Histologic Analysis of the Irradiated Anal Sphincter

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PURPOSE: There is accumulating evidence, both quantitative and qualitative, that pelvic irradiation adversely affects anorectal function. However, histologic evidence of sphincter injury has not been demonstrated. This study was designed to assess the histologic consequences of collagen deposition and nerve alteration in the internal anal sphincters of rectal cancer patients who underwent abdominoperineal resection after adjuvant chemoradiation therapy and to correlate the degree of histologic changes with the time interval between chemoradiotherapy and abdominoperineal resection. METHODS: Anal canal specimens were prospectively collected in patients undergoing abdominoperineal resection. Representative slides were cut transversely at the level of the dentate line. Using trichrome and S-100 protein staining, a single pathologist blinded to the patients’ treatment assessed collagen deposition and nerve fiber densities in the internal anal sphincter, respectively. RESULTS: Twelve patients received radiation for rectal cancer (chemoradiotherapy group) and six were treated by surgery alone, including four patients with rectal cancer (1 leiomyosarcoma) and two with Crohn’s disease (control group). There was a trend toward increased fibrosis (replacement of >10 percent of normal structures by collagen) and nerve density in the chemoradiotherapy group compared with the control group ($P = 0.08$ and $P = 0.05$, respectively). Nerve density significantly increased as chemoradiotherapy to abdominoperineal resection interval increased ($P = 0.04$). CONCLUSIONS: Pelvic irradiation results in damage to the myenteric plexus of the internal anal sphincter of patients with rectal cancer; these alterations seem to be time-dependent. A trend toward increased collagen deposition also was observed. Together, these results provide a morphologic basis, which concurs to previously described physiologic and clinical alterations in the anal sphincter of patients irradiated for rectal cancer. [Key words: Fibrosis; Nerve density; Anal sphincter; Rectal cancer; Radiation; Abdominoperineal resection; Crohn’s disease; Proctectomy] da Silva GM, Berho M, Wexner SD, Efron J, Weiss EG, Nogueras JJ, Vernava AM III, Connor JT, Gervaz P. Histologic analysis of the irradiated anal sphincter. Dis Colon Rectum 2003;46:1492–1497.

It is generally recognized that adjuvant radiotherapy is a major determinant of poor bowel function after restorative proctectomy for rectal cancer.1,2 After this type of procedure, a critical aspect of outcome is the preservation of a functioning anal sphincter. However, recent data have shown that the internal anal sphincter is sensitive to ionizing radiation,3,4 which may subsequently affect both quality of life and continence in patients with colorectal anastomosis or low anterior resection.5,6

The morphologic alterations involved in postradiotherapy anorectal dysfunction remain virtually unknown.7 In normal tissues, the most prominent features of radiation pathology are progressive fibrosis that antedates vascular lesions, seen at the late phase reaction.8,9 Typically radiation-induced fibrosis is not homogenous; some areas have very dense collagen, whereas others have only a few fibrous bands.10 On the molecular level, it is estimated that the increased deposition of collagen results from the release of fibrogenic cytokines from the transforming growth factor (TGF-β) family by atypical fibroblasts.11,12 Nerve abnormalities are another well-known feature of radiation proctitis. In the irradiated rectum of patients treated for prostate cancer, histologic examination has shown marked damage of the myenteric plexus associated with smooth muscle hypertrophy.13,14

Finally, it is crucial to recognize that, morphologically and functionally, radiation-induced damage can greatly vary with time. Therefore, this study was designed to investigate the histologic features of radiation-induced damage to the structures of the anal

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canal, identify the cellular components more likely to be responsible for anorectal dysfunction, and perform a time-dependent evaluation of these alterations.

PATIENTS AND METHODS

From January 2000 to March 2001, the surgical specimens of patients who underwent abdominoperineal resection (APR) were prospectively collected at the time of surgery. Patients who underwent chemoradiation therapy (CRT) before APR comprised the study group (CRT group), whereas patients who did not receive preoperative CRT were used as controls (control group).

Radiation and Chemotherapy

The adjuvant CRT protocol consisted of preoperative administration of 45 Gy dose in 25 fractions administered during a five-week to six-week period (1.8 Gy/fraction), followed by a boost to the primary tumor bed to a total dose of 50.4 Gy for patients with Stage II or III rectal cancer. Typically, a four-field technique was used; the superior border of the tumor bed field is to the L5/S1 junction and the lower limit is the inferior border of the ischial tuberosity. Thus, the anal canal was included in all cases within the field of irradiation. The lateral borders extend 1.5 cm beyond the bony pelvis, and the posterior border encompasses the entire sacrum. Concurrent chemotherapy included a continuous infusion of 5-fluorouracil (5-FU) at a dose of 250 mg/m² per day.

Tissue Processing

After gross pathologic examination, one-third of the circumference of the anal canal was excised en block and fixed in 10 percent formalin for 12 hours. Serial transverse sections at the level of the internal anal sphincter were then obtained. A sample of the internal anal sphincter was subsequently embedded in paraffin, cut at 5 μm, and stained with hematoxylin and eosin, Masson’s trichrome, and S100 immunoperoxidase stain.

Histologic Analysis

One pathologist (M.B), blinded to the patient’s treatment, assessed collagen deposition and nerve fiber densities. Both analyses were performed through a standard light microscope, and the maximum number of fields was evaluated for each slide, on low-power magnification (×1,000).

Fibrosis Analysis

The slides stained with the Masson’s trichrome were used to evaluate the degree of fibrous replacement. An arbitrary score from 0 to III was designed to semiquantitatively assess the fibrous replacement as follows: Grade 0, up to 10 percent replacement; Grade I, 10 to 30 percent replacement; Grade II, 30 to 50 percent replacement; Grade III, >50 percent replacement. The maximum number of fields per slide was evaluated in all the slides available for each case. The final score for each case was reached by calculating the average percentage of fibrous replacement in all slides for each case.

Nerve Analysis

Semiquantitative analysis of the nerve bundles at the level of the sphincter was conducted with an S100 immunoperoxidase stain to highlight these structures. Nerve density was expressed as the mean value of the number of bundle nerves visualized divided by the number of fields evaluated (×1,000).

Statistical Analysis

Statistical analysis of the degree of fibrosis and nerve changes between the two groups was performed by using Wilcoxon’s test, and time correlation between irradiation completion and surgery performing a linear regression using the ANOVA test. For all analysis, \( P < 0.05 \) was considered significant.

RESULTS

Patients

A total of 18 patients (8 males; mean age, 60.9 (range, 39–75) years) who underwent APR were included in this study. Twelve patients who had rectal cancer and received adjuvant CRT formed the CRT group, and six nonirradiated patients composed the control group, including two patients with Crohn’s disease and four with rectal cancer, one of whom had leiomyosarcoma. One patient with Crohn’s disease had a history of colon cancer and had undergone a subtotal colectomy and ileorectal anastomosis. Endoscopic examination revealed ileitis and a concomitant highly dysplastic lesion in the rectum, closed to the...