger W, Hess C, Becker H, Raab HR, Villanueva MT, Witzigmann H, Wittekind C, Beissbarth T and Rödel C. Preoperative versus postoperative chemoradiotherapy for locally advanced rectal cancer: results of the German CAO/ARO/AIO-94 randomized phase III trial after a median follow-up of 11 years. J Clin Oncol 2012; 30: 1926-33.

[4] Dahlberg M, Glimelius B and Påhlman L. Changing strategy for rectal cancer is associated with improved outcome. Br J Surg 1999; 86: 379-84.

[5] MacFarlane JK, Ryall RD and Heald RJ. Mesorectal excision for rectal cancer. Lancet 1993; 341: 457-60.

[6] Horvat N, Carlos Tavares Rocha C, Clemente Oliveira B, Petkovska I and Gollub MJ. MRI of rectal cancer: tumor staging, imaging techniques, and management. Radiographics 2019; 39: 367-387.

[7] Quah HM, Chou JF, Gonen M, Shia J, Schrag D, Saltz LB, Goodman KA, Minsky BD, Wong WD and Weiser MR. Pathologic stage is most prognostic of disease-free survival in locally advanced rectal cancer patients after preoperative chemoradiation. Cancer 2008; 113: 57-64.

[8] Rödel C, Martus P, Papadoupolos T, Füzesi L, Klimpfinger M, Fietkau R, Liersch T, Hohenberger W, Raab R, Sauer R and Wittekind C. Prognostic significance of tumor regression after preoperative chemoradiotherapy for rectal cancer. J Clin Oncol 2005; 23: 8688-96.

[9] Lee YC, Hsieh CC and Chuang JP. Prognostic significance of partial tumor regression after preoperative chemoradiotherapy for rectal cancer: a meta-analysis. Dis Colon Rectum 2013; 56: 1093-101.

[10] Renehan AG, Malcomson L, Emsley R, Gollins S, Maw A, Myint AS, Rooney PS, Susnerwala S, Blower A, Saunders MP, Wilson MS, Scott N and O'Dwyer ST. Watch-and-wait approach versus surgical resection after chemoradiotherapy for patients with rectal cancer (the OnCoRe project): a propensity score matched cohort analysis. Lancet Oncol 2016; 17: 174-183.

[11] Garland ML, Vather R, Bunkley N, Pearse M and Bissett IP. Clinical tumour size and nodal status predict pathologic complete response following neoadjuvant chemoradiotherapy for rectal cancer. Int J Colorectal Dis 2014; 29: 301-7.

[12] Jayanand SB, Seshadri RA and Tapkire R. Signet ring cell histology and non-circumferential tumors predict pathological complete response following neoadjuvant chemoradiation in rectal cancers. Int J Colorectal Dis 2011; 26: 23-7.

[13] Petrelli F, Sgroi G, Sarti E and Barni S. Increasing the interval between neoadjuvant chemo-

radiotherapy and surgery in rectal cancer: a meta-analysis of published studies. Ann Surg 2016; 263: 458-64.
[14] Cui J, Fang H, Zhang L, Wu YL and Zhang HZ. Advances for achieving a pathological complete response for rectal cancer after neoadjuvant therapy. Chronic Dis Transl Med 2016; 2: 10-16.
[15] Rombouts AJM, Hugen N, Verhoeven RHA, Elferink MAG, Poortmans PMP, Nagtegaal ID and de Wilt JHW. Tumor response after long interval comparing 5×5Gy radiation therapy with chemoradiation therapy in rectal cancer patients. Eur J Surg Oncol 2018; 44: 1018-1024.
[16] In: Bosman FT, Carneiro F, Hruban RH, Theise ND, editors. WHO classification of tumours of the digestive system. 4th edition. Geneva: International Agency for Research on Cancer; 2010.
[17] Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET and Carbone PP. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982; 5: 649-55.
[18] Edge SB and Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010; 17: 1471-4.
[19] Lugli A, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H, El Zimaity H, Fléjou JF, Hansen TP, Hartmann A, Kakar S, Langner C, Nagtegaal I, Puppa G, Riddell R, Ristimäki A, Sheahan K, Smyrk T, Sugihara K, Terris B, Ueno H, Vieth M, Zlobec I and Quirke P. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol 2017; 30: 1299-1311.
[20] Rogers AC, Gibbons D, Hanly AM, Hyland JM, O'Connell PR, Winter DC and Sheahan K. Prognostic significance of tumor budding in rectal cancer biopsies before neoadjuvant therapy. Mod Pathol 2014; 27: 156-62.
[21] Reggiani Bonetti L, Lionti S, Domati F, Pagliani G, Mattioli E and Barresi V. Histological grading based on poorly differentiated clusters is predictive of tumour response and clinical outcome in rectal carcinoma treated with neoadjuvant chemoradiotherapy. Histopathology 2017; 71: 393-405.
[22] Taylor FG, Swift RI, Blomqvist L and Brown G. A systematic approach the interpretation of preoperative staging MRI for rectal cancer. AJR Am J Roentgenol 2008; 191: 1827-1835.
[23] Dworak O, Keilholz L and Hoffmann A. Pathological features of rectal cancer after preoperative radiochemotherapy. Int J Colorectal Dis 1997; 2: 19-23.
[24] Kaplan EL and Meier P. Nonparametric estimation from incomplete observations. Journal of the American Statistical Association 1958; 53: 457-81.
[25] Hugen N, Verhoeven RH, Lemmens VE, van Aart CJ, Elferink MA, Radema SA, Nagtegaal ID and de Wilt JH. Colorectal signet-ring cell carcinoma: benefit from adjuvant chemotherapy but a poor prognostic factor. Int J Cancer 2015; 136: 333-9.
[26] Mizushima T, Nomura M, Fujii M, Akamatsu H, Mizuno H, Tominaga H, Hasegawa J, Nakajima K, Yasumasa K, Yoshikawa M and Nishida T. Primary colorectal signet-ring cell carcinoma: clinicopathological features and postoperative survival. Surg Today 2010; 40: 234-8.
[27] Bhoday J, Smith F, Siddiqui MR, Balyasnikova S, Swift RI, Perez R, Habr-Gama A and Brown G. Magnetic resonance tumor regression grade and residual mucosal abnormality as predictors for pathological complete response in rectal cancer post neoadjuvant chemoradiotherapy. Dis Colon Rectum 2016; 59: 925-33.
[28] Chao X, Wang Z, Lu S, Huang Y, Zang S, Ding P, Zhang H and Yun J. Signet ring cell component in pretreatment biopsy predicts pathological response to preoperative chemoradiotherapy in rectal cancer. Int J Clin Oncol 2020; 25: 1653-1662.
[29] Garcia-Aguilar J, Smith DD, Avila K, Bergsland EK, Chu P and Krieg RM; Timing of Rectal Cancer Response to Chemoradiation Consortium. Optimal timing of surgery after chemoradiation for advanced rectal cancer: preliminary results of a multicenter, nonrandomized phase II prospective trial. Ann Surg 2011; 254: 97-102.
[30] Xu BH, Chi P, Guo JH, Guan GX, Tang TL, Yang YH, Chen MQ, Song JY and Feng CY. Pilot study of intense neoadjuvant chemoradiotherapy for locally advanced rectal cancer: retrospective review of a phase II study. Tumori 2014; 100: 149-57.
[31] Caravatta L, Padula GD, Picardi V, Macchia G, Deodato F, Massaccesi M, Sofo L, Pacelli F, Rotondi F, Cecere G, Sallustio G, Di Lullo L, Piscopo A, Mignogna S, Bonomo P, Cellini N, Valentini V and Morganti AG. Concomitant boost radiotherapy and multidrug chemotherapy in the neoadjuvant treatment of locally advanced rectal cancer: results of a phase II study. Acta Oncol 2011; 50: 1151-57.
[32] Machiels JP, Duck L, Honhon B, Coster B, Coche JC, Scalliet P, Humblet Y, Aydin S, Kerger J, Remouchamps V, Canon JL, Van Maele P, Gilbeau L, Laurent S, Kirkove C, Octave-Prignot M, Baurain JF, Kartheuser A and Sempoux C. Phase II study of preoperative oxaliplatin, capecitabine and external beam radiotherapy

in patients with rectal cancer: the RadiOxCape study. Ann Oncol 2005; 16: 1898-905
[33] Merkel S, Mansmann U, Siassi M, Papadopoulos T, Hohenberger W and Hermanek P. The prognostic inhomogeneity in pT3 rectal carcinomas. Int J Colorectal Dis 2001; 16: 298-304.
[34] Chau I, Brown G, Cunningham D, Tait D, Wotherspoon A, Norman AR, Tebbutt N, Hill M, Ross PJ, Massey A and Oates J. Neoadjuvant capecitabine and oxaliplatin followed by synchronous chemoradiation and total mesorectal excision in magnetic resonance imaging-defined poor-risk rectal cancer. J Clin Oncol 2006; 24: 668-74.
[35] Shinde RS, Bhamre R, Niyogi1 D, Mitra A, Desouza A, Ostwal V, Engineer R, Dusane R and Saklani A. Does histology dictate outcomes of locally advanced rectal adenocarcinoma with complete pathological response following neoadjuvant chemoradiation? Journal of Radiation Oncology 2018; 7: 85-89.
[36] Pozos-Ochoa LI, Lino-Silva LS, León-Takahashi AM and Salcedo-Hernández RA. Prognosis of signet ring cell carcinoma of the colon and rectum and their distinction of mucinous adenocarcinoma with signet ring cells. A comparative study. Pathol Oncol Res 2018; 24: 609-616.
[37] Inamura K, Yamauchi M, Nishihara R, Kim SA, Mima K, Sukawa Y, Li T, Yasunari M, Zhang X, Wu K, Meyerhardt JA, Fuchs CS, Harris CC, Qian ZR and Ogino S. Prognostic significance and molecular features of signet-ring cell and mucinous components in colorectal carcinoma. Ann Surg Oncol 2015; 22: 1226-1235.
[38] Lee HS, Soh JS, Lee S, Bae JH, Kim KJ, Ye BD, Byeon JS, Myung SJ, Yang SK, Kim SA, Park YS, Lim SB, Kim JC, Yu CS and Yang DH. Clinical features and prognosis of resectable primary colorectal signet-ring cell carcinoma. Intest Res 2015; 13: 332-8.
[39] Nissan A, Guillem JG, Paty PB, Wong WD and Cohen AM. Signet-ring cell carcinoma of the colon and rectum: a matched control study. Dis Colon Rectum 1999; 42: 1176-80.
[40] Tung SY, Wu CS and Chen PC. Primary signet ring cell carcinoma of colorectum: an age- and sex-matched controlled study. Am J Gastroenterol 1996; 91: 2195-9.
[41] Sasaki S, Masaki T, Umetani N, Futakawa N, Ando H and Muto T. Characteristics in primary signet-ring cell carcinoma of the colorectum, from clinicopathological observations. Jpn J Clin Oncol 1998; 28: 202-6.
[42] Psathakis D, Schiedeck TH, Krug F, Oevermann E, Kujath P and Bruch HP. Ordinary colorectal adenocarcinoma vs. Primary colorectal signet-ring cell carcinoma: study matched for age, gender, grade, and stage. Dis Colon Rectum 1999; 42: 1618-25.