MORPHOLOGY AND PATHOMORPHOLOGY

Morphology of Pararectal Lymph Nodes in Rectal Cancer after Neoadjuvant Therapy

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 2, pp. 213-218, February, 2010
Original article submitted December 21, 2009

Remodeling and cytograms of pararectal lymph nodes were studied in patients with rectal cancer after neoadjuvant therapy by different protocols. Radiotherapy and its combination with chemotherapy lead to an increase in the volume of connective tissue components and significant reduction of the volume density of lymphoid follicles without germinative centers. The counts of dividing cells, immunoblasts, and plasmoblasts in various compartments of the pararectal lymph nodes changed more significantly after radiotherapy, while changes in the count of monocytes and neutrophils were more pronounced after cytostatic therapy combined with exposure to ionizing radiation. These differences can be explained by the systemic toxic effect of chemotherapeutic drugs and primarily local cytotoxic effects of radiotherapy manifesting largely at the site of exposure.

Key Words: *rectal cancer; lymph nodes; chemotherapy; radiotherapy; morphology and morphometry*

Study of structural reorganization of the lymph nodes adjacent to the tumor during antitumor therapy is essential for the development of effective approaches to the prevention of postoperative complications in patients with neoplastic processes. Structural and functional changes in regional lymph nodes during neoadjuvant therapy depend on the type and intensity of antitumor therapy. Chemotherapy and ionizing radiation cause both similar and different changes in the lymph node structure and have different effects on organs and tissues distant from the focus.

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Radiation exposure of the lymph nodes leads to reduction of the lymphoid tissue volume and its replacement with fibrous tissue [9,12], which can be associated with the loss of the filtration function and reduction of the functional capacity. Interphase death of B and T cells resulting from radiation injury impairs local immunity and can be stimulated by mitogens or antigens [4]. Antitumor drugs usually produce systemic toxicity, which limits their use. In addition, drugs used for preoperative chemotherapy (*e.g.* 5-fluorouracyl, 5-FU) can have different effects on the expression of pro- and antiapoptotic proteins and some markers of proliferation in tumor cells and in metastatic cells in the lymph nodes [6-8]. This different sensitivity of cells populations of the tumor and metastases can be responsible for inefficiency of treatment.

Despite the importance of evaluations of the pattern and severity of cytotoxic changes in the lymph

0007-4888/10/1492-0250

nodes during neoadjuvant antitumor therapy according to different protocols, there are virtually no reports about comparative studies of changes in the lymphatic organs after drug therapy and radiation exposure.

We compared structural reorganization of the pararectal lymph nodes in patients with rectal cancer (RC) after neoadjuvant therapy by different protocols.

MATERIALS AND METHODS

The morphology of pararectal lymph nodes of RC patients was studied. The lymph nodes were removed with diagnostic purpose and during radical surgery in 146 patients with stage II RC; according to TNM classification, all patients had $T_2N_0M_0$ or $T_3N_0M_0$. No tumor metastases were detected in the examined lymph nodes. The patients were divided into 3 groups in accordance with the treatment they received: no neoadjuvant therapy (83 patients, 47 men and 36 women, mean age 59.3±11.2 years); after radiotherapy (52 patients, 37 men and 15 women, mean age 57.1±12.1 years); after chemotherapy in combination with radiation exposure (11 patients, 4 men and 7 women, mean age 54.1±15.9 years). All patients received complete symptomatic therapy and correction of concomitant diseases.

Neoadjuvant chemotherapy without combination with radiotherapy for RC is now not carried out in Russia. Preoperative radiotherapy was carried out in the classical fractionation mode: a single dose of 2 Gy 5 times a week (summary dose 40-44 Gy). Neoadjuvant chemoradiotherapy was carried out in the dynamic or classical fractionation modes. Dynamic fractionation mode: 5-FU (500 mg on days 1-5), cisplatine (30 mg on days 8-10), radiotherapy (single dose 4 Gy on days 8-10, then 1 Gy twice daily, 5 days a week to a total dose of 39-40 Gy). Classical fractionation mode: radiotherapy (single dose 2 Gy 5 days a week to a total dose of 40-44 Gy), 5-FU (450 mg/m^2/day 1 h before radiation exposure during the entire course of radiotherapy). The patients were operated on after cessation of radiation reaction within 1 week after the end of neoadjuvant therapy.

Pararectal lymph nodes were fixed in 10% neutral formalin during at least 24 h, processed routinely, and embedded in paraffin. The sections were stained with hematoxylin and eosin, after van Gieson and Romanowskii, and examined under an Axioimager Z1 light microscope (Carl Zeiss) at magnification ×1200. Structural reorganization of pararectal lymph nodes and cytograms of different compartments of the organs were studied using a square test system applied on the monitor with the image obtained by microscope digital videocamera. The final area of the test square for studies of the lymph node structure (×4 objective) was 14,400 μ^2 (120 μ side of the square), area for cytogram evaluation (×40 objective) 144 μ^2 (12 μ side of the square) [1]. Three to five measurements were made for each section. The results were statistically processed using Student's test, the differences between the means were significant at $p \leq 0.05$.

RESULTS

Pararectal lymph nodes of all RC patients receiving no neoadjuvant therapy had no metastases and were hypertrophic. They had a wide paracortical zone and numerous large follicles without germinative centers and with multiplication centers (Fig. 1, *a*, *b*). Hypertrophic follicles were often seen in the cortical matter, forming several layers. These changes were described as "cancer-associated hypertrophy of lymph nodes" [2,14].

Malignant tumor develops against the background of a chronic inflammatory process induced by the tumor, its infiltrative growth impairing the adjacent tissues. Oxygenation disorders in tumor cells often lead to partial necrosis of the tumor. As a result of these events, great amounts of cellular and tissue detritus are released into regional lymph nodes. Many substances from dead cells and tissues possess antigenic activity and stimulate division and differentiation of B cells in lymph nodes. After antigenic stimulation, the cortical plateau B cells start intense division, and follicles of all types appear in this zone.

After radiation exposure and cytostatic treatment combined with radiotherapy, the area of connective tissue components in the pararectal lymph nodes increased, particularly in the cortical matter. The relative volume of the connective tissue in the cortical matter was 14.4 and 21.1 times higher (3.27±0.98 and 4.80±1.47%, respectively) after radiation exposure and combination of cytostatics with radiotherapy, respectively, in comparison with the status without neoadjuvant treatment (0.23±0.06% of the entire node section area). The relative volume of the connective tissue in the lymph node medulla increased after neoadjuvant therapy by 5 and 6.6 times, reaching 2.93±0.96 and 3.87±0.93%, respectively, in comparison with the patients not receiving neoadjuvant therapy (0.59±0.08%).

Neoadjuvant therapy led to a drop in the volume density of lymphoid follicles without germinative centers (from 7.52±0.90% in patients without neoadjuvant therapy to 0.58±0.39 and 0.26±0.02% in patients after radio- and chemoradiotherapy, respectively). The majority of patients examined after preoperative therapy had virtually no nodules without germinative centers in these organs in all groups (Fig. 1, *c*-*e*). We think that the reduction of percent density of lymphoid follicles without germinative centers after radio- and chemo-

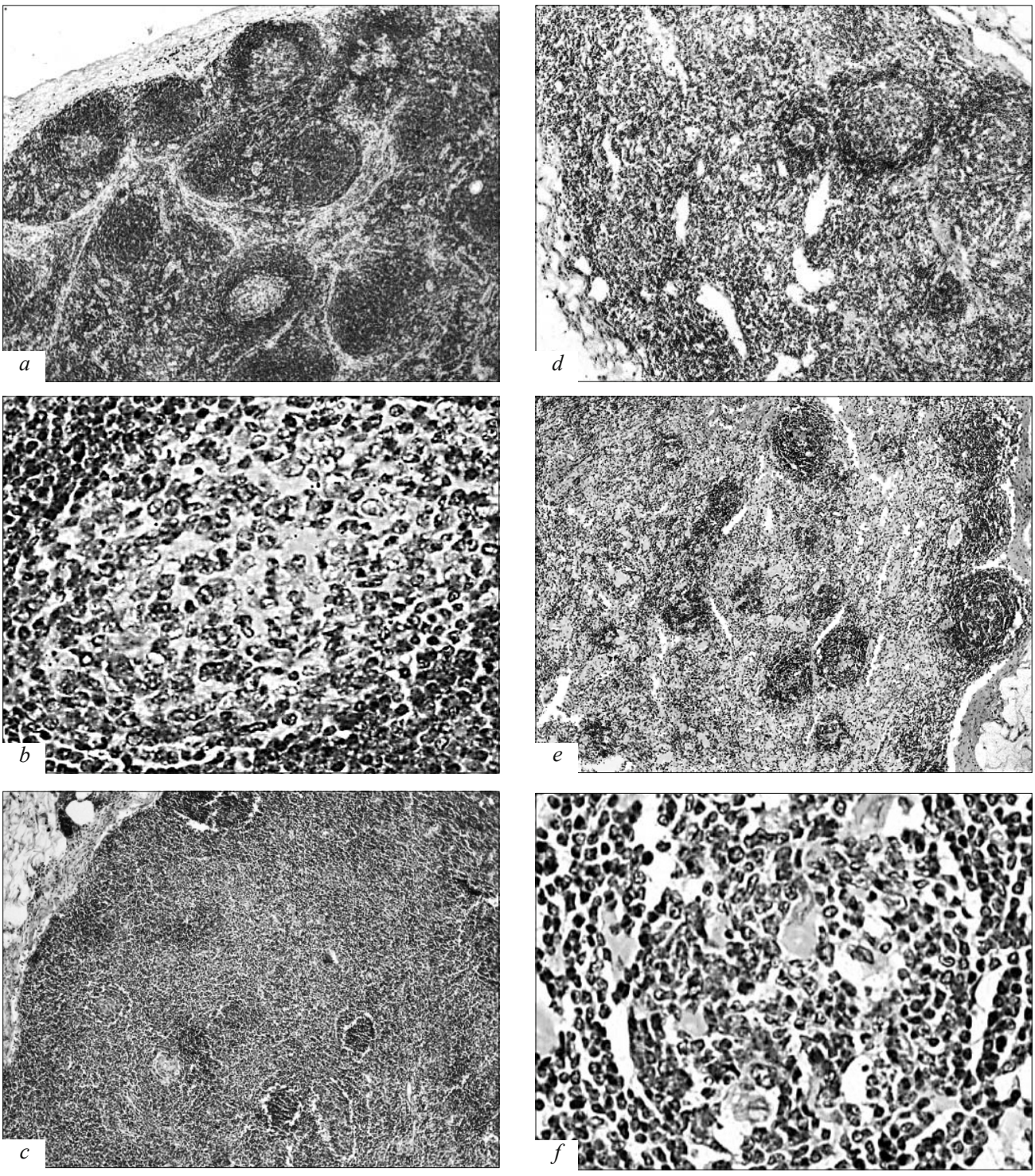

Fig. 1. Pararectal lymph nodes in RC after neoadjuvant therapy (hematoxylin and eosin staining). *a*) lymph node of a patient without neoadjuvant therapy: hypertrophic and hyperplastic lymphoid follicles without and with germinative centers, follicles in the cortical plateau forming several rows; *b*) fragment of picture *a*: immuno- and plasmoblasts predominate in the cytogram of germinative center; many mitotic figures; *c*) small solitary lymphoid follicles in the lymph node cortical matter; *d*) lymph node after radiotherapy: depletion of cellular composition of cortical matter, containing solitary lymphoid follicles with narrow mantle zones; *e*) just solitary small lymphoid follicles in the cortical matter of the lymph node after chemoradiotherapy; sinuses of the cortical matter are dilated and filled by detritus and macrophages; *f*) fragment of picture *e*: significant depletion of cells in the follicle, no mitotic activity, pronounced reduction of the counts of immuno- and plasmoblasts, reticular cells predominate in the cytogram. ×50 (*a*, *c-e*); ×500 (*b*, *f*).

radiotherapy is caused by stimulation of lymph nodes by the tumor antigens.

The effects of drugs used for chemotherapy are directed primarily against actively proliferating and differentiating cells. These processes are inhibited and apoptosis (cell death) is induced [6-8]. Ionizing radiation suppresses mitotic activity and differentiation of B lymphocytes [3,4]. The higher is mitotic activity of cells, the more pronounced is the suppressive effect of ionizing radiation [4].

The development of a malignant tumor is paralleled by the progress of a chronic inflammatory process. The combination of inflammation with tumor process is associated with the release of numerous antigens formed in the involved tissue. Lymph nodes rapidly react to the increase in the volume of antigenic substances in the body. Antigenic stimulation can stimulate cell proliferation and maturation in the nodular follicles, thus causing an appreciable dilatation of the follicles and increase of their number. However, antitumor drugs and radiation exposure inhibit cell proliferation and differentiation. The effects of these drugs are the more pronounced, the more the cells are activated. Lymphoid follicles without germinative centers are the first that suffer after injection of antitumor drugs and irradiation.

After neoadjuvant therapy the cytoarchitectonics of different zones of pararectal lymph nodes in RC patients was characterized by increased counts of reticular cells (Fig. 1, *f*), macrophages, and cells with signs of destruction. This was paralleled by a decrease in the number of mitoses, counts of monocytes, neutrophils, immuno- and plasmoblasts. Monocyte and neutrophil counts after chemoradiotherapy decreased in all structural compartments of the lymph nodes in comparison with patients who received no neoadjuvant therapy. The cortical plateau cytogram showed decreased levels of monocytes (from 3.00±0.68 to 1.26±0.44%), neutrophils (from 1.45±0.59 to 0.57±0.08%); in the paracortical zone the content of monocytes decreased from 3.07±0.55 to 1.10±0.57%, of neutrophils from 2.05±0.75 to 0.80±0.08%; the cytogram of the brain sinuses showed decreased counts of monocytes (from 3.91±0.88 to 0.94±0.68%) and neutrophils (from 3.52±0.85 to 0.94±0.80%).

Decreased counts of mitotically active cells and increase of those with signs of destruction were the most significant after combined chemoradiotherapy. The cytogram of the germinative center of lymphoid follicles showed reduced number of mitotic figures (from 2.98±0.73 to 0.48±0.07%) and increased percent of cells with signs of destruction (from 2.32±0.64 to 4.87± 0.75%). The cytogram of lymphoid follicles without germinative centers showed a decrease of mitotically active cells from 3.34±0.78 to 1.33±0.15% and an increase in the percentage of cells with signs of destruction from 1.11±0.65 to 4.0±1.0%.

Maximally pronounced changes after neoadjuvant therapy were observed in the lymphoid follicles with high counts of mitotically active and differentiating cells. These cells were most sensitive to cytostatics and radiation. The minimum changes were detected in the medullar cords, containing mainly well-differentiated plasma cells poorly reacting to drugs and radiation [10,11].

The decrease in the counts of monocytes and neutrophils is presumably caused by suppression of the myelocytic and monocytic bone marrow stems by antitutmor therapy [5,13]; in other words, by systemic manifestations of cytotoxicity. Radiotherapy of malignant tumors most effectively suppresses the irradiated groups of dividing cells. Lymphocytes, immuno- and plasmoblasts are the most actively dividing and rapidly differentiating cells in the lymph nodes without metastases. These cells are mainly destroyed by ionizing radiation during radiotherapy. This is not paralleled by suppression of the bone marrow myelocytic and monocytic stems, and the concomitant immunosuppression is less pronounced, due to which the intensity of exposure of the pathological focus and adjacent tissues can be the maximum. Hence, irradiation of the tumor and regional lymph nodes should be preferred in cases without multiple distant metastases.

The decrease in the counts of active cells in various structural compartments of the lymph nodes in the presence of unchanged absolute counts of reticular cells and macrophages lead to an increase of their percentage in the cytogram. Increase in the reticular cell percentage can also be due to sclerotic transformation of lymph nodes (increase in the stromal cell counts and coarsening of the connective tissue backbone of the lymph node). Increase in the content of macrophages can be caused by the need to eliminate the remnants of destroyed cells.

These changes in the pararectal lymph nodes after neoadjuvant therapy suggest disorders in their immune functions (suppression of mitotic division and differentiation of B-lymphocytes). These changes are the most pronounced after radiotherapy. Indirect evidence of the red bone marrow myelocytic and monocytic stems suppression after chemotherapy were obtained. These results necessitate the search for approaches to preservation and restoration of impaired functions of the lymph nodes without attenuation of the antitumor effect of neoadjuvant therapy.

Hence, hypertrophy and hyperplasia of the lymphoid follicles with and without germinative centers develop in RC patients receiving no neoadjuvant therapy. Hypertrophic follicles can form several layers in the cortical matter. This phenomenon is caused

by permanent stimulation of the immunocompetent cells in the lymph nodes by antigens (products of tissue degradation caused by infiltrative tumor growth), directly by tumor antigens and metabolites, and by bacterial antigens.

Radiotherapy and its combination with drug therapy in the treatment for RC are associated with a significant reduction of the volume density of lymphoid follicles without germinative centers in the pararectal lymph nodes, which is caused by suppression of cell proliferation and differentiation. Some patients had no follicles of this kind. The death of mitotically active cells after neoadjuvant radiotherapy in combination with chemotherapy led to development of sclerosis in the pararectal lymph nodes.

After neoadjuvant therapy the maximally pronounced changes in the cytogram in the pararectal lymph nodes are observed for the lymphoid follicles with high proliferative activity of cells, paralleled by their rapid differentiation. The minimum changes were noted for the medullar structures, because their main cell populations are well-differentiated cells with a low mitotic potential. Changes in the structure and cell composition of different zones of the pararectal lymph nodes were most pronounced after combined neoadjuvant chemoradiotherapy. The content of dividing cells, immuno- and plasmoblasts in different compartments of the pararectal lymph nodes is changed greater after radiotherapy, while monocytes and neutrophils are more sensitive to cytostatics. These differences are explained by the systemic effects of drugs and local effects (on the cells in the pathological focus) of radiotherapy.

REFERENCES

1. I. V. Maiborodin, D. N. Strunkin, V. I. Maiborodina, *et al.*, *Morfologiya*, **132**, No. 5, 68-73 (2007).
2. D. E. Tsyplakov and S. V. Petrov, *Arkh. Patol.*, **59**, No. 4, 55-60 (1997).
3. A. N. Sharetskii, B. P. Surinov, and M. R. Abramova, *Radiats. Biol. Radioekol.*, **34**, No. 405, 495-501 (1994).
4. R. E. Anderson and N. L. Warner, *J. Immunol.*, **115**, No. 1, 161-169 (1975).
5. C. R. Comereski, W. M. Peden, T. J. Davidson, *et al.*, *Toxicol. Pathol.*, **22**, No. 5, 473-488 (1994).
6. S. A. Ju, S. H. Cheon, S. M. Park, *et al.*, *Int. J. Cancer*, **122**, No. 12, 2784-2790 (2008).
7. A. Khatri, B. Zhang, E. Doherty, *et al.*, *J. Gene Med.*, **8**, No. 9, 1086-1096 (2006).
8. M. Koda, M. Sulkowska, L. Kanczuga-Koda, *et al.*, *Oncol. Rep.*, **18**, No. 1, 113-119 (2007).
9. M. A. Kornev, E. A. Kulikova, and O. S. Kul'bakh, *Neurosci. Behav. Physiol.*, **35**, No. 6, 635-638 (2005).
10. J. J. Miller 3rd and L. J. Cole, *Res. Dev. Tech. Rep.*, 1-18 (1967).
11. J. J. Miller and L. J. Cole, *J. Immunol.*, **98**, No. 5, 982-990 (1967).
12. J. Shvero, R. Koren, G. Marshak, *et al.*, *Oncol. Rep.*, **8**, No. 4, 909-911 (2001).
13. H. Tone, M. Shirai, F. Onoue, and H. Kumagai, *Jpn. J. Antibiot.*, **39**, No. 1, 250-258 (1986).
14. D. E. Tsyplakov, S. V. Petrov, and R. N. Kulagin, *Pathol. Oncol. Res.*, **3**, No. 2, 121-125 (1997).