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• *BRIEF REPORTS* •

Nodal spread and micrometastasis within mesorectum

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Abstract

AIM: To study the distribution of positive lymph nodes within mesorectum and to investigate the possible micrometastasis in negative lymph nodes.

METHODS: Large slice technique combined with tissue microarray was used in the pathologic study of 31 specimens.

RESULTS: A total of 992 lymph nodes were harvested and cancer metastasis was found in 148 lymph nodes. Some positive lymph nodes were located in the outer layer of mesorectum and more at the same site of mesorectum as the primary tumor. Circumferential margin lymph node metastasis was observed in nine cases. No significant difference in occurrence of micrometastasis was observed in different stage tumors.

CONCLUSION: Positive lymph nodes are distributed in mesorectum and micrometastasis can be found in negative lymph nodes.

Key words: Rectal cancer; Mesorectum; Lymph node

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INTRODUCTION

Treatment and prognosis of rectal cancer are mainly determined by the presence of lymph node metastasis in specimens[1,2]. The node status distinguishes TNM stage I and II patients from stage III patients. In general, individuals without evidence of nodal metastasis are at a relatively low risk for developing recurrent disease[3,4].

In this study, we investigated the distribution of metastasized lymph nodes within mesorectum on large slices. Then all negative lymph nodes were collected by tissue microarray technique to search possible micrometastasis. Therefore, the purpose was to examine the largest possible amount of lymph nodes in order to make correct staging, therapeutic decisions and prognosis in patients with rectal cancer.

MATERIALS AND METHODS

Study patients

Specimens from 31 consecutive patients with biopsy-proven adenocarcinoma of the rectum who underwent resection from November 2001 to May 2002 were retrospectively investigated. There were 15 males and 16 females with an average age of 60 years. Preoperative endoscopic evaluation showed tumor 3-15 cm from the anus. None of the patients received preoperative chemotherapy or radiation therapy.

Surgical technique

Twenty-five of the patients underwent laparotomic anterior resection and six patients had laparoscopic total mesorectal excision (TME). The TME procedure was defined as sharp dissection under direct vision, the rectum and mesorectum in the fascia propria were excised[5]. This did not necessarily mean an excision of the entire mesorectum down to the pelvic floor or anal canal, but the standard policy for division of the rectum and mesorectum was 3 cm below the tumor. If possible, 5 cm of rectum and mesorectum was excised.

Specimens' preparation

Mesorectal lymph nodes were never removed so as to preserve the mesorectum. Each specimen was stitched under tension onto a made-to-order board *ex vivo* after a column of sponge was loaded into the intestinal cavity to make sure it conformed to biological dimensions. The boards were floated with the specimens down for fixation in 10% buffered formalin for 48 h. Serial transverse tissue blocks were cut at 5-mm intervals from the distal resection margin to the proximal margin. Each block consisting of full thickness of rectum and mesorectum was embedded in paraffin. Four-micrometer-thick sections were sliced from each block, mounted on a large glass slide (15 cm×6 cm), and stained with hematoxylin-eosin.

Histopathologic diagnosis

All the slices were reviewed to detail the histologic findings according to TNM classification[6]. In addition, we also

examined the direct tumor infiltration and lymph node involvement to assess circumferential margin following the quality control principles formulated by Quirke *et al*[7]. The positive circumferential resection margin (CRM) was defined as tumor growth within 1 mm from the resection margin. The distance of tumor spread was measured using a ruler. During examination and recording, we divided the mesorectum into three regions (i.e., left, right, and rear regions) according to its observed shape on large slices. Each region was further separated into three layers (i.e., outer, middle, and inner layers) on the basis of their distance from the outer limit of serosa. Therefore, we got nine areas and located each focus to one or more of them.

Tissue microarray technique

Negative lymph nodes were noted during examination of the large tissue sections stained with hematoxylin-eosin. Corresponding areas on the sectioned paraffin-embedded block were marked. From each paraffin block, core biopsy specimens of lymph nodes were punched and positioned into a recipient block. Core biopsy from the primary lesions was used as the positive control. In the ensuing paraffin array block, tissue cylinders were aligned, marked for identification, and cut into 4-μm-thick sections for further examination.

Immunohistochemical analysis

Four-micrometer-thick sections were transferred to glass slides disposed with APES. Following deparaffinization in xylene and rehydration in graded ethanol, the sections were treated in citrate buffer (pH 6.0) for antigen retrieval for 3 min, stained immunohistochemically with CK20 (Dako, Glostrup, Denmark) monoclonal antibody using avidin-biotin-peroxidase complex and visualized by diaminobenzidine. Binary antibody was applied to the sections at 37 ℃ for 45 min. Negative control sections for immunohistochemical (IHC) staining were not stained with the primary antibody. Cancer cells positive for CK20 were intensely stained in comparison with other components of lymph nodes.

Statistical analysis

The data were analyzed with the SPSS 10.0 package. Pearson coefficient was chosen in bivariate correlation analysis. The χ^2 and stepwise forward LR tests were used respectively in crosstabs statistics and binary logistic regression. $P<0.05$ was considered statistically significant.

RESULTS

All the operations were considered curative by the surgeons. Distal donuts included in the stapler device were also examined microscopically. All were histologically free of carcinoma.

Examination of lymph nodes

Using large slice technique, 992 lymph nodes were examined from specimens of 31 patients. Tumor involvement was in 148 of them, and there was no tumor involvement in the other 844 lymph nodes. The average number of examined lymph nodes per specimen was 32. The details of harvested nodes are shown in Table 1.

Metastasis rate of lymph nodes, defined as the percent of involved nodes for each specimen, was related to the depth of tumor infiltration ($r = 0.548$, $P = 0.001$) and tumor differentiation ($r = 0.470$, $P = 0.008$). No significant difference in the rate was observed between tumors at different sites.

Table 1 Diameter distribution of examined lymph nodes

	<0.5 mm	<1 mm	<2 mm	<5 mm	<10 mm	≥10 mm	Sum
Uninvolved LN	165	138	245	270	26	0	844
Involved LN	5	7	23	69	32	12	148
Sum	170	145	268	339	58	12	992
Percent (%)	17.1	14.6	27.0	34.2	5.8	1.2	100.0

Distribution of involved lymph nodes

Fifty-one involved lymph nodes were located in the outer layers (including left, right, and rear regions) of mesorectum, accounting for 25.6% of all involved lymph nodes, and 41.7% of the involved lymph nodes were observed in the rear region of mesorectum. Of the 10 stage III patients with primary tumors mainly located on the left or right side of rectal wall, 34.7% of the involved nodes were in the same lateral region of mesorectum, and 6.1% on the opposite side. The difference was significant ($P<0.01$) (Table 2).

Table 2 Mesorectal distribution of tumor involved lymph nodes for 10 stage III patients

Primary tumor location	Involved lymph node			
	Left region	Right region	Rear region	Outer layer
Left	13	1	31	18
Right	5	21	27	22
Others	10	15	25	11

Circumferential margin involvement

Twelve specimens were involved in the circumferential margin and four of them had a real positive margin (i.e., tumor cells in the resection margin). The positive CRM included circumferential margin involvement (CMI) of primary tumor infiltration and lymph node metastasis. If CMI of the latter was included, the number of positive margins would be nine. No relationship between the occurrence of CMI and T stage, tumor location and differentiation was found in logistic regression analysis (Table 3).

Lymph node micrometastasis

A total of 844 negative lymph nodes were marked on tissue blocks and 795 were successfully transferred to recipient blocks using tissue microarray. The other 49 lymph nodes not removed were smaller than 0.5 mm in diameter, accounting for 5.8%. Occult tumor cells in lymph node specimens were found in 9 of 31 patients (29.0%) by IHC analysis. The cells were located in subcapsular sinuses of lymph nodes as single cells or in groups and rarely in

Table 3 Logistic regression analysis of CMI with clinicopathologic characteristics

	Rectal cancer (n = 31)		CMI (n = 12)		Sig.	Exp (B)
	No. of patients	Percent (%)	No. of patients	Percent (%)		
Age (yr)						
<40	3	9.68	3	25.00		
40–59	12	38.71	5	41.67	0.069	0.908
⩾60	16	51.61	4	33.33		
Primary tumor location						
Lower	9	29.03	4	33.33		
Middle	17	54.84	6	50.00	0.828	0.858
Upper	5	16.13	2	16.67		
Differentiation						
Poor	11	35.48	8	66.67		
Moderate	18	58.06	3	25.00	0.564	0.587
Well	2	6.45	1	8.33		
Infiltration						
T1	2	6.45	1	8.33		
T2	6	19.35	0	0.00	0.654	1.517
T3	21	67.74	9	75.00		
T4	2	6.45	2	16.67		
Involved LN						
0	15	48.39	3	25.00		
1–9	11	35.48	5	41.67	0.242	1.082
⩾10	5	16.13	4	33.33		

parenchyma. We rarely detected a stromal reaction around the tumor cells. No significant difference in incidence rate of micrometastasis was obtained between tumors at different T stages, N status, and differentiation (Table 4).

Table 4 Lymph node micrometastasis with clinicopathologic characteristics

	Micrometastasis		P
	Yes	No	
T			
1	1	1	
2	1	5	0.11
3	5	16	
4	2	0	
N			
0	2	11	
1	1	5	0.124
2	6	6	
TNM stage			
1	1	6	
2	1	5	0.267
3	6	11	
4	1	0	
Differentiation			
Well	0	2	
Moderate	3	15	0.06
Poor	6	5	

DISCUSSION

Radical surgery remains the first choice of treatment for rectal cancer and the prognosis primarily depends on the stage of tumor at the time of diagnosis. As diagnostic assessment is performed more and more precisely and surgical techniques have been greatly improved in the last decade, the rate of curative resections and sphincter-saving procedures for rectal cancer is significantly increased and the mortality rate is decreased[8]. However, numerous factors such as characteristics of primary tumor and presence of lymph node metastasis affect the survival of patients with carcinoma of the rectum[9,10]. Detection of lymph node metastasis and determination of pN status are essential for prediction of prognosis and planning therapeutic modalities. Hida *et al*[11], and Greenson *et al*[12], reported that the number of lymph nodes identified at the time of pathologic examination of surgical specimens might vary not only in patients but also in the extent of pathologic exploration. However, because the surgical stuff was stable during the study period and the operations were performed following the principles of TME, we believe surgical technique was unlike to affect our data. Therefore, the result mainly reflected the significance of pathological examination.

The number of lymph nodes that need to be examined to accurately stage individuals with rectal cancer is controversial and ranges between 12 and 17[13-16]. In this study, we combined large tissue slides with tissue microarray technique in examining lymph node status of patients with rectal cancer. Metastasis detected in our study by the integration of HE staining with IHC process provides an absolutely new method for maximizing the number of lymph nodes. It detected a higher average number of harvested lymph nodes compared with other studies. Furthermore, 583 lymph nodes had a diameter of less than 2 mm, which could not be achieved by conventional pathologic test. Scott and Grace[13] demonstrated that 80% of lymph node metastases are found in lymph nodes smaller than 5 mm in diameter. In this study, 70.3% metastasized nodes were smaller than 5 mm in diameter, those bigger than 10 mm in diameter just accounted for 8.1%. Clinical studies suggested that the number of positive lymph nodes

with mural penetration is the most valuable predictive factor for survival[11,13]. In this study, correlation test did confirm the relation between metastasis rate of lymph nodes and depth of tumor infiltration.

Providing a holistic view of rectum, together with surrounding mesorectum, the large slice technique used in this study promoted us to study the pattern of tumor spread. The result of our study showed that 41.7% of the involved lymph nodes were observed in the rear region of mesorectum and the rate was 25.6% in outer layers (including left, right, and rear regions). Concerning the principles of TME, dissection between the relatively avascular plane outside the visceral fascia and complete removal of mesorectum from behind are mandatory. Therefore, lymphatic drainage from the rectum is eliminated by TME. When conventional blunt procedure is used, metastasis in the outer layers would be left in the pelvis of patients and gives rise to further local recurrence. Furthermore, as shown in Table 2, in tumors located on the left and right side of the rectum, nearly one-thirds of the positive nodes were located in the same lateral region of the mesorectum, significantly more than those in the opposite region, suggesting that the lateral discrepancy in dissection of the hypogastric plexus can guarantee clear lateral margin in these areas.

Quirke *et al*[7], de Haas-Kock *et al*[17], Adam *et al*[18], and Cawthorn *et al*[19] have introduced the conception of CMI. They found that CMI is a surgery-related factor that increases the risk for local recurrence. It was reported that CRM influences the rate of local recurrence, distant metastases and survival[20]. A large multicenter trial in Netherland[21] even advised that 2 mm should be used as the criterion for CRM in predicting local recurrence. Logistic regression test in this study did not prove the relation of CMI with tumor location and differentiation.

It has been observed that as the number of examined lymph nodes increases, the number of lymph node positive specimens increase[15,16,22]. Theoretically, sampling of the entire perirectal fat would be an accurate method for total lymph node examination[23-25]. Koren *et al*[26] introduced the lymph node revealing solution method and showed that stage of the disease can be determined more accurately by it. However, 20-30% of patients with no histopathologically detectable lymph node metastasis die of a local tumor relapse or distant metastases, which might be explained by an early dissemination of tumor cells into lymphatic system that cannot be detected using conventional pathological techniques. Gusterson[27] first reported that up to 20% of cases negative for lymph node metastasis on routine sections have micrometastasis. In the current study, we collected all negative lymph nodes observed on large slices using tissue microarray technique for further investigation. IHC analysis discovered micrometastasis from the negative lymph nodes of nine specimens. No statistical difference was found in detected tumor cells, suggesting that the submucosa lymphatic spread of tumor cells occurs once the infiltration reaches this layer.

In conclusion, positive lymph nodes are distributed in mesorectum, and micrometastasis can be discovered in cancers at different stages and causes future recurrence. TME is rather a good procedure for rectal cancer.

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