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World J Gastroenterol 2004;10(22):3369-3373
World Journal of Gastroenterology
 ISSN 1007-9327

• BRIEF REPORTS •

Tumor micrometastases in mesorectal lymph nodes and their clinical significance in patients with rectal caner

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Received: 2004-03-09 **Accepted:** 2004-04-05

Abstract

AIM: To investigate the number, size, and status of lymph nodes within the mesorectum and to explore the prognostic significance of lymph node micrometastases in patients with rectal cancer.

METHODS: Thirty-one patients with rectal cancer undergone total mesorectal excision between October 2001 and October 2002 were included. Mesorectal nodes retrieved from the resected specimens were detected with a combination of haematoxylin and eosin (HE) staining and immunohistochemistry (IHC). The relations between lymph node metastases, micrometastases and postoperative recurrence were analyzed.

RESULTS: A total of 548 lymph nodes were harvested, with 17.7±8.2 nodes per case. The average number of metastatic nodes in HE-positive patients and micrometastatic nodes in IHC-positive patients was 5.2±5.1 per case and 2.2±1.3 per case, respectively. The mean size of all nodes and metastatic nodes was 4.1±1.8 mm and 5.2±1.7 mm in diameter, respectively. The mean size of micrometastatic nodes was 3.9±1.4 mm in diameter. The size of the majority of mesorectal nodes (66.8%), metastatic nodes (52.6%), and micrometastatic nodes (79.5%) was less than 5 mm in diameter. During a median follow-up period of 24.6±4.7 mo, 5 patients (16.7%) had recurrence, of them 2 died and 3 survived. Another case died of tumor unrelated cause and was excluded. All 5 recurrent cases had 3 or more nodes involved, and one of them developed only lymph node micrometastases. The mean number of both metastatic and micrometastatic nodes per case differed significantly between the recurrent and non-recurrent groups ($P<0.01$ and $P = 0.01$, respectively).

CONCLUSION: The majority of lymph nodes, metastatic, and micrometastatic lymph nodes within the mesorectum are smaller than 5 mm in diameter. The nodal status and the number of lymph nodes involved with tumor metastases and micrometastases are related to the rapid postoperative recurrence.

Zheng YC, Tang YY, Zhou ZG, Li L, Wang TC, Deng YL, Chen DY, Liu WP. Tumor micrometastases in mesorectal lymph nodes and their clinical significance in patients with rectal caner. *World J Gastroenterol* 2004; 10(22): 3369-3373
http://www.wjgnet.com/1007-9327/10/3369.asp

INTRODUCTION

Lymph node involvement is one of the most important factors in determining the prognosis of patients with rectal cancer[1-6]. Patients with lymph node involvement were found to suffer from more advanced diseases according to the tumor-node-metastasis (TNM) classification system[6], and up to 30% of them eventually developed recurrences after a potentially curative resection[7,8]. In contrast, some patients without positive lymph nodes also had postoperative recurrences or developed metastatic diseases, which were reported to be associated with lymph node micrometastases missed by conventional pathological examination[9-13].

Lymph node spread of rectal cancer generally follows an anatomical route from the proximity to distance along the main supplying vessels of the rectum[14,15]. Lymph nodes contained in the mesorectum are thereby the earliest and most frequent ones that might be involved when tumor spread occurs[14-16]. Although reports have emphasized the importance of regional lymph nodes in the prognosis of rectal cancer[1,2,17-21], few studies available have investigated the number and size of lymph nodes within the rectal mesentery[22,23]. The clinical significance of mesorectal lymph node metastases and micrometastases remains to be fully acknowledged[22,23]. This study was conducted to examine the number, size, and status of lymph nodes within the mesorectum, and to investigate the prognostic value of lymph node metastases, especially lymph node micrometastases, in patients with rectal cancer.

MATERIALS AND METHODS

Patients

From October 2001 to October 2002, 31 patients with rectal cancer undergone total mesorectal excision (TME) at the Department of Gastroenterological Surgery of West China Hospital were included. There were 18 males and 13 females, with an average age of 55 (32-72) years. Of them, 7 cases were patients with high rectal cancer (above the peritoneal reflection) and 24 cases were patients with lower rectal cancer (below the peritoneal reflection). Three tumors were highly differentiated, 20 moderately differentiated, and 8 poorly differentiated. All patients received standard preoperative examination and the diagnosis of rectal adenocarcinoma was made by fibrocolonoscopy and confirmed by pathological biopsy before surgery. All operations were carefully performed by skillful surgeons following the principle of TME[24], and the rectal specimens resected were collected prospectively.

Specimen processing

After removal, the specimens were routinely processed with neutral buffered 100 mL/L formalin solution for 24 h, and immersed in lymph node revealing solution (LNRS) for 6 h or more[25]. Later, they were washed thoroughly with running

water. Fat tissues in the specimens were dissected carefully at intervals of 2-3 mm from upward down to the level of distant transaction along the runway of the superior rectal artery (SRA). Lymph nodes stood out as white, and chalky nodules against the background of yellow fat were harvested and recorded.

Pathological examination

After retrieval, each lymph node was embedded separately in paraffin. The block was then sectioned serially at intervals of 20-40 μm, with each section of 4 μm in thickness. Two sections sampled randomly from 10 representative levels were subjected to haematoxylin and eosin (HE) staining. For nodes diagnosed negatively by HE staining, another 3 sections were further singled out, one for HE re-examination, the other two for immunohistochemistry (IHC).

Immunohistochemistry was performed using labelled streptavidin biotin method (LsAB). Briefly, all sections were deparaffinized and rehydrated. Then, they were immersed in 30 mL/L H_2O_2 for 20 min to block the endogenous peroxidase activity. Sections were then incubated with 100 mL/L normal goat serum for 20 min, followed by incubation with mouse monoclonal anti-human cytokeratin (CK) 20 antibody (Neomarkers, Lab Vision Corporation, CA, USA) (1:50) at 4 ℃ overnight. After that, the sections were washed with phosphate buffered solution (PBS) (0.01 mol/L, pH 7.2) and sequentially incubated with biotinylated goat anti-mouse IgG, and streptavidin biotin horseradish peroxidase complex following the manufacturer's instructions (Histostain™-SP Kits, Zymed laboratories Inc., San Francisco, CA, USA). Staining was developed by immersing slides in 0.5 g/L 3,3'-diaminobenziding tetrahydrochloride (DAB) with 3.3 mol/L H_2O_2. All slides were counterstained with haematoxylin, dehydrated and mounted. Yellowish staining of the tumor cell cytoplasm was taken as positive. Previously confirmed rectal adenocarcinoma tissue served as positive control, and substitution of the primary monoclonal antibody with PBS was used as negative control. Tumor micrometastases were occult diseases generally missed by HE staining while detected by IHC, and were characterized as single cell or small cluster of cells showing malignant morphology.

Follow-up

All patients were regularly followed up to now. The follow-up interval was every 3 mo after surgery during the first year and every 6 mo thereafter. The time of recurrence and the cause of death were inquired and recorded.

Statistical analysis

The *t* test for difference in mean values and χ^2 test for difference in frequencies were performed using SPSS 10.0 software package. $P<0.05$ was considered statistically significant.

RESULTS

Micrometastases and number of lymph nodes

A total of 548 mesorectal lymph nodes were retrieved from the 31 specimens, with 17.7±8.2 lymph nodes per case. HE staining detected 114 lymph nodes positive in 22 patients (71.0%). The average number of metastatic lymph nodes in HE-positive patients was 5.2±5.1 per case. IHC re-examination of the remaining negative nodes revealed 39 lymph nodes (9.0%) positive with tumor micrometastases from 18 cases (58.1%). Among them, 5 cases (16.1%) with 10 nodes positive with IHC staining were previously assumed free of metastatic diseases. The average number of micrometastatic lymph nodes in IHC-positive patients was 2.2±1.3 per case.

Micrometastases and size of lymph nodes

The average diameter of all the 548 lymph nodes was 4.1±1.8 mm. The mean size of 114 metastatic lymph nodes (5.2±1.7 mm in diameter) was significantly larger than that of 39 micrometastatic lymph nodes (3.9±1.4 mm in diameter) ($P<0.01$). Of all the lymph nodes, 92 (16.8%) were ≤2 mm in diameter, with 4 metastatic and 3 micrometastatic lymph nodes; 182 (33.2%) ≥5 mm, with 54 metastatic and 8 micrometastatic lymph nodes; and 274 (50.0%) with a size between 2 mm and 5 mm in diameter, with 56 metastatic and 28 micrometastatic lymph nodes (Table 1). It was noted that the majority of lymph nodes (66.8%) were <5 mm in diameter, and 52.6% of the metastatic nodes and a higher proportion of the micrometastatic nodes (79.5%) were <5 mm in diameter also.

Lymph node metastases and micrometastases and prognosis

All the 31 patients were successfully followed-up. Patients with lymph node metastases received regular chemotherapy after surgery. However, during a median follow-up period of 24.6±4.7 mo, one

Table 1 Size of lymph nodes (LNs) and status of tumor metastases and micrometastases

Size of LNs (mm)	No. of LNs				Positive ratio (%)
	Total	HE-positive	IHC-positive	Sum of positive	
≤2	92	4	3	7	7.6[b]
2-5	274	56	28	84	30.7
≥5	182	54	8	62	34.1[d]
Total	548	114	39	153	27.9

[b]$P<0.01$, *vs* 2-5; [d]$P<0.01$, *vs* ≤ 2.

Table 2 Clinicopathological features of five recurrent patients with lower rectal cancer

Case No.	Sex	Age (yr)	Characteristics of tumor		No. of LNs			Site of recurrence	Outcome
			Differentiation	Invasion	Total	M	MM		
1	Female	35	Well	Muscle	15	3	2	Lung	Surviving
2	Female	65	Poor	Serosa	22	22	0	Pelvis+liver	Dead
3	Male	50	Moderate	Muscle	27	0	3	Pelvis	Surviving
4	Male	45	Poor	Serosa	30	17	4	Pelvis	Dead
5	Male	32	Poor	Muscle	21	5	5	Pelvis	Surviving

LNs: lymph nodes; M: metastatic; MM: micrometastatic.

Table 3 Comparison of recurrence between patients with different nodal status

Variables	HE-positive		HE-negative		Total
	IHC-positive	IHC-negative	IHC-positive	IHC-negative	
No. of case	13	8	5	4	30
Recurrence	3	1	1	0	5
Recurrence rate (%)	23.1	12.5	20	0	16.7

Table 4 Comparison of nodal status between recurrent and non-recurrent group

Grouping	LNs (mean±SD)				Positive ratio (%)
	Total	HE-positive	IHC-positive	Sum of positive	
Recurrent group	23.0±5.8	9.4±9.6	2.8±1.9	12.2±8.9	53.0
Non-recurrent group	17.0±8.2	2.6±2.5	1.0±1.2	3.6±3.0	21.4
P	0.135	0.003	0.01	0.000	0.000

patient died of tumor unrelated cardiovascular disturbance 24 mo post operation and was excluded from the analysis. Among the five cases (16.7%) with lower tumor recurrences, 3 cases were localized in the pelvic floor, one case spread to the lung, and one case with both pelvic and liver involvements. Two of them died and the other three remained alive. All the recurrent patients shared the characteristics of having 3 or more lymph nodes involved with tumor metastases and/or micrometastases. Of them, one case with mere lymph node micrometastases also developed local recurrence 17 mo after operation (Tables 2, 3). Comparison analysis showed that both the number of metastatic and micrometastatic lymph nodes between the recurrent and non-recurrent groups differed significantly ($P<0.01$ and $P = 0.01$, respectively), although such a statistical difference was not found between them in reference to the total number identified per case (Table 4).

DISCUSSION

The nodal status is the single powerful predictor of survival in rectal cancer[1,2,20,21]. Both the number and location of lymph nodes involved have significant impacts on the outcome of patients with rectal cancer[6,15,21,26]. However, the nodal staging accuracy is not easy to make[27-29]. Factors contributing to this dilemma include the mount of mesentery resected, diligence for search of nodes paid, number of histological slices investigated, and methods of pathological examination employed[30-33]. There are still wide variations regarding the minimum number of lymph nodes to be examined for a reliable node-negative diagnosis, the reported recommendations were 6 to 17 lymph nodes[34-36]. The non-uniform extent of lymph nodes collected for investigation made the results incomparable between authors, and the absence of consensus on the number of lymph nodes contained in the mesentery worsened the situation further[36,37].

It was established that the lymphatic spread of rectal cancer followed an anatomical way along which the normal lymphatic fluid drains[14,15]. As a consequence, lymph nodes contained in the mesorectum are the earliest and the most frequent ones that might be involved when the dissemination of tumor cells takes place[14-16]. Focused examination of the mesorectal lymph nodes, thereby, could identify most metastases and micrometastases in the first place[15], though skip metastases might exist in a few cases[38,39]. However, available data have provided little information on the lymph nodes enveloped within the mesorectum[22,23]. The number and size of lymph nodes, and their tumor status remain to be defined.

Recently, Canessa *et al*.[22] recovered 168 mesorectal lymph nodes from 20 cadavers and found that the mean number was 8.4 per specimen. They searched the lymph nodes from the division of the superior rectal artery which excluded the lymph nodes that lie above it, i.e., the lymph nodes along the main trunk of it, that should otherwise be referred to 'mesorectal' nodes by strict definition[40]. Also in an another study on cadavers, an average of 24.9 pelvic lymph nodes were identified from 7 fresh specimens, which included 13.6 mesorectal lymph nodes per case[23]. Their study employed modified LNRS to facilitate the identification of lymph nodes in the mesorectum. Therefore, the lymph nodes they retrieved outnumbered those in previous study markedly[23]. In the current study, the LNRS was also used to help identify lymph nodes embedded in the mesorectal fat. A mean number of 17.7 lymph nodes per case were dissected. The majority of them (82.2%) were located along the superior rectal artery. The sizes of 66.8% of the total lymph nodes, 59.8% of the metastatic lymph nodes and 79.5% of the micrometastatic lymph nodes were <5 mm in diameter. The minimal size of lymph nodes found was 0.7 mm in diameter. These lymph nodes, especially those with a diameter $\leqslant$2 mm, were at great risk of being missed in routine pathological sampling with only naked speculation and manual palpitation[41-43]. By further examining the HE-negative nodes with IHC staining, we identified 39 nodes positive with tumor micrometastases from 18 cases (58.1%). Of interest, 10 nodes with micrometastatic diseases in 5 cases (16.1%) were previously assumed free of tumor spread by routine pathological examination. Thus this group of patients was upstaged.

The superior sensitivity of IHC to HE staining in detecting micrometastases in colorectal cancer has been well recognized[44-47]. Although its sensitivity remained ten times lower compared to reverse transcriptase polymerase chain reaction (RT-PCR) techniques, IHC is probably more specific and reliable, in that it allows examining the morphology of stained cells and differentiating from that of non-specific background staining[44-46]. There are still many controversies regarding the prognostic significance of micrometastases in colorectal cancer[10,11,47-54]. It was argued that although the application of IHC technique had the added ability to identify overt diseases missed by HE staining, it offered little prognostic information on the postoperative recurrence and long year survival[10,49-52]. On the contrary, authors favoring the detection of lymph node micrometastases stated that it was not only one of the major concerns for the proper staging of diseases, but also of prognostic relevance to the outcome of patients with rectal cancer, thus suggesting helpful in the early planning of a multimodality treatment protocol for indicated patients[11,53,54].

Choi *et al*.[50] retrospectively reviewed 1 808 lymph nodes from 93 Dukes' B colorectal tumors with IHC staining and found that 54 lymph nodes (3.0%) from 29 cases (31.2%) harbored micrometastatic diseases. However, the five-year survival analysis showed no significant difference between the micrometastatic group and non-micrometastatic group. Yasuda *et al*.[11] employed

IHC method and examined a total of 1 013 lymph nodes from 12 recurrent and 30 non-recurrent patients with histologically determined Dukes' B colorectal cancer. Micrometastases were confirmed in 59 lymph nodes (16%) from 11 cases (92%) in the recurrent group, and 77 lymph nodes (12%) from 21 cases (70%) in the non-recurrent group. They further demonstrated that micrometastases in four or more lymph nodes occurred more frequently in the recurrent group than in the non-recurrent group (58% *vs* 20%, $P<0.05$), and micrometastases to N2 or higher nodes occurred more frequently in the recurrent group also (92% *vs* 47%, $P<0.01$). Afterwards they concluded that the number and level of positive micrometastatic lymph nodes were both significantly correlated with postoperative recurrence of histologically determined Dukes' B colorectal cancer[11].

In the present study, 5 patients with rectal cancer developed recurrences. The rate of recurrence was 16.7% within two years after curative operation. All patients suffering from recurrent diseases had three or more mesorectal nodes involved with tumor metastases and/or micrometastases, one patient with lymph node micrometastases developed recurrence also. It was noted that both the number of metastatic and micrometastatic lymph nodes differed significantly between the recurrent and non-recurrent groups ($P<0.01$ and $P = 0.01$, respectively), implying that lymph node micrometastases and the number of lymph nodes involved might also correlate with the outcome of patients with rectal cancer, while the prognostic value of lymph node metastases was confirmed.

In summary, by using the LNRS to facilitate identifying lymph nodes, and with a combination of HE and IHC to detect tumor metastases and micrometastases, we have provided a detailed description of both the number and size of lymph nodes enveloped in the mesorectum, as well as their tumor status with metastases and micrometastases in rectal cancer. The rapid recurrence of tumor after a cure-intended surgery is related to the tumor status of mesorectal lymph nodes. The presence of lymph node micrometastases and the number of lymph nodes involved might have some value for the prediction of tumor recurrence in rectal cancer[11,47,48,53,54], now that the importance of lymph node metastases has been widely acknowledged[1-6,19,21]. However, it is too cursory to draw a convincing conclusion, concerning the small samples included and only short-term outcome investigated in the current study. Future multi-central large-sample controlled studies are needed to clearly define the role of lymph node micrometastases in the prognosis of patients with rectal cancer.

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Edited by Kumar M and Wang XL **Proofread by** Xu FM