

Histopathological Assessment of Rectal Carcinomas after Neoadjuvant Chemoradiotherapy

Abstract

Background: Neoadjuvant chemoradiotherapy followed by definitive surgery has been well established as the standard treatment for patients with locally advanced rectal cancer. The aims of the study were to assess the various histopathological changes in tumor cells and stroma, to assess the tumor regression grade (TRG) and tumor downstaging after neoadjuvant chemoradiotherapy. **Materials and Methods:** Ninety cases of carcinoma rectum which received neoadjuvant chemoradiotherapy followed by definitive surgery were included. Pretreatment biopsy slides were assessed for tumor type and differentiation. In postsurgical specimens, pathological assessment of morphological changes, tumor downstaging, and TRG was done. **Results:** Out of the 90 cases in the study group, the peak incidence was in the age group of 61–70 years (35 cases, 38.9%). The male-to-female ratio was 1.25:1. Moderately differentiated adenocarcinoma was the most common histologic type on biopsy samples, accounting for 86.6% of cases. Complete disappearance of tumor cells or TRG0 was seen in 11 cases (12% of total). TRG1 was seen in 32% of cases, TRG2 in 34% of cases, and TRG3 in 22% of cases. Tumor downstaging was noted in 68% of cases. The most striking histopathological features observed were increased cytoplasmic eosinophilia (58.9%) and marked nuclear pleomorphism (78.9%). The predominant type of stromal response was fibroinflammatory type (53.3% of cases). **Conclusion:** Pathological evaluation remains the gold standard for assessing the tumor response to neoadjuvant therapy. Accurate assessment of therapy-induced morphologic changes and tumor downstaging is important in further treatment and prognostication of patients.

Keywords: Carcinoma rectum, neoadjuvant therapy, tumor regression grade

Introduction

Colorectal cancer is one of the commonly diagnosed cancers in both sexes, after carcinomas of lung, breast, and prostate. It is one of the leading causes of cancer deaths (9.2% of total cancer deaths) after lung cancer. It is the seventh leading cancer in India although the incidence is less than in western countries. There have been 27,605 new cases and 19,548 deaths due to colorectal cancer in India in 2018, with a 5-year prevalence of 53,700 cases for all ages.^[1]

The current standard of management of locally advanced rectal cancer is neoadjuvant chemoradiotherapy followed by total mesorectal excision (abdominoperineal resection or anterior resection). This has resulted in improved disease-free survival and decreased overall recurrence

rate.^[2] There has been significant tumor downstaging and improved local disease control with neoadjuvant therapy. Tumor regression and downstaging enable curative resection and even sphincter preservation.^[3,4]

The increasing use of preoperative therapy creates new challenges to surgical pathologists which include assessment of tumor response to preoperative treatment and analysis of therapy-induced morphologic changes. Pathologic evaluation is more difficult when there is no residual tumor on macroscopic evaluation.^[5] In such scenario, the accuracy of pathologic stage depends on meticulous search for residual tumor. Preoperative chemoradiation may significantly reduce the size and number of retrieved lymph nodes, which in turn will lead to underestimation of nodal status. Histopathological assessment of the extent of tumor, tumor regression grade (TRG), lymphovascular tumor emboli and

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Rahul Rajagopal,
Renu Sukumaran,
Choondal Devan
Sivanandan¹,
Jayasree Katoor

Departments of Pathology
and ¹Radiation Oncology,
Regional Cancer Centre,
Thiruvananthapuram, Kerala,
India

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Address for correspondence:

Dr. Renu Sukumaran,
Department of Pathology,
Regional Cancer Centre,
Thiruvananthapuram - 695 011,
Kerala, India.
E-mail: renu.sukumaran@gmail.com

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perineural invasion, and surgical margins and lymph nodes are important in prognostication and further management.

The therapy-induced histomorphological changes described include cytoplasmic changes, nuclear changes, necrosis, apoptosis, extracellular mucin pools, stromal fibrosis, and inflammatory infiltrates.

The response of the tumor after neoadjuvant therapy ranges from pathological complete response (no residual tumor) to no response (extensive tumor with no regressive changes). Various systems have been suggested for grading tumor response to preoperative chemoradiotherapy.^[6-8] Consistency regarding correlation with prognosis and reproducibility of grading varies in different systems. The CAP guidelines recommend modified Ryan system for reporting tumor regression grading.^[9]

Materials and Methods

In this prospective observational study, ninety cases of rectal carcinoma treated with neoadjuvant chemoradiotherapy followed by surgery (anterior resection/abdominoperineal resection) were included. The study period was 2 years from August 2017 to July 2019. Patients with histological diagnosis of rectal carcinoma on biopsy and pretreatment evaluation of tumor stage by imaging modalities were included in the study. The patients underwent neoadjuvant chemoradiotherapy followed by surgery. Patients who underwent surgery without neoadjuvant chemoradiotherapy were excluded.

Pretreatment biopsy samples were assessed for histological type and differentiation. Level of tumor invasion and nodal status were assessed by imaging modalities. As per the standard treatment protocol, patients were treated with neoadjuvant chemoradiotherapy (50.4 Gy 28 fraction radiotherapy and oral capecitabine) followed by surgery (anterior resection/abdominoperineal resection).

Specimens were fixed in 10% neutral-buffered formalin and adequately sampled. Multiple sections were taken from the tumor bed area, margins (proximal, distal, and circumferential resected margins), and adjacent mucosa. Lymph nodes were sampled from perirectal fat.

Histological features such as cellularity, alteration in tumor cells, and changes in tumor bed area were assessed. The above findings were compared with the pretreatment biopsy. Pathological response (pCR) or TRG was classified according to the College of American Pathologists protocol for rectal carcinoma as: No viable cancer cells – TRG0 (complete response), single cells or small groups of cancer cells – TRG1 (near-complete response), residual cancer outgrown by fibrosis – TRG2 (partial response), minimal or no tumor kill showing extensive residual cancer – TRG3 (poor response).

The cytomorphologic features and stromal changes were analyzed. The cytoplasmic features included increased

cytoplasmic eosinophilia, vacuolation, and clear cell change. Nuclear changes assessed were nuclear pleomorphism, enlargement, shrinkage, pyknosis, and multinucleation. Other features evaluated were apoptosis, necrosis, mucin pools, and endocrine differentiation.

The inflammatory infiltrates in the stroma were analyzed, whether lymphoplasmacytic or mixed inflammatory infiltrate. The predominant stromal reaction was noted (fibrotic or fibroinflammatory). Features such as tumor deposits, lymphovascular tumor emboli, and perineural invasion were also assessed. The lymph nodes were evaluated for the presence of metastasis. The presence of treatment associated changes such as fibrosis, necrosis, and mucin pools was evaluated.

The posttreatment pathological staging of tumor and lymph nodes was done and compared with the pretreatment stage.

Statistical analysis

The continuous variables were represented by mean and standard deviation. The categorical variables were reported using frequency and relative proportion. The comparison between TRG and clinicopathologic variables was done using the Chi-square test or Fisher's exact test.

Results

Out of the ninety cases in the study, the peak incidence was seen in the age group 61–70 years with 35 cases, forming 38.9% of the total. Age range was from 15 to 83 years, with a mean age of 59.97 years. The minimum number of cases was in the age group <40 years with six cases, forming 6.7% of the total cases [Figure 1].

There was a slight male preponderance with 50 cases (55.6% of total). The male-to-female ratio was 1.25:1.

The pretreatment small biopsy samples were assessed for tumor type and differentiation. Out of the 90 cases of adenocarcinoma, 78 cases were moderately differentiated. Six cases were well-differentiated, and three cases were poorly differentiated. The remaining three cases were constituted by mucinous adenocarcinoma with signet ring

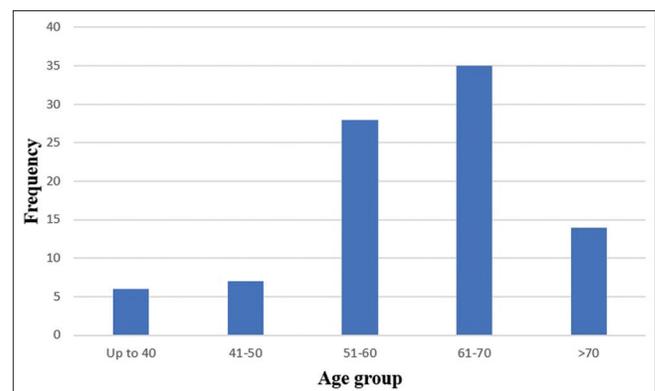


Figure 1: Age distribution (in years)

cells (two cases) and poorly differentiated adenocarcinoma with signet ring cells (one case).

Morphological changes following neoadjuvant therapy were divided into cytoplasmic and nuclear. Cytoplasmic changes observed were cytoplasmic eosinophilia (58.9% of the total cases), vacuolation (58.9%), and clear cell change (24.4%). Nuclear changes observed were nuclear enlargement (75.6%), nuclear shrinkage (36.7%), multinucleation (18.9%), pyknosis (25.6%), and pleomorphism (78.9%). Other changes noted were apoptosis, necrosis, extracellular mucin pools, and endocrine differentiation [Figure 2].

Analysis of the type of inflammatory infiltrate in the stroma was done [Figure 3]. Lymphoplasmacytic infiltrate was noted in 54.4% of cases, and a mixed infiltrate composed of eosinophils, neutrophils, lymphocytes, and histiocytes was seen in 33.3% of cases. Foreign body giant cells were noted in 12.2% of cases.

A predominant fibrotic stromal reaction was seen in 34.4% of cases and fibroinflammatory stromal reaction in 53.3% of cases.

Other changes assessed included lymphovascular invasion (24.4% cases, $n = 22$), perineural invasion (12.2% cases, $n = 11$), and tumor deposits (5.6% cases, $n = 5$).

The pretreatment T stage (cT) was assessed using radiologic investigations. Out of ninety cases, 67 cases were of cT3 stage (74.4% of total cases) [Table 1].

Following surgery, the pathologic assessment of the T stage was done. The majority of the posttreatment T stage (ypT) was constituted by ypT2 stage, accounting for 51.1% of total cases [Table 2].

The pretreatment and posttreatment T stages were compared [Table 3]. It was found that downstaging was

present in 61 out of 90 cases (68%). cT2 stage cases had no downstaging after treatment ($n = 4$). The majority of the cT3 stage cases were downstaged to ypT2 stage after therapy (47.8%). 52.6% of cT4 stage cases were downstaged to ypT2 stage.

Out of the ninety cases, 60% of the cases were in the pretreatment cN1 stage, 30% of cases were in cN2 stage, and 10% of cases were in cN0 stage [Table 4].

The posttreatment N stage (ypN) was assessed from the lymph nodes sampled from the surgical specimen. 73% of the cases were ypN0 stage, followed by ypN1 and ypN2 (17% and 10%, respectively) [Table 5].

The comparison of the pretreatment N stage (cN stage) and posttreatment N stage (ypN stage) of cases was done [Table 6]. 88.9% of cN0 stage cases had no N stage variation, 70% of cN1 stage cases had downstaging to ypN0, and 74.1% of cN2 stage cases had downstaging to ypN0.

After neoadjuvant therapy, nodal downstaging was noted in 61 cases, whereas 24 cases showed no change in nodal status. Five cases showed upstaging of nodal status.

TRG scoring was done in all the ninety cases according to the College of American Pathologists Protocol for Examination of Colorectal specimens (Modified Ryan score). Complete pCR (no residual tumor, TRG0) was present in 11 cases (12%) and no response or TRG3 was present in 20 cases (22%) [Table 7 and Figure 4].

The TRG score was compared with various clinicopathologic variables such as age, gender, histologic grade, and pretreatment T and N staging (cT and cN). There was no statistical significance in the comparison of TRG with these variables.

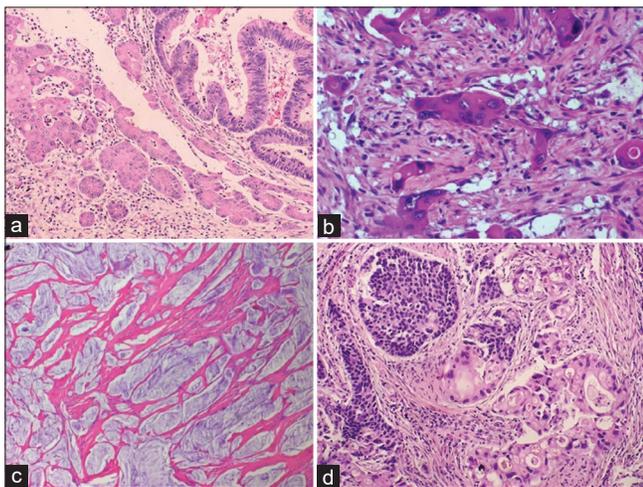


Figure 2: (a) Cytoplasmic eosinophilia and vacuolation in posttreatment rectal adenocarcinoma (H and E, $\times 200$), (b) tumour cells showing multinucleation and bizarre nuclei (H and E, $\times 400$), (c) mucin pools dissecting rectal wall (H and E, $\times 100$), (d) tumour with regressive changes and adjacent areas showing neuroendocrine differentiation (H and E, $\times 100$)

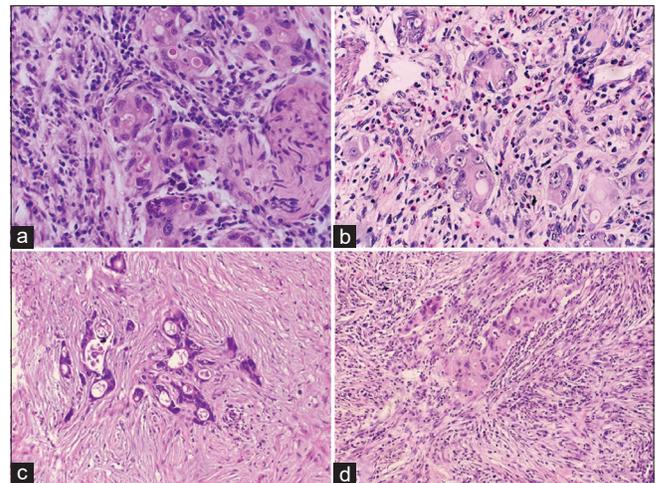


Figure 3: (a) Predominant lymphoplasmacytic infiltrate in the stroma (H and E, $\times 400$), (b) tumour with surrounding mixed inflammatory infiltrate (H and E, $\times 400$), (c) predominant fibrotic stromal reaction around tumour (H and E, $\times 200$), (d) predominant fibroinflammatory stromal response (H and E, $\times 200$)

The TRG score and T stage variation were compared in our study and were found to be statistically significant [$P = 0.001$, Table 8].

Out of the ninety cases, there was macroscopic perforation in two cases (2.2%). CRM was involved in four cases (4.4%) and involvement of distal margin was noted in one case (1.1%). The proximal margin was free in all the cases.

From the 90 cases, a total of 1030 lymph nodes were sampled. 95 lymph nodes showed viable tumor cells. The changes in lymph nodes in posttreatment cases were assessed including fibrosis, mucin, and necrosis [Figure 5]. Fibrosis was present in 55.6% of cases ($n = 50$), mucin was present in 11.1% of cases ($n = 10$), and necrosis was present in 26.7% of cases ($n = 24$).

Discussion

Neoadjuvant therapy applied before surgery can result in the pathological downstaging. There can be reduction in the level of invasion or even complete disappearance of tumor cells. The response of tumor cells to chemoradiation can be assessed by analyzing such downstaging effects at the histopathological level.

Comparison of pretreatment and posttreatment T stages

In the present study, 75% of the cases were in the pretreatment cT3 stage ($n = 67$). There were 19 cases in the pretreatment cT4 stage (21%). Four cases were in the pretreatment cT2 stage. These four cases received preoperative chemoradiotherapy based on the adverse pathology findings on biopsy and positive nodal status on imaging.

In 2016, Zhang *et al.* did an assessment of the AJCC Tumor Regression Grading System in locally advanced

rectal cancer. In their series, the majority of cases had a pretreatment cT4 stage (53%), 44% of cases had a cT3 stage, and 3% of cases had a cT2 stage.^[10] Of the 215 cases studied by Reggiani Bonetti *et al.*, the majority of cases had a cT3 stage (80%), 15% cases had a cT4 stage, and 5% cases had a cT2 stage, which is in par with the cT stage distribution of our study.^[11]

In the present study, T stage downstaging was noted in 68% of cases after neoadjuvant therapy. After therapy, 51.1% of the cases were in ypT2 stage and 31.1% were in ypT3. ypT0 stage was obtained in 11 cases (12% of total). In the study by Reggiani Bonetti *et al.*, T stage downstaging was observed in 57% of cases after therapy, 38% of cases had a ypT3 stage, and 16% of cases had a ypT0 stage.^[11] In their study, Zhang *et al.* noted that the majority of cases after treatment were in ypT3 stage (46%) and 27% cases had a ypT0 stage.^[10]

Comparison of pretreatment and posttreatment N stages

Out of the ninety cases in our study, 60% of cases (54 cases) had a pretreatment cN1 stage. After treatment, 73% of cases were in ypN0 stage (66 cases).

In their series, Reggiani Bonetti *et al.* observed 28% of cases had N stage downstaging, whereas 13% of cN0 cases had N stage upstaging.^[11] In the study by Zhang *et al.*, the majority of cases had a pretreatment cN+ stage (71%), while after neoadjuvant therapy majority of cases had ypN0 stage (76%).^[10]

In our study, N stage upstaging was noticed in 5 cases (5.5%), which could be due to the false-negative pretreatment N stage which can occur in clinical nodal staging in occasional cases. This was shown in studies by Kuo *et al.*, and they mainly attributed this false-negative staging to the limitation of imaging modalities in detecting small metastatic nodes.^[12]

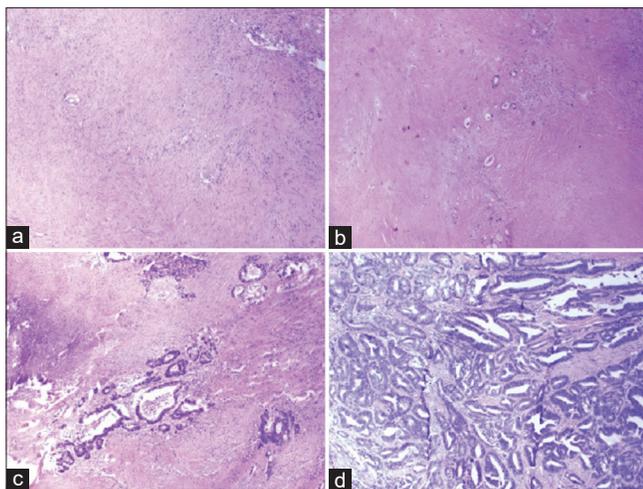


Figure 4: (a) Tumour regression grade score 0 or complete response (H and E, $\times 40$), (b) tumour regression grade score 1 or near-complete response (H and E, $\times 40$), (c) tumour regression grade score 2 or partial response (H and E, $\times 40$), (d) tumour regression grade score 3 or no response (H and E, $\times 40$)

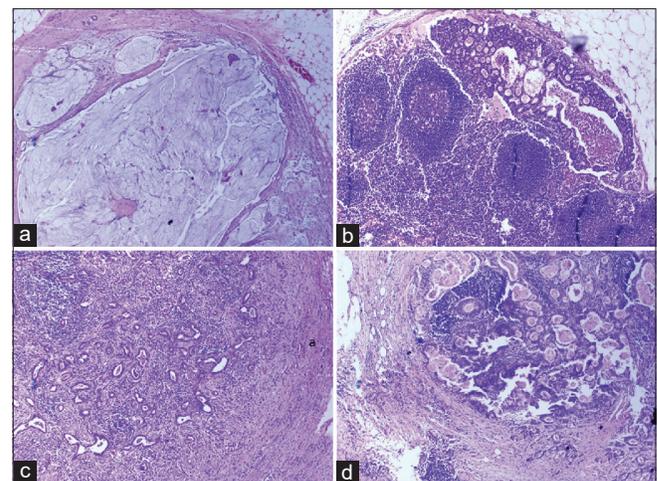


Figure 5: (a) Lymph node showing mucin pools with tiny foci of viable tumour cells (H and E, $\times 40$), (b) small lymph node showing metastasis (H and E, $\times 40$), (c and d) metastatic foci showing tumour cells with surrounding fibrosis (H and E, $\times 40$)

Table 1: Distribution of pretreatment T stage (cT)

Pretreatment T stage (cT)	Frequency, n (%)
cT2	4 (4.4)
cT3	67 (74.4)
cT4	19 (21.1)

Table 2: Distribution of posttreatment T stage (ypT)

Posttreatment T stage (ypT)	Frequency, n (%)
ypT0	11 (12.2)
ypT1	3 (3.3)
ypT2	46 (51.1)
ypT3	28 (31.1)
ypT4	2 (2.2)

Table 3: Comparison of pretreatment T stage (cT) and posttreatment T stage (ypT)

Pretreatment T stage (cT)	Posttreatment T stage (ypT)				
	ypT0	ypT1	ypT2	ypT3	ypT4
cT2, n (%)	0	0	4 (100.0)	0	0
cT3, n (%)	10 (14.9)	2 (3.0)	32 (47.8)	23 (34.3)	0
cT4, n (%)	1 (5.3)	1 (5.3)	10 (52.6)	5 (26.3)	2 (10.5)

Table 4: Distribution of pretreatment N stage (cN)

Pretreatment N stage (cN)	Frequency, n (%)
cN0	9 (10.0)
cN1	54 (60.0)
cN2	27 (30.0)

Table 5: Distribution of posttreatment N stage (ypN)

Posttreatment N stage (ypN)	Frequency, n (%)
ypN0	66 (73.3)
ypN1	15 (16.7)
ypN2	9 (10.0)

Table 6: Comparison of pretreatment N stage (cN) and posttreatment N stage (ypN)

Pretreatment N stage (cN)	Posttreatment N stage (ypN)		
	ypN0	ypN1	ypN2
cN0, n (%)	8 (88.9)	0	1 (11.1)
cN1, n (%)	38 (70.4)	12 (22.2)	4 (7.4)
cN2, n (%)	20 (74.1)	3 (11.1)	4 (14.8)

Table 7: Distribution of tumor regression grade score

TRG	Frequency, n (%)
0	11 (12.2)
1	29 (32.2)
2	30 (33.3)
3	20 (22.2)

TRG: Tumor regression grade

Assessment of tumor regression grade

In the present study, out of the 90 cases, complete pCR or TRG0 was seen in 12% of cases, TRG1 or near-complete

response in 32% of cases, TRG2 or partial response in 34% of cases, and TRG3 or no response in 22% of cases. Zhang *et al.* noted pathological complete response or TRG0 in 27% of cases, TRG1 in 19% of cases, TRG2 in 45% of cases, and TRG3 in 7% cases.^[10]

In the current study, no statistical significance was observed in the comparison of TRG with clinicopathologic variables such as age, gender, histologic grade, pretreatment T and N staging (cT and cN). Rödel *et al.* and Reggiani Bonetti *et al.* in their studies observed no statistical significance in the comparison of TRG with the above-mentioned variables, which is in par with the findings in our study.^[3,11]

Comparison of tumor regression grade with cT stage and T stage variation

Out of the four cases with pretreatment cT2 stage, none had TRG0, one case had TRG1, two cases had TRG2, and one case had TRG3.

Out of 67 cases with pretreatment cT3 stage, 14.9% of cases had TRG0, 31.3% of cases had TRG1, 32.8% of cases had TRG2, and 20.9% of cases had TRG3.

Out of 19 cases with pretreatment cT4 stage, 5.3% of cases had TRG0, 36.8% of cases had TRG1, 31.6% of cases had TRG2, and 26.3% cases had TRG3.

The TRG score and T stage variation were compared in our study and were found to be statistically significant ($P = 0.001$). Out of the 61 cases with T stage downstaging, 33 cases had significant regression (54%). A similar comparison between TRG and T stage variation was done by Reggiani Bonetti *et al.* and was found to be statistically significant ($P < 0.0001$). In their study, significant regression was seen in 42 cases with T stage downstaging.^[11]

Assessment of histopathological features of tumor cells and stroma

We analyzed the various histopathological changes in the tumor cells and stroma in the surgical specimens after chemoradiation. The presence of residual viable tumor cells in deep layers of rectal wall and perirectal tissue, even when the superficial tumor was completely regressed, points toward the importance of thorough and meticulous sampling. Residual malignant cells showed a variety of cytoplasmic and nuclear changes due to the effect of chemoradiation.

Cytoplasmic changes observed were increased cytoplasmic eosinophilia (58.9% of cases), vacuolation (58.9% of cases), and clear cell change (24.4% of cases).

Nuclear changes seen in posttreatment specimens included nuclear pleomorphism (78.9%), nuclear enlargement (75.6%), shrinkage (36.7%), multinucleation (18.9%), and pyknosis (25.6%).

Apoptosis was noted in 76 cases (84.4%), necrosis in 59 cases (65.6%), extracellular mucin pools in 34 cases (37.8%),

Table 8: Comparison of tumor regression grade score with stage and stage variation

	TRG				P
	TRG0	TRG1	TRG2	TRG3	
Pretreatment T stage (cT), n (%)					
cT2	0	1 (25.0)	2 (50.0)	1 (25.0)	0.94
cT3	10 (14.9)	21 (31.3)	22 (32.8)	14 (20.9)	
cT4	1 (5.3)	7 (36.8)	6 (31.6)	5 (26.3)	
Pretreatment N stage (cN), n (%)					
cN0	0	4 (44.4)	3 (33.3)	2 (22.2)	0.747
cN+	11 (13.6)	25 (30.9)	27 (33.3)	18 (22.2)	
T stage variation, n (%)					
None	0	7 (24)	14 (48)	8 (28)	0.001
Downstaging	11 (18)	22 (36)	16 (26)	12 (20)	
N stage variation, n (%)					
None	0	9 (37.5)	10 (41.7)	5 (20.8)	0.235
Upstaging	0	1 (20.0)	2 (40.0)	2 (40.0)	
Downstaging	11 (18.0)	19 (31.1)	18 (29.5)	13 (21.3)	

TRG: Tumor regression grade

and endocrine differentiation in 4 cases (4.4%). The stromal reaction was predominantly fibrotic in 34.4% of cases and fibroinflammatory in 53.3% of cases.

In our study, the most striking features were increased cytoplasmic eosinophilia and marked nuclear pleomorphism. Studies by Shia *et al.* which assessed the patterns seen in residual rectal cancer treated with preoperative chemoradiation observed marked nuclear atypia and cytoplasmic eosinophilia as the most common findings (29.8% of the cases).^[13]

Similar findings were seen in the study by O'Neil and Damjanov in which the tumor cells showed increased eosinophilia or oncocytic differentiation. This can be due to dense packing of mitochondria which can be demonstrated by immunohistochemical staining. Some cells even showed a squamoid appearance. Other changes noted in their study were nuclear hyperchromasia, nuclear atypia, and rarity of mitotic figures.^[14]

Another important feature observed in our study was the presence of mucin pools (37.8% of cases, $n = 34$). In few cases, extensive sampling was needed to detect scattered viable tumor cells within dissecting pools of mucin. The study by Shia *et al.* noted mucin pools in 21.2% of the cases in their series.^[13]

Conclusion

A wide range of histomorphological changes occur in carcinoma rectum after neoadjuvant therapy. Pathologist plays a major role in assessing the tumor downstaging and scoring of TRG. This helps clinician to know about the response of tumor to chemoradiation which is important in further treatment and prognostication of patients.

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Conflicts of interest

There are no conflicts of interest.

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