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Early results of ex-vivo sentinel lymph node mapping in patients with early-stage colorectal carcinoma

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ABSTRACT

Correct determination of lymphatic nodal statement is necessary to stage accurately and to predict survival. As it is so critical to make an evaluation about the adjacent lymph node(s) this study was designed to make a sensitive detection on the sentinel lymph nodes (SLN) indicating tumoral lymphatic basin by using ultrastaging pathologic examination. From June 2002 to June 2003, this prospective study was performed in 46 patients undergoing standard resection for colorectal cancer. In this study, we employed the ex-vivo SLN mapping technique. At least one SLN in 37 of 41 patients was identified (90.2%). The lymph nodes (LN) from those patients were utilized by Hematoxylin and Eosin dye (H&E) and multisectioning. Then 20/37 patients with trace of the metastasis were found. Remaining 17 patients without any metastatic LN by H&E were applied to clarify micrometastases (MM) by using immunohistochemical (IHC) staining technique. 2 patients (11.7%) had MM in the SLN(s). Then upstaging was evaluated in those 2. The sensitivity of SLNs was obtained as 90%. Two patients with no metastatic SLN had metastasis in the non-sentinel LNs. MM in the SLN identified by IHC in the patients with CRC is still not obvious to display bad prognosis. But upstaging and the need for treatment alteration in those patients were obvious. [Turk J Cancer 2005;35(1):12-18].

KEY WORDS:

Colorectal cancer, lymphatic mapping, ultrastaging, immunohistochemical staining

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in the world and represents the sixth most frequent reason of cancer death in Turkey (1). As a treatment modality a different type of surgical resection and end-to-end anastomosis is selected in the first step. But in rectal tumors neoadjuvant chemotherapy or chemo-radiotherapy are employed as the first step treatment with significant decrease in local recurrences (4-11%) (2). Chemotherapy alone could increase the 5-year survival about 20-30%. Lymph node involvement is a foremost prognostic factor affecting the stage of CRC. Five year survival rate is as high as 90% after Stage I (AJCC; American Joint Committee of Cancer) but decreases substantially as 50% in stage III (3). The manifestation of lymphatic basin has vital effect on the prognosis. It was reported that CRC even in early stage without lymphatic invasion in the basin has nearly 20% of recurrence (4). Furthermore 5 year survival was also

shortened. So it is so critical to make an evaluation about the adjacent lymph node(s). Sensitive detection methods and reporting of significant micrometastases (MM) in the sentinel lymph node(s) (SLN) are necessary to make true staging of colorectal cancers. Previous studies have demonstrated that lymph node micrometastases documented by ultrastaging correlate with poorer prognosis (5,6).

The lymph nodes harvested by the surgical procedure are almost 15-20 nodes per case. Pathologists take 1 or 2 section for each lymph node and stained with Hematoxylin and Eosine (H&E) stain. It is not feasible to screen all lymph nodes by using advanced pathologic techniques. Thus the researchers try to find sentinel lymph node(s) popularized by Giuliano (5) in melanomas. Sentinel lymph node is the first node to collect the lymphatic drainage from primary tumor and is most likely to include metastasis. Bilchik et al. (6) and Saha et al. (7) have utilized the SLN mapping in CRCs. The aim of this study is to show whether viability of lymphatic mapping in CRC improves staging by advanced pathologic techniques.

PATIENTS AND METHODS

From June 2002 to June 2003, this prospective study was performed in consecutive 46 patients undergoing surgical resection for colorectal cancer. As first five patients were admitted in the training period they were not included in this study. Eighteen patients were female and 23 were male. Mean age of the patients was 63 (31-86). The cases with advanced stage CRC according to Astler-Coller classification, presenting mechanical bowel obstruction due to the bulky lesion into lumen, the cases with long distance organ metastases and the cases operated in emergency condition for perforation of the bowel were also excluded. All patients were instructed to get the standardized preoperative assessment including digital rectal exam with abdominal ultrasonography (US), computed tomography (CT) and colonoscopy and rigid rectosigmoidoscopy. Then under tomographic evaluation, an attempt was made to stage CRC preoperatively. All patients were approached via open surgical procedures such as low anterior resection, abdomino-perineal resection and segmental resection + end-to-end anastomosis (Table 1). Informed consent form was obtained in the preoperative period from each patient in accordance with the rules of local Ethics Committee of Istanbul University.

Ex-vivo SLN mapping technique

Ex-vivo sentinel lymphatic mapping was admitted as the main attempt of this study. Our technique was similar to that of Wood et al. (8). After the surgical procedure was completed, the specimen was instantly taken to an extra table in the operating room. It was performed just after the specimen was taken out. The colonic specimen was incised longitudinally on the antimesenteric side. The rectal specimen was incised on the anterior border across the mesorectum. Lymphatic mapping was employed on the specimen by using 1 ml 1% Patent blue V dye (Guerbet Lab., France) subserosally and submucosally around the tumor (peritumoral site was employed) by using tuberculin syringe. After 5-7 minutes of massage with little circulatory movements on the lesion, the dye moved into the lymphatic paths to the SLN(s) in the mesentery. By low level diathermy, sharp dissection of lymphatic path(s) to the SLN(s) was existent under more care. Each sentinel lymph node was removed from the basin and marked before the specimen was submitted for pathologic appraisal (Figure 1).

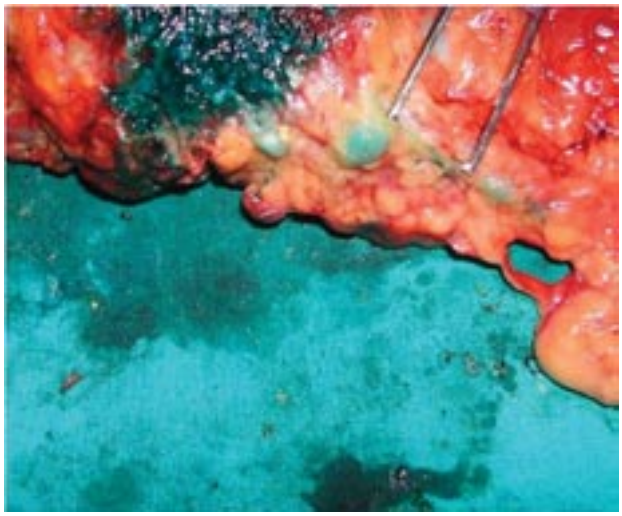

Fig 1. A photograph of the specimen which was prepared with dying

Histopathologic procedure

Pathologic analysis entailed routine microscopic examination of the tumor, margins and LNs. Lymph nodes were manually dissected from the mesenteric fat. No chemical clearance method was employed. Each identified LN and SLN more than 5 mm was bisected and embedded in paraffin. Single section was routinely performed. Slices

Table 1
The patients' operational characteristics and tumor localization

Localisation of the lesions	No. of Patients	Types of Operations	SLNs & MM (IHC)	Postoperative staging (Astler-Coller) (H&E)	(IHC)	Postoperative staging (TNM) (H&E)	(IHC)
Ascending colon	3	A	2/4	B2	C2	T4N0M0	T4N1M0
			0/3	B2	B2		
			0/2	B1	B1		
Transverse colon	4	A	-	B1	B1		
			0/3	C1	C1		
			0/4	B1	B1		
			0/2	B2	B2		
Descending colon	7	A	0/2	B2	B2		
			0/3	B2	B2		
			0/6	C1	C1		
			0/3	C2	C2		
			0/7	B1	B1		
			0/4	B2	B2		
			0/2	B2	B2		
Sigmoid colon	11	A	-	C2	C2		
			0/3	C1	C1		
			0/2	B1	B1		
			0/1	C1	C1		
			0/2	B2	B2		
			0/4	C1	C1		
			0/6	C1	C1		
			0/2	B1	B1		
			0/5	B1	B1		
			0/3	B2	B2		
Rectum							
Upper 1/3	8	B	0/4	B1	B1		
			0/10	C1	C1		
			2/5	B2	C2	T3N0M0	T3N1M0
			0/4	B2	B2		
			0/4	B1	B1		
			0/1	B2	B2		
			-	B2	B2		
			0/5	B1	B1		
Mid 1/3	3	B	0/4	B2	B2		
			0/1	C1	C1		
			0/2	B2	B2		
Lower 1/3	5	C	0/4	C2	C2		
			0/1	B2	B2		
			0/1	B2	B2		
			0/3	B2	B2		
			0/3	B2	B2		

TME: Total mesorectal excision

A: Hemicolectomy + ileotransversostomy, Segmenter resection + end-to-end anastomosis, Total colectomy + ileorectal anastomosis

B: Sphinckter preserving resection +, anastomosis/w stapler +TME, Abdomino-Perinal Resection (APR)

C: Abdomino-Perinal Resection (APR)

were stained by H&E staining. If the result (after two faces of the LN bigger than 5 mm and only one face for LN smaller than 4 mm was observed) was negative all SLN's paraffin blocks were sectioned in multiple slices of 4 microns thick. Slices apart from each other 200 microns in length were also stained by H&E stain in second step of pathological evaluation. When no metastasis was experienced in multi-sectioned slices further analysis as immunohistochemical (IHC) staining was utilized to search metastasis and/or MM.

Immunohistochemical staining

A single paraffin section was stained with antibodies (Pan-Keratin AE1/3, CAM 5.2®, Beckton-Dickinson, San Jose, California - 35 bH11; prediluted Vantana Medical System Inc. Tuscon, AZ).

Then H&E and IHC staining and multi-sectioning were employed for LNs harvested as SLN. Isolated tumor cells, micrometastasis and any metastatic foci was evaluated in the thinner level of the SLNs. A false negative SLN was described as a SLN containing no tumor cell while one or more LNs in the specimen were positive for tumor. Upstaging was determined as pN1 in the patients with SLN stained by IHC while those LNs had no metastatic deposit by using H&E stain.

RESULTS

In 25 patients primary tumors were localized in the colon (3 in cecum and ascending colon, 4 in transverse colon, 7 in descending and 11 in sigmoid colon) while only sixteen were found in the rectum (Table 1). From 41 specimens 351 LNs were harvested during the pathologic clearance. One-hundred and twenty-five LNs were defined as SLNs. Three SLN per patient and 5.5 non-SLNs per patient was gathered. In 37 of 41 patients SLN(s) was identified (90.2%). No SLN was harvested in the remaining 4 patients (9.8%). Those mostly were in the first 10 patients. In the first step of pathological staining technique, all SLNs cut out 1-2 slices were colored by H&E stain. Twenty patients demonstrated metastases in the SLN(s). The other 17 had no metastasis on SLNs by using H&E stain. The SLNs from 17 patients were evaluated by multi-sectioning, which did not reveal any trace of metastasis. The last step of pathological staining techniques which we used in this study was the anti-cytokeratin antibody for IHC staining (Pan-Keratin AE 1/3, CAM 5.2) applied to clarify metastasis and/or MM.

By using IHC staining technique 2 patients had MM in the SLN(s) despite of the fact that there was no metastasis and MM after H&E staining and multi-sectioning. The exact stage of CRC in 2 patients was upstaged (11.7%). Then the chemotherapy protocol was changed. In the 4 patients with no SLN harvested one had no LNs in the tumoral lymphatic basin. In the remaining 3 patients, two without SLN had metastasis in the non-SLN (from tumoral basin via H&E stain), which was evaluated as a false negative result (10%) (Table2).

DISCUSSION

In even early CRC with no LN metastasis, about 20 percent of local recurrence was appeared in first two years after surgery. Several studies showed that the metastatic lesion or tumor cells remained in the tumoral lymphatic basin which was the reason for unexpected local recurrences (3,9,10).

Joseph et al. (11) stated that about 40 LNs in the tumoral lymphatic basin should be evaluated to make a true staging for T1-T2 CRC. Furthermore it was declared by UICC that 12 LNs must be examined in depth in a specimen's lymphatic basin for this purpose (12). Some authors such as Bilchik (12), Joseph (11) and Koren (13) stated that all LNs taken from the basin were not evaluated enough in routine pathologic manner. Because there have been more than 15-20 LNs coming with each specimen detailed examination of those requires more time and that is not cost effective. In routine pathologic examination about 85-90% of harvested LNs set down not investigated (6,11-13). Joosten et al. (14) in 1997 declared the sentinel lymph node mapping in CRC in the meeting of Society of Surgical Oncology. The authors such as Wood (8) and Feig (15) dealing with the SLN biopsy (SLNB) then suggested that SLNB has been a sensitive and analytical process of pathologically staging patients with CRC. SLNB of the basin achieved 93-95% of accuracy by using advanced pathologic techniques. Though multiple sectioning and IHC staining are too time-consuming and expensive for examination of

Table 2
The overall results in this study

Characteristics of lymph nodes (LNs)	n	%
Number of whole LNs	351	
Number of SLNs	125	35.6
Mean number of SLNs per patient	3	
Mean number of non-SLNs per patient	5.5	
Mean number of SLN with metastasis per patient	2.1	
Patients with no LNs	1	2.4
SLN and upstage characteristics		
Patients with SLN	37	90.2
Number of patients without harvested SLN	4	9.8
Number of SLNs with metastasis by H&E	74/125	59.2
Number of SLNs without metastasis by H&E	51	40.8
Number of SLNs with MM by IHC	4/51	7.8
Number of patients cancer stage upgraded after using IHC	2	11.7
Number of patients without SLN metastasis, but with non-SLN metastasis by IHC (False negativity)	2	10

SLN: Sentinel lymph nodes
H&E: Hematoxylin and Eosin dye
LN: Lymph nodes
MM: Micrometastases
IHC: Immunohistochemistry

all LNs, these ultrastaging pathologic techniques could be cost effective for 2 to 4 SLNs. But debate on regarding both accuracy and significance of SLNB is present. In CRC, SLNB is used to improve staging unlike in breast cancer and melanoma in which SLNB is used to evade unnecessary radical lymphatic dissection. The advantages achieved in patients with breast cancer and melanoma that abolishing the morbidity of regional LN dissection besides improved staging of disease with MM may not have a significant impact in patients with CRC (14-16). More recent studies, in CRC, have reported decreasing survival in the patients with MM, which were appraised as pN1 but isolated tumor cell (ITC) was received as pN0 (3,4,12,17,18). At the same time, the authors such as Adell, Broll, Cutait and Jeffers (19-22) stated the presence of MM had no significant effect on survival. But survival in true node negative stage II CRC could be better than that with MM.

Pathological assessment of regional LNs gives the most important knowledge for decision making regarding adjuvant therapy in CRC providing a survival benefit for patients with positive LNs but no benefit to those with negative LNs. SLNB for CRC provides an efficient means of scrutinizing the regional lymph node basin more in the patients with CRC. Thus, it better evaluates the stage of the CRC and determines the patients having benefit from additional therapy (10, 23,24).

In this study we did perform SLN mapping by using blue dye. SLNs were exposed in 37 patients (90.2%). The mean number of SLN was 3. Upstaging of CRC was revealed in 2 patients by IHC staining (11.7%). False negative result were also revealed in 2 patients (10%) (Table 3). The reasons of false negativity may depend on small number of patients in this series, atypical localization of SLNs, or whole invasion of SLN by tumor cells that does not allow blue dye. Four patients in first 10 cases in the study had no SLN exposed. In fact no LN was assessed in 1 of 4 patients. That should be correlated with inattentive surgical techniques or erroneous pathological cleansing methods. SLNB indicating the basin with 90% of accuracy was found in this study.

Table 3
The comparison of the outcomes between several studies and ours

Study	No. Pt.s	Technique	Accuracy of SLN (%)	Mean No. SLN	Up-staged (%)	Sensitivity of SLN (%)	IHC	Multi- section	RT-PCR
Joosten (1999)	50	Hybrid	70	3	-	66	+	-	-
Saha (2000)	86	In vivo	98.8	1.6	8.2	96	+	+	-
Bilchik (2001)	40	In vivo	100	2	17.5	100	+	+	+
Wong (2001)	26	Ex-vivo	92	2.8	16.7	96	+	+	-
Wood (2001)	75	Ex-vivo	96	2	17	94	+	+	-
Fitzgerald (2002)	26	In vivo/Ex-vivo	88	2.5	8.7	91	-	+	-
This study	41	Ex-vivo	90.2	3	11.7	90	+	+	-

CONCLUSION

SLNB can be performed in an easy way in CRC with high degree of success rate similar to that achieved in breast cancer and melanoma. As it is known, SLNB in CRC is not intended to minimize extent or morbidity of dissection as in those diseases. SLNB currently helps to concentrate on pathologic examination of SLN more accurately indicating the tumoral basin to detect evidence of micrometastases which is missed by usual pathologic techniques in a significant percentage of patients with early-staged CRC. Upcoming studies are needed to conclude the prognostic evidence of nodal micrometastatic invasion in CRC.

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