Improving lymph node harvest in rectal cancer by intra-arterial injection of methylene blue: A randomized trial

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Abstract: Background: Prognosis in colorectal carcinoma is related to the state of lymph node involvement. Studies demonstrate that both survival and prognosis are significantly influenced by the number of lymph nodes harvested, particularly in node-negative disease. **Aim**: The aim of this study was to evaluate the usefulness of injecting methylene blue into the main artery/ arteries of resected colorectal specimens in terms of the total number of lymph nodes identified. **Patients and methods**: The study included 30 patients randomly divided into two groups: group 1 (15 patients), in which resected specimens of colorectal carcinoma were injected with methylene blue, and group 2, in which no injection was carried out. **Results**: The total number of lymph nodes per patient in group 1 was 19.5 (17–39) [median (range)] and that in group 2 was 16.5 (8–19). The difference was statistically highly significant ($P < 0.001$). We also noticed that the best improvement in lymph nodes harvest was among the very small and small lymph nodes. **Conclusion**: Methylene blue injection into the main artery/arteries is an effective and simple method for improving the lymph node harvest in resected specimens of colorectal carcinoma.
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1. Introduction

Prognosis in colorectal carcinoma is clearly related to the degree of tumor infiltration through the bowel wall and the presence or absence of lymph node involvement. These two factors are the basis of the staging systems established for this disease. Today, almost 80 years after Dukes published his pioneer classification; all attempts to improve his proposal are mostly based on refinements of these two data [1].

Many authors have advocated that a minimum of 12 lymph nodes are required for accurate colorectal cancer staging [2–4].

Most studies suggest that node positivity rates increase with increased nodal harvest [5–8].

In other words, the higher the number of lymph nodes we examine, the higher the number of positive lymph nodes we find [9].

Studies demonstrate that both survival and prognosis are significantly influenced by the number of lymph nodes harvested, particularly in node-negative disease [10–12].

In addition, the current paradigm of neoadjuvant chemoradiation for the treatment of rectal cancer complicates this issue because radiation is known to result in a decrease in the number of assessable lymph nodes [13].

Hence, the concept of improving lymph node identification to facilitate further pathological assessment was introduced.

Immunohistochemical staining and fat clearance methods using alcohol, acetone, and other substances seem to be efficient techniques for upgrading lymph node identification [14–16].

However, these methods are time-consuming, need additional equipment and expertise, and entail the use of hazardous materials [14].

Methylene blue injection is found to be superior to other dyes because of higher availability and affordability [17, 18].

This method was originally introduced by Märkl *et al*. [19]. They injected the superior rectal artery with methylene blue solution to improve lymph node harvest in rectal carcinoma [19].

In this study we carried the technique one step ahead by injecting methylene blue into the inferior mesenteric artery in rectal carcinoma cases only.

2. Patients and methods

This study was carried out between October 2016 and october2018 in Nasser Institute Hospital (Cairo, Egypt). The study included 30 consecutive patients who had undergone surgeries for colorectal carcinoma with a curative intent, both laparoscopic and open.

The patients were randomly divided into two groups: group 1, in which specimens were injected with methylene blue, and group 2, in which no injection was carried out. In group one, at the end of surgery and before removal of the specimen, the surgeon cannulated the main artery (arteries) with a

20-G plastic cannula and injected 15 ml of methylene blue dye (Merck, Darmstadt, Germany) (50 mg methylene blue in 5 ml distilled water, diluted in 10 ml 0.9% NaCl solution). The artery was then ligated, stained lymph nodes dissected and the specimen sent for histopathological evaluation following standard techniques (i.e. no special handling was done by the pathologist). Group 2 represented the control group, in which patients were operated upon by the same surgeons and the specimens were processed in the same way as group 1 and examined by the same pathologist.

Both groups were compared for age, sex, weight, total number of lymph nodes recovered, number of lymph nodes with malignant deposits, and the size of lymph nodes (≤1 mm, between 1 and 5 mm, and >5 mm). We recorded whether the patients received radiotherapy, the type and length of each specimen, and the TNM stage of each tumor.

Statistical analysis

Results were statistically analyzed using statistical package SPSS (version 16; SPSS Inc., Chicago, Illinois, USA). Student's *t*-test and the Mann–Whitney test were used for quantitative variables. The χ^2 and *Z* tests were used for qualitative variables. *P* values less than 0.05 were considered significant.

3. Results

Fifteen patients were assigned to group 1 (injection group) and 15 patients to group 2 (no injection group). Table 1 shows a summary of the findings from both groups. Both groups were homogenous with regard to age, grade, sex, length, prior chemoradiotherapy, and type of resection. None of our cases had undergone a total colectomy. In group 1, we encountered no case with a lymph node count less than 12; however, in group 2 all three cases who had received prior radiotherapy had a lymph node count of less than 12.

4. Discussion

Insufficient lymph node harvest is commonly attributed to an incomplete resection by the surgeon. Moreover, it is an indication for expensive chemotherapy with known side effects [20].

It is demonstrated that the total number of resected lymph nodes in colorectal cancer surgeries is an independent prognostic factor, as it is used as an indicator of the quality of surgery itself [10].

Table (1): The findings in the two groups

Point of comparison	Group one (*n* = 15)	Group two (*n* = 15)	*P*-value
Age (mean ± SD) (years)	55.26 ± 9.06	57.42 ± 6.61	0.319
Sex Male	8 (53.8)	8 (53.8)	–
Female	7 (46.2)	7 (46.4)	
Weight (mean ± SD) (kg)	76 ± 11.73	74 ± 12.54	0.228
Prior chemoradiotherapy	4 (15.4)	3 (10.7)	0.913
Length of specimen (mean ± SD) (cm)	23.48 ± 6.19	22.52 ± 7.30	0.605
TN stage T1	0 (0.0)	0 (0.0)	–
T2	2 (15.4)	4 (10.7)	0.913
T3	7 (53.4)	6 (71.4)	0.277
T4	6 (19.2)	5 (17.9)	0.819
N0	5 (53.8)	8 (42.9)	0.597
N1	4 (26.9)	5 (35.7)	0.687
N2	6(19.2)	2 (21.4)	0.890
Total number of lymph nodes recovered/patient [median (range)]	19.5 (17–39)	16.5 (8–19)	<0.001 (S)
Number of positive nodes/patient [median (range)]	6.40 (2–12)	3 (2–10)	0.043 (S)
Lymph node ratio	6/23 = 26	4/14 = 28.5	0.829
Size of lymph nodes (mm) <1	5 (1–8)	2.5 (1–3)	<0.001 (S)
Between 1 and 5	15 (11–22)	8 (3–11)	<0.001 (S)
>5	6 (3–8)	6 (3–8)	0.349

S, Significant.

It is understood that the accuracy of lymph node status assessment increases with an increase in the number of lymph nodes examined [3,21,22], and most studies suggest that node positivity rates increase with increased nodal harvest [2].

Methylene blue (methylthioninium chloride) when injected intra-arterially stains the arteries and capillaries, and, because the lymph nodes harbor a higher content of vessels in relation to the surrounding fat, they stain deeper than the surrounding fat and become easily identified [23].

It is not entirely clear how the solution passes into the lymph nodes when injected into the specimens, but the most likely mechanism could be the increase in interstitial pressure caused by the injection and therefore induced lymphatic flow [24]. That is why we injected the specimens with methylene blue just after ligation of the pedicle (s) of the specimen.

Methylene blue solution is often found to be superior to other dyes because of its relative safety, higher availability, and affordability [25].

In our study, the total number of lymph nodes recovered per patient was significantly higher in the methylene blue group compared with the control group ($P < 0.001$) (Table 1). Both groups were homogenous regarding other factors that could have affected the lymph node harvest (Table 1).

The American Society of Anesthesiologists stated that grade, older age of the patient, and neoadjuvant radiotherapy are commonly found to be the main factors associated with a low lymph node harvest. Moreover, transverse colectomy and abdominoperineal resection are the methods of surgery associated with the poorest lymph node harvest in the histopathological examination of the surgical specimens [26]. Patients with higher BMI usually have a bigger mesocolon with a larger number of lymph nodes.

We noticed significant improvement in the identification of smaller lymph nodes: that is, less than 1 mm and between 1 and 5 mm Table 1. This is in accordance with other studies [23].

This could be explained by the improved visual identification of smaller lymph nodes when using methylene blue because smaller lymph nodes are more likely to be mistaken for fat lobules if not stained. Overlooking small-sized lymph nodes is dangerous as lymph node size is not a reliable marker for lymph node metastases [27]; that is, very small and small lymph nodes may contain metastases and large lymph nodes may be free and the enlarged size may be due to an immune response rather than due to metastases. Large lymph nodes in stage I/II disease might indicate a favorable outcome [27].

Better identification of smaller lymph nodes is a good point in favor of the use of methylene blue.

Upstaging of colorectal cancer with improved nodal harvest is a controversial issue. Some authors claim that collecting and examining more lymph nodes will lead to upstaging of cases because of better identification of metastases that would otherwise be missed [28]. Others deny this [29].

Extensive studies were carried out on the lymph node ratio [30–32] (i.e. the ratio between the number of positive lymph nodes resected and the total number of lymph nodes harvested) being a better prognostic factor than the more presence of positive lymph nodes.

In our study, we noticed that the total number of lymph nodes harvested increased significantly with the use of methylene blue; yet simultaneously the number of positive lymph nodes per patient also increased from four to six. Thus, the lymph node ratio did not differ significantly ($P > 0.05$) (Table 1). Also, the treatment plans in three of our patients were changed with the finding of additional lymph nodes.

Preoperative use of chemoradiotherapy is known to reduce the number of lymph nodes identified in the specimens [33,34]. In a recent study, complete absence of recovered lymph nodes in resected specimens after neoadjuvant chemoradiation was observed in 7.6% of specimens [35]. Th us, the use of methylene blue in such cases is more clearly indicated [36].

We also noticed significant improvement in the number of lymph nodes with deposits identified in the methylene blue group compared with the control group (Table 1). This can be attributed to the larger number of lymph nodes examined, but further study may be needed to evaluate the hypothesis that blue staining of lymph nodes makes the identification of micrometastases easier. Recently, Markl *et al.* [20] studied this hypothesis and concluded that identification of lymph node metastases was not improved using methylene blue.

Conclusion

We recommend the routine use of methylene blue intra-arterial injection in rectal cancer, especially in patients treated with neoadjuvant therapy.

Conflicts of interest: None declared.

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