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Histopatholgical Spectrum of Changes in Post Neoadjuvant Chemotherapy Treated Rectal Carcinoma: Single Center Study

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Abstract

Background: Rectal carcinomas are one of ten leading causes of death in India. There is gradual rise in their incidence which has prompted the greater research into their behavior. Now world wide all locally advanced rectal carcinomas are treated with preoperative chemo or radiotherapy. *Aims and objectives:* To observe the post chemotherapy histopathological changes in pretreated rectal carcinoma. *Material and Methods:* the present was both prospective and retrospective study conducted at Department of Pathology, on the 36 cases who had received pre-operatives NACT (Neoadjuvant chemotherapy). *Results:* Out of these 36 cases none showed complete remission of tumor. Both cytoplasmic and nuclear changes were observed and documented. The most common findings was cytoplasmic eosinophilia (98% of cases) and stromal dense fibrosis was seen in TRG3 tumors. The residual tumors were classified as TRG 0,1,2,3 as according to Dworak, Rodel and AJCC TNM system. The Rodel system was better as it was more objective and reducing the subjective bias in resected specimens. Conclusion -Hence the pathologist awareness towards the assessment of therapeutic response including the evaluation of residual tumor burden or tumor response grading (TRG) and cytomorphology including the stromal changes has become the hour of need.

Keywords: Rectal Carcinoma; Neoadjuvant Chemotherapy; Post Chemotherapy Changes; Tumor Regression Grading (TRG).

Introduction

Neoadjuvant treatment has become the standard of care for locally advanced rectal tumors. Preoperative radiation (RT) and/ or chemotherapy (CT) have been shown to improve outcome in patients with locally advanced rectal tumors. An increasing number of patients with stage IV rectal cancers are also being managed with neoadjuvant chemotherapy, followed by resection when down staging allows. In addition, 15-25% of patients with rectal cancer have liver metastases at the time of presentation and the 5-year survival can be improved if the liver metastases are reduced in size by chemo therapy, thereby allowing initially unresectable patients to become resectable [1].

It is for these reasons that the pathologists are increasingly encountering treated rectal carcinoma and the assessment of the therapeutic response and the evaluation of residual tumor burden are important. Various studies are also shown that the histological response to therapy or tumor response grade (TRG), correlate with survival of the patients. In this study we try to observe the cytoplasmic and stromal histopathological changes seen in post-chemotherapy treated rectal carcinoma [2].

Materials and Methods

The present retrospective study was carried out in Department of Pathology, MS Ramaiah Medical College. All the cases between years January 2013 to May 2015 were collected. Out of all the 45 cases rectal

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carcinoma, 36 cases had received NACT pre operative. An inclusion criterion was patients with an established histopathological diagnosis of rectal adenocarcinoma on biopsy and who had received at least two cycles of neoadjuvant chemotherapy. An exclusion criterion was other cases with one cycle of chemotherapy or without biopsy diagnosis before the surgery and squamous cell carcinoma. All these patients were given 45 grey external beam radiotherapy as boost along with 5 FU (Fluorouracil) and underwent surgical resection six weeks post NACT induction. All these cases were in clinical staging T2 level during the induction.

The preoperative biopsy was fixed in 10% neutral buffer formalin and processed like regular formalin fixed paraffin embedded sections cut at 4 um thick. Post surgical resection specimens were opened and fixed overnight in buffered formalin. Grossing was carried out according to a standard in-house grossing protocol that included blocking of the complete tumor/ residual lesion [3]. Distances between the tumor circumferential margins (CRM) and resected surgical margins were measured. Separate paraffin blocks representing extramural residual tumor and the inked circumferential margin were taken in all cases. Mesorectal resection was assessed as complete or partial for rectal carcinomas. The mesorectal fat was dissected carefully for maximum harvest of lymph nodes. Perineural invasion and lymphovascular invasion was specifically looked for and were noted. Surgical pathology reports carried staged the tumors according to the TNM system and also the residual tumor was graded according to three TRGs i.e Rodel, AJCC TNM and Dworak tumor regression grading (TRG).

All tumors were reviewed by two pathologists (RK, CW) to determine tumor regression grade and staging of the tumors according to the seventh edition of the AJCC. TRGs of the primary tumors were also re-assessed using the Dworak, Rodel, and AJCC TRG systems. Since the Mandard and Dworak TRG systems have similar grading criteria, with the only difference being the reverse order of TRG number tumor assessment using the Mandard TRG system was not performed like Kim et al study [4].

Statistical Analysis

Frequency tables, Pearson Chi-Square and likelihood ratio was utilized for this study.

Results

In this study the age range observed was 28-80 years, the youngest being 28 years and oldest was 80 years. Majority of the cases were between 61-70 years closely followed by 51-60 years. The males were predominant with male to female ratio 2:1. The location of tumor was middle rectum in 55% of cases and lower or distal rectum in 38% cases. For all these resected tumor was assessed for residual tumor burden using TRG grading of Dworak, Rodel and AJCC TNM grading (Table 1). Histopathological sub classification showed majority of the cases were moderately differentiated adenocarcinoma accounting for 77% of all cases (28 out of 36). The mucinous carcinoma was seen in rest of others. The AJCC grading showed majority of the cases belong to primary tumor PT3 and regional lymphnode status was no lymphnode metastasis (N0). The residual tumor staging with TNM staging showed the primary tumor was in T3, tumor invades the muscularis propria into perirectal tissue (26 out of 36 cases). The rest of the cases were in T2 stage, tumor invades muscularis propria. The average lymphnode retrieval was 8.6. The lymphnododе metastasis was seen in 14 cases and was in N1 stage. Lymphnode was N0 in majority of cases (22 out of 36 cases). All these cases had no evidence of distant metastasis on PET scan (M0). The stage grouping was stage 1 (55.6%) and rest in stage II (44.4%).The distance from circumferential margin was graded as <1mm, 1-3 mm and >3mm (Table 2). The majority of the cases were 1-3 mm from circumferential resected margins (77.8%). The residual tumor showed evidence of lymphatic invasion in 13(36.1%) cases and vascular invasion in 16 (44.1%) cases respectively.

The stromal hyalinization was classified as loose, hypocellular and dense. The loose stroma indicates loose myxoid stroma with no keloid like areas. Hypocellular areas indicate keloid like scarring with occasional stromal fibroblasts. Dense stroma indicates irregular fascicles of collagen with numerous reactive fibroblasts. The loose, hypocellular and dense fibrosis was seen in 04, 11 and 22 cases respectively. The grade TRG3 was associated with dense stromal hyalinization.

The TRG grading of Dworak, Rodel and AJCC TNM were used in this study. All the cases were assessed for these three grading systems. None of the cases showed complete regression post neoadjuvant therapy. A 50 % of cases showed >50 % regression of tumor burden (TRG3), in according to AJCC it is TRG2 (Moderate response). There was the young patient included in our study she was diagnose to have mucinous adenocarcinoma, responded well with neoadjuvant therapy with TRG3. The tumors with TRG1 (Figure1) had 10 each case in stage I and II each. TRG2 response was seen in stage I and II of 5 and 1 case respectively. The good response TRG3 was seen

in 20, 30 cases belonging to stage I and II respectively. The lower rectal tumors had responded better to neoadjuvant chemotherapy TRG3 (figure 2) (70%) however the p value was not significant.(p >0.001). These patients are being followed-up for local recurrence and distant metastasis every three months for the first two postoperative years, then every 6-12 months thereafter. Follow-up included physical examinations; serum carcinoembryonic levels (CEA), chest X-ray and abdominal ultrasound or computed tomography (CT).

Table 1: Various tumor regression grading (TRG) used in this study

TRG Grade	Dworak	Rodel	TNM	No. of cases
0	No regression	No regression	No viable cancer cells	05(13%)
1	Predominantly tumor with significant fibrosis/ vascularity	Regression of <25 % of tumor mass	Minimal or no tumor cells killed (TRG 3: poor response)	04(11%)
2	Predominantly fibrosis with scattered tumor cells	Regression of 25-50 % of tumor mass	Residual cancer outgrown by fibrosis with few tumor cells cells (TRG 2: minimal response)	10(27%)
3	Only scattered tumor cells in fibrosis with/without acellular mucin pools.	Regression of >50 % of tumor	Single or small groups (TRG 1: moderate response)	18(50%)
4	No vital tumors detected	Complete regression	--------	0(0%)

Table 2: Distance from circumferential resected margin (CRM)

CRM	No. of cases
<1mm	6
1-3mm	28
>3mm	2

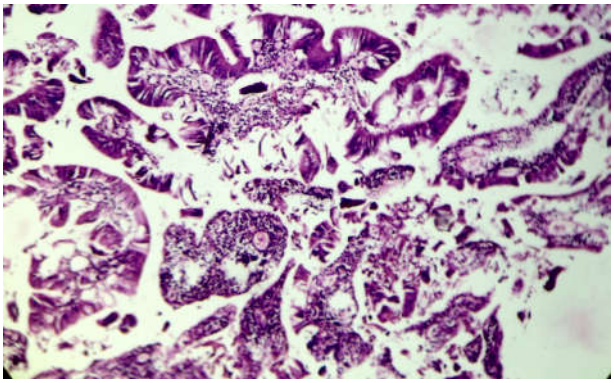

Fig. 1: TRG 1- Predominantly tumor with minimal fibrosis, cells have increased eosinophilia. (H& E stain, x 40)

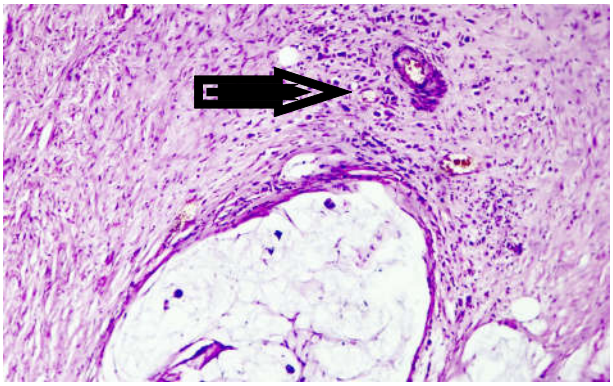

Fig. 2: TRG 3 – Predominantly fibrosis with scattered tumor cells (in arrow) the stroma shows dense fibrosis. (H& E stain, x 40)

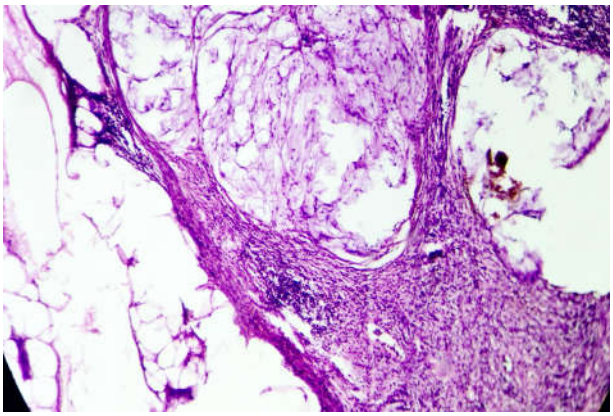

Fig. 3: Acellular mucin pools in perirectal tissue which was considered as one of the response to NAC. The stroma shows moderate amount of inflammatory response. (H& E stain, x 40)

Discussion

In the present study, a large spectrum of histopathological changes was observed in post NACT (Neo Adjuvant Chemotherapy) specimens. The effects have been divided into two, as pathologic changes in residual tumor cells and changes in the stroma.The tumor cells showed nuclear enlargement, nuclear shrinkage, necrosis, vacuolation of nucleus, cytoplasm, pyknotic nuclei and degenerative changes which are also been described in few studies in the literature [2]. The tumor cells showed cytoplasmic eosinophilia is a well-established chemoradiation related change and has been well documented in colorectum was seen in 98% of cases. This increased

eosinophilia in the cells is regarded as one of a group of changes serving as the diagnostic indication of irreversible cellular injury, which occurs in unison with one or more of the nuclear features: nuclear as described above. As such, it can be presumed that the eosinophilia is a degenerative feature directly induced by chemoradiation. The oncocyte-like change was seen in 16% of cases, which differs from the cytoplasmic eosinophilia in that they are larger with more voluminous [5]. In the stroma, fibrosis, collagenization, hyalinization, microcalcification and neovascularization were observed. In our study, we also saw stromal fibrinoid necrosis and acellular mucin pool. With all these changes the stroma in addition also shows moderate amount of inflammatory cells rich in lymphocytes and plasma cells. Focally hemosiderin laden macrophages were noted. The stromal hyalinization was classified as loose, hypocellular and dense. The loose stroma indicates loose myxoid stroma with no keloid like areas. Hypocellular area indicates keloid like scarring with occasional stromal fibroblasts. Dense stroma indicates irregular fascicles of collagen with numerous reactive fibroblasts (Figure 3).

In the present study majority of rectal cancers are adenocarcinomas of no special type like Hav M study [6]. According to their article histological subtypes, has no stage-independent prognostic significance. The exceptions include the nongland forming tumors such as signet-ring cell carcinoma, small- cell carcinoma, and undifferentiated carcinoma, which are prognostically unfavorable. The medullary carcinoma is prognostically favorable. A review of literature suggests that mucinous adenocarcinoma has adverse prognostic factor, however larger studies didn't confirm mucinous histology to be a stage-independent predictor of poor outcome [6]. Even in our study eight out of 36(22%) cases were mucinous carcinoma. Majority of this mucinous carcinoma showed TRG2 response. On the other hand, mucinous carcinoma tends to be prognostically favorable when associated with microsatellite instability (MSI) [6]. There were no cases of signet ring cell carcinoma in the present study.

The three TRG grading used have different advantages and disadvantages. The major limitation of all the current TRG systems is that grading is imprecise and the criteria, particularly for near complete regression, may be very subjective. For example, Dworak TRG 3 was scattered few tumor cells in fibrotic tissue with or without acellular mucin and TNM grade 3 is also similar to Dworak, however the Rodel criteria is more objective as >50% tumor regression is considered as TRG3 [4].

Another disadvantage of these TRG, endpoint of pathologic complete response has been defined as ypT0N0, however, current TRG systems evaluate only the primary tumor. Even though regional LN status after NAC is the most important prognostic factor, current TRG systems do not consider regional LN metastasis. Thus, the TRG systems may be inaccurate in predicting prognosis, particularly in ypT0N+ patients. Even though ypT0N+ patients have residual tumors, they would be classified as having achieved complete response using the current TRG systems. RFS and OS ates were significantly lower in ypT0N+ patients than in ypT0N0 patients [4].

Despite multiple trials of various TRG systems, none was found to be a better predictor of prognosis than yp Stage. Pathologic staging after neoadjuvant therapy is more objective and more predictive of prognosis and therapeutic response. Futures studies may up with ideal TRG system or use of multiple TRG for better predictor of outcome in theses set of patients. Thus the pathologic evaluation of surgically resected specimens after neoadjuvant therapy is an extremely important for surgical pathologists.

Drawbacks – the major drawback is the single center study and numbers of cases are comparatively less. But this study is conducted as pilot study, as many future studies are expected to come from this study.

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