

LYMPH NODE REVEALING SOLUTION: A PROSPECTIVE STUDY ON 35 PATIENTS WITH COLORECTAL CARCINOMAS

S. Saleki and H. Haeri

Department of Pathology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract- Lymph node dissection is of prime importance for accurate staging of colorectal carcinomas. Since a great number of small lymph nodes are missed in the traditional method, several fat clearing solutions have been introduced for easier detection of smaller lymph nodes. In this study we evaluated the efficacy of a new fat clearing solution so-called Lymph Node Revealing Solution (LNRS) in colectomy specimens of patients with colorectal carcinoma from year 2000 till 2002, thirty five colectomy specimens with less than 4 metastatic lymph nodes received in the pathology department of IKMC, were selected and re-evaluated using the LNRS. By using this solution 456 additional lymph nodes and 16 more metastatic lymph nodes were detected resulting in upstaging of 3 patients from Dukes' B to C. In all patients categorized as Nx (regional lymph nodes not assessed) by the traditional method, lymph nodes were detected after employment of LNRS. The mean diameter of dissected lymph nodes was significantly smaller than the traditional method (0.268 versus 0.429, $p < 0.000001$). LNRS in comparison with previous solutions is inexpensive, rapid and easy to use. We propose use of this solution in all Dukes' B and patients with no lymph node detection by traditional method.

Acta Medica Iranica, 40(4); 223-225: 2002

Key Words: Lymph node revealing solution, colorectal carcinoma, metastatic lymph node

changeably (3). In both of these systems, the status of lymph nodes is of paramount importance. It is essential to obtain as many lymph nodes as possible (3). For accurate staging the cancer Committee of the College of American Pathologists recommends that if fewer than 12 nodes are found with the traditional methods (serial sectioning, inspection, and palpation), then the use of "visual enhancement techniques" should be considered (4). The traditional method is laborious and lymph nodes may be missed especially those smaller than 5 mm (5). Several solutions have been used to increase lymph node yield. These methods result in detecting more and smaller lymph nodes (5-11). It has been shown that 44% to 78% of lymph nodes containing metastasis measure 5 mm or less (5,12). So by using these solutions some of the patients with colorectal carcinomas have been upstaged, especially from Dukes' B to C (5,7,9). Most of the fat clearing methods are time-consuming and difficult. Recently an ether-based solution (called Lymph Node Revealing Solution [LNRS]) has been introduced which is inexpensive, easy, rapid and innocuous. This solution is composed of 65% of 95% ethanol, 20% diethyl ether, 5% glacial acetic acid, and 10% buffered formalin (9). In this study we have evaluated the efficacy of LNRS in upstaging patients with colorectal carcinomas.

INTRODUCTION

Colorectal carcinoma is one of the most frequent malignancies of the gastrointestinal tract (1). It is the second cause of death in the western countries (2). Pathologic staging is the most important prognostic factor in colorectal carcinomas (1). There are two major staging systems for colorectal carcinomas: Dukes and TNM (Nx= regional lymph nodes not assessed; N0=no metastatic lymph nodes; N1= one to three positive nodes; N2= four or more positive nodes). These two systems can be used inter-

MATERIALS AND METHODS

In this analytical prospective study, which was conducted in Imam Khomeini Hospital from July 2000 until January 2002, the colectomy specimens of about 80 patients with colorectal tumors were evaluated. Since we wanted to determine the effect of LNRS on upstaging the patients with colorectal carcinomas, patients with four or more metastatic lymph nodes were excluded. Because based on TNM classification, cases with more than 3 metastatic lymph nodes being classified as N2 and detecting more lymph nodes will not change the "N", patients with lymphoproliferative disorders, mesenchymal and carcinoid tumors- that are not classified by TNM or Dukes'-were also excluded. All patients were clinically free from distant metastasis (Mo). All specimens were first routinely handled. Its lumen was opened, the tumor and the mesocolic fat

Correspondence:

S. Saleki, Department of Pathology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 2583942
Fax: +98 21 8008837
E-mail: sepiddeh_saleki@yahoo.com

Lymph node revealing solution

sectioned on the day of arrival and fixed in 10% formalin overnight. The gross examination was performed the next day. All lymph nodes in the pericolic fat were dissected by serial sections, inspection, and palpations (traditional method). After studying the slides, cases that fulfilled the inclusion and exclusion criteria of our study, were entered into the study. In these cases, the entire pericolic fat was immersed for six hours in approximately three times its volume of LNRS. The LNRS composed of 65 percent of 95 percent ethanol, 20 percent diethyl ether, 5 percent glacial acetic acid, and 10 percent buffered formalin. Subsequently, the pericolic fat was washed and sectioned again. The lymph nodes appeared as white chalky nodules in a background of yellow fat. All additional detected lymph nodes were submitted and stained as usual.

RESULTS

After consideration of inclusion and exclusion criteria 35 cases were enrolled in our study. The mean age of the patients was 54.2 years with the standard deviation of 16.5 years. In the traditional method a total number of 219 lymph nodes were found in all of the cases, with an average and standard deviation of 6.26 and 5.90 lymph nodes per case, respectively. The total number of metastatic lymph nodes in the traditional method was 22 (0.622 metastatic lymph nodes per case). After application of LNRS, 456 additional lymph nodes and 16 additional metastatic lymph nodes were detected. The metastatic rate (number of patients with metastatic lymph node divided by the total number of patients) was 31.4% in the traditional method and 42.8% after consideration of both methods (traditional method followed by application of LNRS). Although the metastatic rate increased, the difference was not statistically significant ($p=0.322$). The metastatic incidence defined, as the number of metastatic lymph nodes divided by the total number of dissected lymph nodes, in the traditional method was about 10%. When considering both methods (traditional followed by LNRS method) this incidence decreased to 5.6% ($p=0.023$). The significant decrease in metastatic incidence is due to larger number of dissected lymph nodes. The average of diameters of dissected lymph nodes in the traditional and LNRS methods were 0.429 and 0.268 mm, respectively ($p<0.0000001$). The minimum diameters of dissected and metastatic lymph nodes were the same in both methods (0.1 and 0.2 mm, respectively). The maximum diameter of the lymph nodes dissected in the LNRS method was 0.9 mm. It means that all the lymph nodes larger than 0.9 mm were successfully detected in the traditional method. The mean diameter of metastatic lymph nodes in the traditional method was 0.568 mm and that of the LNRS method was 0.350mm ($p=0.00572$). As it has been shown LNRS is useful for

detecting more and smaller lymph nodes. Figure 1 shows that only 26% of lymph nodes smaller than 5 mm could be detected by the traditional method in our study, and the rest of the small lymph nodes were detected by using LNRS. Although metastatic incidence is higher in the large lymph nodes (13% versus 4%), more than half of the metastatic lymph nodes are small (Fig. 2). It is because of the large number of small lymph nodes. To evaluate the effect of LNRS on accurate staging of the patients, we compared the results of TNM staging before and after application of LNRS (Tables 1 and 2).

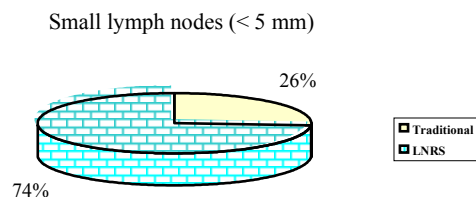


Fig. 1. Frequency of the small lymph nodes detected by the two methods

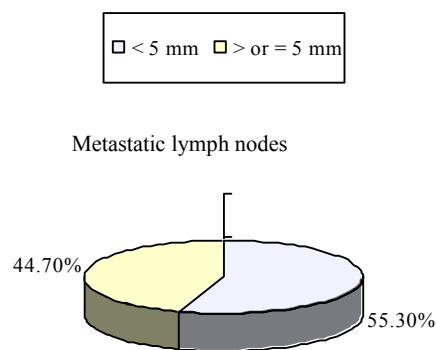


Fig. 2. Frequency of metastasis in small lymph nodes

Table 1. Comparison of the “N” of the TNM staging before and after LNRS application

		Primary N			
		Nx	N0	N1	Sum
Final N	N0	4	16	0	20
	N1	1	3	9	13
	N2	0	0	2	2
	Sum	5	19	11	35

Table 2. Results of TNM staging before and after LNRS employment

Final TNM	Primary TNM				
	1	2	3	Unclassified (Nx)	Sum
	1	5	0	0	1
2	0	11	0	3	14
3	0	3	11	1	15
Sum	5	14	11	5	35

DISCUSSION

Our study proved that LNRS could result in detection of significantly more and smaller lymph nodes as in other similar studies which used the same or different fat clearing methods (5-11). Only in one recent study LNRS failed to show statistically significant difference from the formalin in the numbers of total lymph nodes, positive lymph nodes, or minute (less than 1 mm) lymph nodes found (13). Three of our patients were upstaged from TNM stage 2 to 3 (i.e., from Dukes' B to C). Another study which was conducted by Romelia Koren (9) using the same solution, eight of the patients were upstaged from Dukes' B to C which is much higher than our results. In that study all the Nx cases were considered as N0, and that seems to be the cause of the observed differences between these two studies. We categorized the Nx cases as unclassified in the TNM staging. After application of LNRS we did not have any Nx cases. So all of our patients could be accurately staged by TNM or Dukes' classifications. More than half of the metastatic lymph nodes in this study were smaller than 5 mm. It calls attention to the importance of small lymph nodes, which can harbor metastasis. Since more than 70% of the small lymph nodes were detected only after employment of LNRS, this solution is valuable in detection of metastatic lymph nodes. Lymph node revealing solution is an easy to apply, rapid and inexpensive solution. The LNRS does not alter the quality of H&E slides. We recommend this method for accurate staging of colorectal carcinomas especially in Dukes' B stages and also when no lymph node is detected in the traditional method.

REFERENCES

- Morson BC, Dawson IP, Morson, Dawson's. Gastrointestinal pathology, Third edition, Blackwell Scientific Publications 1991; p: 597-619.
- Crissman JD, Zabro RJ. Histopathologic parameters and DNA analysis in colorectal adenocarcinomas. *Pathol Annu* 1989; 24: 103-147.
- Beahrs OH. Staging of cancer of the colon and rectum. *Cancer Supplement* 1992; 70: 1393-1396.
- Compton CC. Updated protocol for the examination of specimens from patients with carcinomas of the colon and rectum, excluding carcinoid tumor, lymphomas, sarcomas, and tumors of vermiform appendix. *Arch Pathol Lab Med* 2000; 124: 1016-1025.
- Newell KJ, Sawka BW. An inexpensive, simple, and effective aid for retrieval of lymph nodes from colorectal cancer resection. *Arch Pathol Lab Med* 2001; 125: 642-645.
- Gilchrist RK, David VC. Lymphatic spread of carcinoma of the rectum. *Ann Surg* 1938, 108: 621-642.
- Scott KW, Grace RH. Detection of lymph node metastasis in colorectal carcinoma before and after fat clearance. *Br J Surg* 1989 Nov; 76(11): 1165-1167.
- Hida J, Mori N. Metastasis from carcinoma of the colon and rectum detected in small lymph nodes by the clearing method. *J Am Coll Surg* 1994 Mar; 178(3): 223-228.
- Koren R, Annette Siegal, Lymph node-revealing solution, Simple new method for detecting minute lymph nodes in colon carcinoma. *Dis Colon Rectum* 1997; 40: 407-410.
- Koren R, Kyzer S. Lymph node revealing solution: a new method for lymph node sampling: results in gastric adenocarcinoma. *Oncol Rep* 1998 Mar-Apr; 5(2): 341-344.
- Utsun MO, Onal G. Lymph node revealing solution: is it effective on detecting minute lymph nodes? *Adv Clin Path* 1999 Oct; 3(4): 135-138.
- Herrera OL. Metastasis in small lymph nodes from colon cancer. *Arch Surg* 1987 Nov; 122(11): 1253-1256.
- Jan YJ, Huang PC. Lymph node revealing solution and traditional 10% buffered formaldehyde for detecting lymph nodes in colorectal carcinoma. *Chung Hua I Hsueh Tsa Chih (Taipei)* 2000 Feb; 63(2): 131-137.