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## Karyometric analysis of radiation-induced colitis

**KEYWORDS:** Karyometry; Colitis; Radiation Injuries; Colorectal Neoplasms; Neoplasms, Radiation-Induced

### ABSTRACT

Even though the colon and rectum are relatively radioresistant, they exhibit a high incidence of radiation damage due to both the large radiation doses used to treat tumors arising in the pelvic area and the fixed position of the sigmoid colon. The aim of this study was to investigate histological and karyometrical changes of colonic mucosa after pelvic irradiation for other malignant or benign lesions. This study included 51 men and 45 women aged from 28 to 79 years. Endoscopic biopsies of radiation-induced colitis (10), chronic colitis (n=25), colonic carcinoma (n=42), and mucosa surrounding carcinoma (n=42) were analyzed. The mean volume-weighted nuclear volume of epithelial cells was estimated by point-sampled nuclear intercept method, by original test system, using objective 100x and total magnification of 1200x (3). Nuclear area, volume, perimeter, mean chord, circularity, and integrated optical density (IOD) were estimated by image analyzer Lucia M 3.52 ab. The difference in nuclear size between chronic and radiation colitis was not statistically significant, but in radiation colitis the nuclei were significantly more elongated and dense ( $p < 0.05$ ). The significantly highest values of integrated optical density (IOD) were found in radiation colitis. Our results suggest that early radiation damage is more elongated nuclear shape of epithelial cells of colonic mucosa, with increased integrated optical density (IOD). This early changes are probably not connected to malignant alteration of colonic mucosa.

### INTRODUCTION

Even though the colon and rectum are relatively radioresistant, they exhibit a high incidence of radiation damage due to both the large radiation doses used to treat tumors arising in the pelvic area and the fixed position of the sigmoid colon. About 75% of patients develop clinical symptoms, usually by the middle of the second week of therapy. Mucosal biopsies are generally performed in cases of suspected radiation injury in order to confirm the presence of the colitis or exclude the presence of recurrent tumor, the reemergence of a new tumor, or the presence of an opportunistic infection. Acute effects consist of cryptitis, prominent submucosal edema, variable ulceration, inflammatory polyps, and sometimes ischemic changes. More chronic features include

architectural distortion with variable atrophy, vascular ectasia, and thickening of the collagen layer beneath the surface and crypt epithelium. The vascular lesions are typically random and focal in nature, and consist of variable degrees of myointimal proliferations, including collections of macrophages within the intima. Foam cells deposited within the vessels are fairly characteristic of radiation damage (1). As a result of chromosome fragmentation after irradiation, the micronuclei can be found (2). The aim of this study was to investigate histological and karyometrical changes of colonic mucosa after pelvic irradiation for other malignant or benign lesions.

### MATERIAL AND METHODS

This study included 51 men and 45 women aged from 28 to 79 years. Endoscopic biopsies of radiation-induced colitis (10), chronic colitis (n=25), colonic carcinoma (n=42), and mucosa surrounding carcinoma (n=42) were analyzed. After standard fixation and paraffin embedding, 4 (m thick sections were stained with hematoxylin and eosin. The mean volume-weighted nuclear volume of epithelial cells was estimated by point-sampled nuclear intercept method, by original test system, using objective 100x and total magnification of 1200x (3). Nuclear area, volume, perimeter, mean chord, circularity, and integrated optical density (IOD) were estimated by image analyzer Lucia M 3.52 ab (Nikon, Tokyo, Japan), using objective 40 (N.A. = 0.65), after manual editing of binary image. In each case a hundred nuclei were measured. The significance of differences between groups was estimated by Mann-Whitney test.

### RESULTS AND DISCUSSION

The nuclei were significantly larger in cancer cells than in other groups ( $p < 0.01$ , Table 1). The difference in nuclear size between chronic and radiation colitis was not statistically significant, but in radiation colitis the nuclei were significantly more elongated and dense ( $p < 0.05$ ). The significantly highest values of IOD were found in radiation colitis.

**Table 1.** Karyometric variables in different pathological states of colonic mucosa (mean (SD))

	Area ( $\mu\text{m}^2$ )	Volume ( $\mu\text{m}^3$ )	Perimeter ( $\mu\text{m}$ )	Mean Chord ( $\mu\text{m}$ )	Circularity	Integrated Optical Density (a.u.)
Radiation Colitis	22.56	133.89	19.34	3.92	0.80	388.33
Chronic Colitis	19.77	79.02	16.9	3.63	0.86	254.62
Carcinoma	34.22	182.28	22.42	4.48	0.81	356.67
Mucosa Surrounding Carcinoma	24.23	158.73	19.22	3.86	0.81	281.36

### CONCLUSION

Carcinomas and sarcomas are known to develop after radiation therapy. Radiation-associated rectal cancer has a tendency to be diagnosed in the advanced stage and to have a poor prognosis. The average interval between irradiation and diagnosis of the rectal cancer was 15.2 years, the range being from one year, two months to 33 years (4). Our results suggest that early radiation damage is more elongated nuclear shape of epithelial cells of colonic mucosa, with increased IOD. This early changes are probably not connected to malignant alteration of colonic mucosa.

### REFERENCES

1. Fenoglio-Preiser CM, Stemmermann GN, Lantz PE, Listrom MB, Rilke FO. Gastrointestinal pathology, 2nd ed. Philadelphia: Lippincott-Raven; 1999.
2. Tofilon PJ, Vines CM, Bill CA. Enhancement of radiation-induced DNA double-strand breaks and micronuclei in human colon carcinoma cells by N-methylformamide. *Radiat Res* 1989;119:166-75.
3. Mihailovic D, Djordjevic B, Mihailovic V. Nuclear volume in type I gastric intestinal metaplasia. *Analyt Quant Cytol Histol* 1999; 21(2):143-6.
4. Martins AA, Sternberg SS, Attiyyeh FF. Radiation-induced carcinoma of the rectum. *Dis Colon Rectum* 1980;23:572-5.

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