Using low concentration sodium hypochlorite to improve colorectal surgical specimen lymph node harvest

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Received February 1, 2019; Accepted January 1, 2020

DOI: 10.3892/mco.2020.2018

Abstract. Lymph node (LN) retrieval is a critical procedure to determine the pathological stage and adjuvant therapy for colorectal cancer. The present study aimed to recommend a novel method by using sodium hypochlorite to improve colorectal surgical specimen LN harvest. Dissolving time of mesenteric LNs and fat tissue was firstly investigated in different concentrations of sodium hypochlorite. In the sodium hypochlorite group, 65 patients with colorectal cancer who underwent curative surgery were included. After standard manual gross dissection, the mesenteric tissue was subsequently immersed in 1% sodium hypochlorite for ~30 min, and then manual dissection was again applied for additional LN harvest. In the manual method group, 68 patients with colorectal cancer were selected and only manual dissection method was applied for LN harvest. The number of LNs in both groups were recorded for each case. Sodium hypochlorite could dissolve fat tissue significantly faster than LNs and the low concentration sodium hypochlorite had the maximum dissolving time difference between fat tissue and LNs (P<0.001). After sodium hypochlorite treatment, more LNs were identified when compared with the manual dissection method (28.2±12.1 vs. 16.5±8.7; P=0.010), whereas the number of positive LNs had no significant statistical difference between the two groups (P=0.181). After sodium hypochlorite immersion, 818 additional LNs (12.5±4.8 per case) were identified and LNs ≤2 mm were 58.4% (478/818). Moreover, 16 additional metastatic LNs were found in 10 patients. A total of 7 of them were upstaged, including 2 initially N0 cases. Using sodium hypochlorite at low concentrations may be the most simple, rapid, cost-saving, nontoxic and effective technique to improve LN harvest in colorectal carcinoma specimens so far. This method should be used routinely regardless of whether the number of LNs is <12 or not.

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Key words: sodium hypochlorite, colorectal cancer, lymph nodes

Introduction

The lymph node (LN) status largely dictates prognosis and adjuvant therapy strategy for colorectal cancer patients. The NCCN guidelines recommend that a minimum of 12 LNs should be dissected to accurately stage colorectal cancer (1). Even though larger LNs are more prone to having metastatic deposits, the presence of metastases could also be identified in small measuring nodes (1 mm, 6.5%; 2 mm, 12.4%; 15.3%, 3 mm) (2). These small LNs are hard to be found and may lead to downstage of tumors. Some patients who might benefit from adjuvant therapy were misclassified as node-negative due to incomplete sampling of LNs (3). Therefore, researchers suggested that more nodes should be examined to increase the likelihood of proper staging (3-6). The number of LNs retrieved from colorectal cancer specimens are influenced by multiple factors, including age of the patient, sex, tumor grade, tumor site, neoadjuvant radiotherapy, operating surgeon and examining pathologist (7-9). One of the most important reasons is that very small LNs are difficult to find, especially amid large amounts of pericolic/perirectal fat.

Various methods mainly concerning fat clearance have been recommended to increase LN harvest (10-14). However, these traditional fat clearance techniques are noxious, time-consuming, costly and troublesome. It cannot be widely used in clinical practice, only for cases in which few LNs are initially identified.

Sodium hypochlorite is the most commonly used irrigating solution in endodontics because of its antimicrobial effect and tissue dissolution capacity. The antimicrobial activity is related to bacterial essential enzymatic sites promoting irreversible inactivation and the chloramination reaction. The dissolution action can be observed in the saponification reaction when sodium hypochlorite degrades lipids and fatty acids resulting in the formation soap and glycerol (15). In this study, we intend to investigate the use of sodium hypochlorite to clear pericolic/perirectal fat and improve harvest of LNs.

Materials and methods

Dissolving time of LNs and fat tissue in different concentrations of sodium hypochlorite. The mesentery is now recognised as an organ composed by the tissues of vessels, lymphatic, nerve and adipose (16). Since sodium hypochlorite dissolved organic tissue unselectively, we firstly investigated the dissolving time of different tissues from mesocolon or mesorectum. LNs and fat tissue were obtained from fresh colorectal surgical specimens. LNs diameter (length x width) measuring about 3x2 and 10x5 mm were separately chosen. Because the exposed surface area had a great impact on the dissolving capability of sodium hypochlorite, pericolic/perirectal fat were cut to produce samples of similar size and shape.

Sodium hypochlorite in concentrations of 1, 3 and 5.25% were commercially purchased and respectively tested at room temperature. Specimens from each group were individually immersed in plastic specimen bags filled with 50 ml of the test solution, then placed without mechanical agitation. The time of complete dissolution of LNs and fat were recorded. These procedures were repeated 5 times.

Properties of LNs after sodium hypochlorite treatment. Alterations of the chemical composition of the dentin have been reported after exposure to sodium hypochlorite (17). Here, we also focused on whether sodium hypochlorite would change the properties of LNs. LNs measuring about 5x5 mm with possible metastases was immersed in 1% sodium hypochlorite for 30 min and then was fixed in 10% formalin overnight. Afterwards, 4 μm sections were cut from the paraffin-embedded blocks for hematoxylin and eosin (H&E) examination.

Case selection and specimen treatment. Between January 2018 and June 2018, 65 colorectal cancer patients who underwent either open or laparoscopic radical surgery at the Affiliated Cancer Hospital and Institute of Guangzhou Medical University were included in this study. All cases were without distant metastases and had not received preoperative chemoradiotherapy. Patients with recurrent tumors were excluded. The surgical procedure was conducted according to the standard of total or complete mesocolic excision.

LNs of these colorectal resection specimens were firstly harvested by traditional manual gross dissection method. After standard manual gross dissection, the bulk of the mesentery was dissected from the bowel wall and tumor. The mesentery immediately related to the tumour was left *in situ* since this was to be examined for gross evidence of circumferential resection margin. The tumor/bowel was fixed in 10% buffered neutral formalin solution for 24 h before embedded, whereas the entire remaining mesenteric tissue was separately immersed in approximately three times its volume of 1% sodium hypochlorite for 30 min. After the fat was 'washed' by sodium hypochlorite, manual dissection method was again applied for the visible LN harvest. In order to reduce opportunity for operator bias in the dissection process, only one experienced staff, S.C., performed dissections both before and after immersion in sodium hypochlorite. The number and size of LNs were recorded for each case. All of the LNs were fixed in 10% buffered neutral formalin as mentioned above. After paraffin embedding, 4 μm thin sections were cut, stained with H&E and then scanned for metastases.

In addition, 68 patients who were treated for colorectal cancer from July 2017 to December 2017 were identified from our collected database. All of these patients were neither with distant metastases nor given chemoradiotherapy before radical operation. Patients with recurrent tumors were also excluded. The manual dissection method without fat clearance was applied to the surgically removed specimens for LN harvest. For these selected patients, the number but not the size of LNs had been recorded.

The present study protocol was approved by The Affiliated Cancer Hospital and Institute of Guangzhou Medical University Ethical Committee. All selected patients provided written statements of their informed consent and the Ethical approval was granted.

Statistical analysis. Statistical analysis was conducted using the statistical software SPSS 17.0 (SPSS, Inc.). Measurement data were compared by the independent-samples t-test or the one-way ANOVA followed by Student-Newman-Keuls post hoc test. The associations between categorical data were performed using the χ^2 test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Sodium hypochlorite dissolved fat tissue significantly faster than LNs. The complete dissolving time of small fat tissue (3x2 mm) in 1, 3 and 5.25% sodium hypochlorite was 13.6±1.1, 6.8±0.83 and 5.6±0.54 min respectively, while it was 120.6±5.6, 48.0±1.8 and 29.0±3.8 min respectively for small LNs (Table I). Moreover, the complete dissolving time of bigger fat tissue (10x5 mm) in 1, 3 and 5.25% sodium hypochlorite was 27.0±4.0, 18.6±1.1 and 14.8±0.83 min, while it was 474.0±19.4, 118.0±8.3 and 97.0±6.7 min for bigger LNs (Table II). These results indicated that sodium hypochlorite dissolved fat tissue significantly faster than LNs ($P<0.001$).

Though high concentration sodium hypochlorite dissolved fat tissue and LNs more quickly than low concentration sodium hypochlorite, the low concentration sodium hypochlorite had the max dissolving time difference between fat tissue and LNs ($P<0.001$, Table I). So 1% sodium hypochlorite was chosen for further study in order to avoid rapid destruction of LNs.

Sodium hypochlorite would not change the properties of LNs. H&E staining examination showed that LNs immersed in 1% sodium hypochlorite would not change their biological characteristics in a short period of time (Fig. 1).

Sodium hypochlorite significantly improved LN harvest. Clinicopathological details in relation to the two different dissection methods were summarized in Table II. There were no significant differences in sex, age, body mass index (BMI), tumour size or tumour location between the two groups ($P>0.05$). Moreover, no significant association was found when histologic types, T and N stage were taken into account ($P>0.05$). The very small LNs became easily visible

Table I. Dissolving time of lymph nodes and fat tissue in different concentrations of sodium hypochlorite (min).

Different concentrations of sodium hypochlorite	Lymph node, mean ± SD (n=5)	Fat tissue, mean ± SD (n=5)	P-value[a]
3x2 mm			
1% Sodium hypochlorite	120.6±5.6	13.6±1.1	<0.001
3% Sodium hypochlorite	48.0±1.8	6.8±0.83	<0.001
5.25% Sodium hypochlorite	29.0±3.8	5.6±0.54	<0.001
P-value[b]	<0.001	<0.001	
10x5 mm			
1% Sodium hypochlorite	474.0±19.4	27.0±4.0	<0.001
3% Sodium hypochlorite	118.0±8.3	18.6±1.1	<0.001
5.25% Sodium hypochlorite	97.0±6.7	14.8±0.83	<0.001
P-value[b]	<0.001	<0.001	

[a]t-test; [b]one-way ANOVA.

Table II. Clinicopathological features of patients included.

Variables	Sodium hypochlorite group (n=65)	Manual method group (n=68)	P-value
Sex			0.929
Male	32 (49.2%)	34 (50.0%)	
Female	33 (50.8%)	34 (50.0%)	
Age, years	56.6±11.3	56.2±11.8	0.568
BMI	21.2±4.7	20.7±4.6	0.851
Tumor size, cm	4.3±1.8	4.1±1.6	0.414
Location			0.832
Right colon	16 (24.6%)	20 (29.4%)	
Transverse colon	3 (4.6%)	4 (5.9%)	
Left colon	6 (9.2%)	9 (13.2%)	
Sigmoid colon	20 (30.8%)	17 (25.0%)	
Rectum	20 (30.8%)	18 (26.5%)	
Histologic type			0.651
Well differentiated adenocarcinoma	27 (41.5%)	23 (33.8%)	
Moderately differentiated adenocarcinoma	24 (36.9%)	29 (42.6%)	
Poorly differentiated adenocarcinoma	14 (21.5%)	16 (23.5%)	
T stage			0.818
T1	5 (7.7%)	5 (7.4%)	
T2	13 (20.0%)	16 (23.5%)	
T3	23 (35.4%)	19 (27.9%)	
T4	24 (36.9%)	28 (41.2%)	
N stage			0.166
N0	14 (21.5%)	16 (23.5%)	
N1	24 (36.9%)	34 (50.0%)	
N2	27 (41.5%)	18 (26.5%)	
Total lymph node harvest	28.2±12.1	16.5±8.7	0.010
Positive lymph node harvest	3.0±2.3	2.3±2.1	0.181

after sodium hypochlorite treatment (Fig. 2). When the total number of LNs was compared, the sodium hypochlorite group increased more LN harvest than manual method group (28.2±12.1 vs. 16.5±8.7, P=0.010). However, the number of

Table III. Changes in the staging of 10 patients after sodium hypochlorite treatment.

Case	Number of LNs (+) before sodium hypochlorite treatment	Initial N stage	Number of extra LNs (+) after sodium hypochlorite treatment	Final N stage
1	3	N1b	1	N2a
2	3	N1b	3	N2a
3	4	N2a	1	N2a
4	0	N0	1	N1a
5	0	N0	2	N1b
6	6	N2a	2	N2b
7	6	N2a	1	N2b
8	5	N2a	2	N2b
9	4	N2a	2	N2a
10	5	N2a	1	N2a

LNs, lymph nodes.

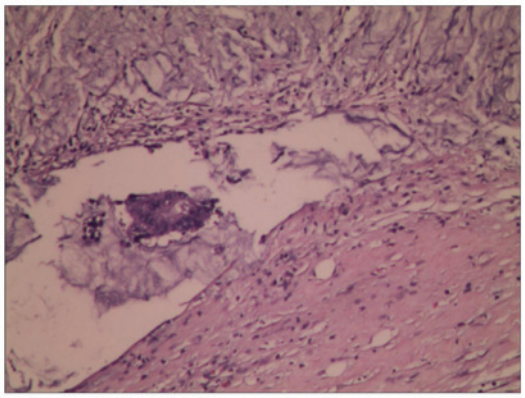

Figure 1. H&E examination indicated tumor cell infiltration in the lymph nodes after 1% sodium hypochlorite treatment for ~30 min. Magnification, x100. H&E, hematoxylin and eosin.

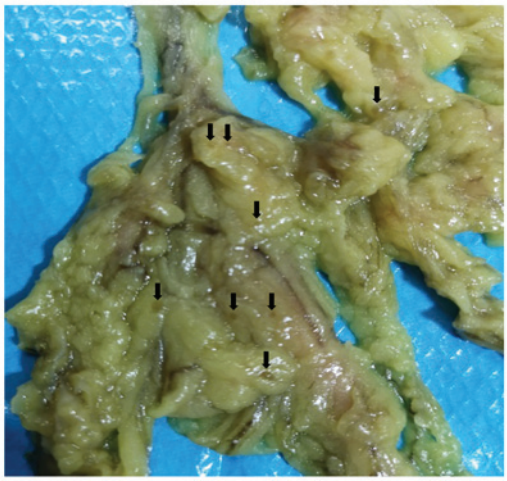

Figure 2. Very small lymph nodes were easily visible after 1% sodium hypochlorite treatment for ~30 min. The black arrows indicate very small lymph nodes.

positive LNs did not increase in the sodium hypochlorite group when compared with manual method group (P=0.181).

Within the sodium hypochlorite group, a total of 1,020 LNs (15.6±8.7 per case) were revealed in initial inspection. After treated in sodium hypochlorite for 30 min, 818 additional LNs (12.5±4.8 per case) were identified, which account for 44.5% of all the LNs revealed in this group (Fig. 3). LNs ≤2 mm were 9.6% (98/1,020) and 58.4% (478/818) separately before and after sodium hypochlorite treatment. Use of sodium hypochlorite method could obviously increase LN harvest, especially those smaller than 2 mm (Fig. 3).

After treated with sodium hypochlorite, 16 additional metastatic LNs were found in 10 patients. Seven of the 10 patients were upstaged, including 2 initially N0 cases (Table III).

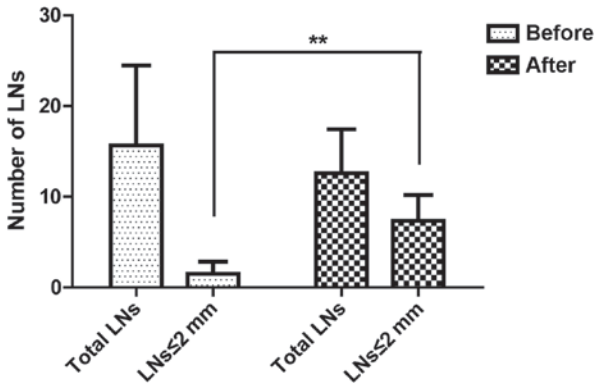

Figure 3. Sodium hypochlorite increased LN harvest, especially those <2 mm. **P<0.01. LN, lymph node.

Discussion

LN size may not be a reliable indicator for LN metastasis. Studies had showed that most nodal metastases in colorectal cancer were found in small LNs (5 mm or less in diameter) which were often missed during routine dissection of specimens (2,18-21). Brown *et al* even reported that 75% of all positive nodes were under 2 mm in size (6). Furthermore, the total number of LNs investigated was considered an independent risk factor no matter metastases were present or not (22).

Thus, the detection of the largest possible number of LNs and a complete pathologic assessment of nodal status are essential for accurate staging, therapeutic decisions, and prognosis of patients (3,23).

Manual LN dissection is the current standard method at most institutions and it depends on visual isolation and palpation of the mesenteric tissue. It is certainly that smaller LNs could be found using manual techniques by experienced personnel who take appropriate time. Sampling the entire pericolic fat is theoretically the most accurate method for total lymph node examination. However, very small lymph nodes are rarely found and it requires time, money and is not practical in routine surgical pathology (11,24,25). So, many researchers suggested that additional techniques combined with manual dissection could help to increase small LN harvest more easily. Fat clearance method has been recently recommended for the detection of small or inconspicuous LNs. However, this technique requires the use of sequential immersions of the mesenteric tissue in baths of alcohol, xylene, followed by methyl salicylate. These solutions are noxious and the fat has to be dissected in a ventilated cabinet using transillumination to locate the LNs. In addition, the clearance process takes dozens of hours or even weeks and causes an unacceptable reporting delay (10,26). Furthermore, serial sectioning of the cleared mesenteric tissue at 1-2 mm intervals is necessary to reveal small LNs. Nodes may be probably cut into 2 halves during multilevel step sectioning process, resulting in error of LN count. The disadvantages of fat clearance precludes its use in routine pathological assessment, only for cases in which an unacceptably low number of LNs are retrieved (27).

Other techniques such as LN revealing solution (11,28), sentinel node procedures with methylene blue injection (29), and fat-dissociation method using enzymes (25) are applied to increase the yield of LNs. However, these methods are not used routinely largely because they are hazardous, time-consuming and expensive and do not provide rapid pathology report.

Sodium hypochlorite is still the most commonly used irrigation solution for endodontic procedures because of their characteristics such as wide-spectrum antimicrobial activity and organic tissue dissolution capacity (17). The tissue-dissolving capability of sodium hypochlorite relies on its concentration, volume, contact time of the solution, the surface area of the exposed tissue, pH, temperature, and mechanical agitation (30,31). Here, we showed that sodium hypochlorite could easily dissolve fat tissue when compared with LNs even in a low concentration at room temperature without agitation.

High concentrations may be more toxic and dissolve the whole mesenteric tissue with no dissolving time difference, while the low concentration sodium hypochlorite has the maximum dissolving time difference between fat tissue and LNs. In addition, H&E examination indicated that low concentration sodium hypochlorite avoided changes in tissue composition of LNs. This study found that the use of sodium hypochlorite at lower concentration, such as 1%, demonstrated to be effective in promoting a suitable dissolution of mesenteric fat and preventing a pronounced damage to LNs.

We had showed that 1% sodium hypochlorite had the maximum dissolving time difference between fat tissue and LNs, and the complete dissolving time of bigger fat tissue (10x5 mm) in 1% sodium hypochlorite was about 30 min (27.0±4.0 min, Table I) without change in the properties of LNs. So, we considered that 30 min would be the appropriate processing time. After 1% sodium hypochlorite immersion for about 30 min, the perienteric adipose tissue was cleared and the LNs were easily visible. The sodium hypochlorite group detected more LNs in total than manual dissection method group. More LNs harvest means more accurate pathological staging. Though the number of positive LNs did not significantly increase after sodium hypochlorite treatment, this method is still meaningful because it is not a question about 'significant statistical difference' but a question about 'have or have no'. Even a single additional LN metastasis identified will be sufficient to upstage the malignancy from pN0 to pN1 when adjuvant therapy is required.

Furthermore, 818 additional LNs were revealed after sodium hypochlorite treatment, which account for 44.5% of all the LNs found in sodium hypochlorite group. In line with other assistant techniques (6,32), sodium hypochlorite solution was greatly helpful in detecting small LNs and most of these additional LNs (58.4%) were smaller than 2 mm. Besides, 16 additional metastatic LNs were found in 10 cases after sodium hypochlorite treatment and the stage of the disease was upgraded in 7 of the 10 cases. More importantly, additional metastatic LNs had been found in 2 initially pN0 cases, resulting in upstaging from TNM stage II to stage III, implying that postoperative adjuvant chemotherapy had to be given.

However, there were still some limits in our research. The sample number of this study was small. Also, because it was a retrospective study, the size of lymph nodes for the control group had not been recorded before and the data cannot be gathered. In order to reduce opportunity for operator bias in the dissection process, only one experienced person performed dissections both before and after immersion in sodium hypochlorite.

For the first time, this study indicates that sodium hypochlorite at low concentration can be used to reveal LNs in colorectal carcinoma specimens. As we know, it may be the most simple, rapid (30 min), cost-saving (US $1.0), nontoxic and effective method to improve LN harvest so far. We suggest that sodium hypochlorite after manual dissection should be used routinely regardless of whether the number of LNs is less than 12 or not.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

NY and HL conceived and designed the present study, and gave final approval. JL and SC contributed to the data analysis and

interpretation. SC was responsible for provision of the study material and wrote the manuscript. All of the authors revised the manuscript critically. All authors read and approved the final draft of the manuscript.

Ethics approval and consent to participate

The present study protocol was approved by The Affiliated Cancer Hospital and Institute of Guangzhou Medical University Ethical Committee. All selected patients provided written statements of their informed consent and the Ethical approval was granted.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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