Preoperative Colonoscopic Tattooing Using a Direct Injection Method with Indocyanine Green for Localization of Colorectal Tumors: An Efficacy and Safety Comparison Study

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Purpose: Endoscopic tattooing is used to mark colorectal lesions for subsequent surgery. As a tattooing agent, India ink has been widely used but is not currently available in Korea. Indocyanine green (ICG) can be applied as an alternative agent. However, studies on colonoscopic tattooing by the direct injection of indocyanine green are lacking. This study aimed to compare the efficacy and safety between an ICG direct injection method and an India ink saline test injection method.

Methods: A total of 227 patients who underwent preoperative endoscopic tattooing for colorectal neoplasm (149 patients in the ICG direct injection group and 78 patients in the India ink saline test injection group) were included in the study. The efficacy of the two methods was compared by visualization and safety was compared by evaluating the perioperative tattooing complications.

Results: The visualization of lesions in the ICG group was not different from that of the India ink group (p=0.42, 96.0% vs 98.7%, respectively). Only one patient in the ICG group had abdominal pain related to tattooing, but no complications developed in the India ink group.

Conclusion: Considering the good visualization and low complication rate, the direct injection of ICG can be used as an alternative tattooing method.

Keywords: Colorectal neoplasm, Colonoscopy, Tattooing, Indocyanine green, Ink

INTRODUCTION

Primary tumor resection is the main treatment for colorectal cancer. For the optimal resection of colorectal neoplasms, identifying the exact location of the lesion is essential. Unlike advanced colorectal neoplasms, early colorectal cancer or adenoma cannot be easily detected during surgery. Moreover, the detection of these lesions can be more difficult during minimally invasive surgery because of the lack of tactile sensations to locate the tumor. Inaccurate localization of the lesion may lead to an unexpected procedural change during surgery or resection of the wrong segment.

The rates of laparoscopic surgery for colorectal cancer have increased in Korea, as has the proportion of colorectal cancer detected early. With these trends, the need to locate primary colorectal lesions has also increased.

Several methods have been used for the localization of colorectal neoplasms. Colonoscopic tattooing, colonoscopic metallic...
clipping, colon study, and computed tomography colonography can be used depending upon the clinical situation. Among these methods, colonoscopic tattooing is one of the popular approaches because it can accurately localize lesions and detect small or non-palpable lesions.

Methylene blue, indigo carmine, indocyanine green (ICG), and India ink can be used for colonoscopic tattooing. An initial study reported that ICG was effective based on persistency and tolerable adverse reactions. However, a limitation of ICG is the tendency to fade with time. A randomized animal study showed that India ink was superior to ICG in terms of durability. Currently, India ink is the most effective and widely used agent for tattooing.

For tattooing, India ink has been supplied as a prepackaged sterile suspension consisting of purified carbon particles. However, this premixed package is not currently available in Korea. Thus, a recent study on endoscopic ICG tattooing investigated the usefulness of ICG using a saline test injection method.

In the saline test injection method, the saline is injected into the submucosal layer before and after tattooing, and was shown to improve the accuracy and safety of India ink tattooing. However, this method requires more manipulations than the conventional direct injection method. The direct injection method can be used with ICG because ICG is a liquid solution, which does not contain particles like those in India ink. However, studies comparing the outcomes between colonoscopic tattooing using the direct injection of ICG and the India ink method are still lacking. This study aimed to compare the efficacy and safety between the direct injection method of ICG and the saline test injection method using India ink.

**MATERIALS AND METHODS**

This study included patients who underwent primary tumor resection after preoperative endoscopic tattooing for colorectal neoplasm at Seoul National University Hospital Study from January 2009 to December 2017. The protocol was approved by the Seoul National University Hospital Institutional Review Board (IRB No.: H-1907-043-1046). Patients in whom both agents were used were excluded from the study.

The patients were divided into two groups according to the tattooing agent used, the ICG group and the India ink group. India ink was used before October 2013, and after that, ICG was used as a tattooing agent. In the ICG group, the endoscopists injected 0.5 cc of ICG (Diagnogreen Injection, Daiichi Sankyo Co., Ltd., Tokyo, Japan) directly, without saline loading, into the submucosal layer using a 23-gauge injection needle (Olympus Medical Systems Co, Ltd., Tokyo, Japan) (Fig. 1). In the cases of ICG, tattooing was usually performed a day before operation day. If there was a public holiday or weekend just before operation day, tattooing was performed a day before a public holiday or weekend. In the India ink group, 1 to 1.5 ml of India ink (SPOT® Endoscopic Marker, GI Supply, Camp Hill, PA, USA) was injected into the submucosal layer using the same gauge injection needle, after 1 ml of saline was injected to form a proper submucosal elevation. An additional 1 ml of saline was injected to flush out the remaining agents. In both groups, the agents were injected at three circumferential sites 120° apart around the tumor site.

The data were collected retrospectively from the surgical and anesthesia records, pathologic reports, and medical charts. Visualization was based on the serosal color changes and localization of the main lesion. The visualization was identified in the records of the operation notes and gross pathologic images. The information on intraoperative colonoscopy events, complications from tattooing, and the perioperative outcomes were collected. The χ² test or Fisher’s extract test and the t-test were used to compare the characteristics and outcomes of the two groups using SPSS software (SPSS Inc., Chicago, IL, USA). p values of less than 0.05 were considered significant.

**RESULTS**

Two hundred-thirty patients underwent primary tumor resection after tattooing. Three cases were excluded. One patient

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Fig. 1. (A) Endoscopic view after tattooing with indocyanine green. (B) Serosal appearance during laparoscopic surgery after tattooing with indocyanine green. (C) Serosal appearance during open surgery after tattooing with indocyanine green.
was tattooed twice with ICG and both agents were used in two patients. Finally, a total of 227 patients were included in this study, 149 patients in the ICG group and 78 patients in the India ink group. The baseline characteristics are shown in Table 1. There was no difference between the two groups in terms of age, gender, body mass index, previous endoscopic treatment, ASA(American Society of Anesthesiologists) classification, tumor stage, and histologic type. The India ink group had more left-sided colon tumors than the ICG group. Laparoscopic resection was performed more often in the India ink group than in the ICG group. The interval between tattooing and surgery was shorter in the ICG group than in the India ink group.

Table 2 shows the results of endoscopic tattooing. There was no difference between the ICG and India ink groups in visualization (96.0% vs 98.7%, respectively, p=0.42). One patient in the ICG group underwent intra-operative endoscopy because of the non-visualization of a tattooed lesion. The rate of visualization was not significantly different between ICG and India ink in both open and laparoscopic surgery group (Open: 98.3% vs 100%, p=1.00; Laparoscopic: 94.4% vs 98.3%, p=0.40). There was one complication in the ICG group, which was mild abdominal pain that improved with conservative treatment. There was no intra-operative endoscopy or tattooing complications in the India ink group.

The perioperative outcomes are shown in Table 3. The mean operation time and hospital stay were not different between the two groups. There was no mortality in either group within 30 days.

| **Table 1. Baseline characteristics between indocyanine green and India ink group** |
|-------------------------------|-------------------|------------------|-----------|
|                               | ICG (n=149)      | India ink (n=78) | p value  |
| Age (year, Mean±SD)           | 62.4±9.8         | 60.1±10.6        | 0.118    |
| Gender                        |                  |                  | 0.666    |
| Male                          | 91 (61.1)        | 52 (66.7)        |          |
| Female                        | 58 (38.9)        | 26 (33.3)        |          |
| BMI (kg/m², Mean±SD)          | 24.3±2.9         | 24.2±2.9         | 0.716    |
| Previous endoscopic treatment |                  |                  | 0.076    |
| Yes                           | 49 (32.8)        | 35 (44.8)        |          |
| No                            | 100 (67.2)       | 43 (55.2)        |          |
| ASA classification            |                  |                  | 0.319    |
| I/II                          | 141 (94.6)       | 77 (97.4)        |          |
| III/IV                        | 8 (5.4)          | 2 (2.6)          |          |
| Tumor stage                   |                  |                  | 0.594    |
| 0/1                           | 91 (61.1)        | 49 (62.8)        |          |
| 2                             | 22 (14.7)        | 9 (11.6)         |          |
| 3                             | 34 (22.8)        | 17 (21.8)        |          |
| 4                             | 2 (1.3)          | 3 (3.8)          |          |
| Histologic type               |                  |                  | 0.890    |
| Low grade                     | 130 (86.1)       | 69 (87.3)        |          |
| High grade                    | 7 (4.6)          | 4 (5.1)          |          |
| Other                         | 12 (7.9)         | 5 (6.3)          |          |
| Tumor lesion                  |                  |                  | 0.024    |
| Right-side colon              | 42 (28.2)        | 11 (14.1)        |          |
| Left-side colon               | 68 (45.6)        | 49 (62.8)        |          |
| Rectum                        | 39 (26.2)        | 18 (23.1)        |          |
| Operative type                |                  |                  | 0.005    |
| Laparoscopic                   | 90 (60.4)        | 62 (79.5)        |          |
| Open                          | 59 (39.6)        | 16 (20.5)        |          |
| Interval between tattooing and operation (day, Mean±SD) | 1.4±0.9 | 6.2±7.4 | 0.000 |

Values are presented as number (%). Histologic type—other: 13 intramucosal carcinoma, 1 adenosquamous carcinoma, 2 adenocarcinoma (unknown differentiation), 1 neuroendocrine carcinoma. ICG = indocyanine green; BMI = body mass index; ASA = American Society of Anesthesiologists.

| **Table 2. The results of endoscopic tattooing between indocyanine green and India ink and group** |
|-------------------------------|-------------------|------------------|-----------|
|                               | ICG (n=149)      | India ink (n=78) | p value  |
| Visualization                 | 144 (96.0)       | 77 (98.7)        | 0.42     |
| Open                          | 58 (98.3)        | 17 (100)         | 1.00     |
| Laparoscopic                  | 85 (94.4)        | 60 (98.3)        | 0.40     |
| Intraoperative endoscopy      | 1 (0.6)          | 0 (0)            | 1.00     |
| Complication of tattooing    | 1 (0.6)          | 0 (0)            | 1.00     |

Values are presented as number (%). ICG = indocyanine green.

| **Table 3. Postoperative outcomes between indocyanine green and India ink group** |
|-------------------------------|-------------------|------------------|-----------|
|                               | ICG (n=149)      | India ink (n=78) | p value  |
| Operative time (min, mean±SD) | 137.9±74.4       | 141.9±74.7       | 0.747    |
| Overall postoperative         |                  |                  | 0.178    |
| complication                  | 32 (21.4)        | 11 (14.1)        |          |
| Hospital stay (day, mean±SD)  | 8.6±3.8          | 8.0±7.6          | 0.413    |
| Mortality within post         |                  |                  | 1.000    |
| operation 30 days             | 0 (0)            | 0 (0)            |          |

Values are presented as number (%). ICG = indocyanine green.
days after surgery.

**DISCUSSION**

This study demonstrated that the direct injection of ICG could be a safe and effective approach for colorectal endoscopic tattooing. Compared with the current gold standard method (a saline test injection method with India ink), the direct ICG injection method had good and non-inferior visualization (96.0%) and mild complications from tattooing (0.6%). A systemic review reported no differences in the incidence of localization errors between India ink and ICG tattooing (7.7% vs 3.6%, respectively). Miyoshi et al. also reported that ICG could be an alternative tattooing agent to India ink. However, the visualization time of ICG was limited and did not persist over eight days in most cases. Another study reported that the visualization rate was only 40% when ICG tattoos were made more than three days before surgery. Because of this limitation of ICG, most patients in the ICG group underwent surgery within three days after tattooing in this study. In contrast to ICG, India ink persists semi-permanently, so does not have a time limitation between tattooing and surgery.

The saline test injection method was reported to improve the accuracy of localization and lower the incidence of adverse events. This technique also has the advantage of reducing the leakage of agents. A study using this method with ICG showed good visualization (93.5%) and no complications. Because ICG has no particles, unlike India ink, it can be manipulated more easily than India ink and injected directly into the submucosa without a saline test injection. The advantage of the direct injection method is simple handling without the need to change syringes. Our study is meaningful in that it demonstrated the feasibility of the direct injection method using ICG for colorectal tattooing.

There were no severe complications related to tattooing in this study. One patient in the ICG group felt abdominal pain upon tattooing, but there were no other symptoms or sequelae after tattooing and no abnormal findings during surgery. Other studies have reported low complication rates related to tattooing. Nizam et al. reviewed studies using India ink tattooing, and reported only a 0.22% complication rate. The complications included peritonitis and inflammatory bowel disease, and it was clear that leakage of the agent beyond the serosa of the colon could present problems. In other ICG studies in animals and humans, there were no major complications associated with tattooing. Because of the nature of tattooing agents, India ink contaminates operative field with carbon pigmentation when it is spilled into peritoneal cavity. Even though ICG is sustainable in the colorectal tissue for a limited time, ICG is less likely to disturb surgical field in the case of leakage. This is one of the benefits when using ICG as a tattooing agent. However, this study did not investigate the exact rates of leakage because of the lack of data.

Other approaches for the localization of colorectal tumors, such as endoscopic tattooing with autologous blood and endoscopic marking with near-infrared fluorescent clips, have been suggested. The visualization of tattooing using 6-12 ml of autologous blood was good (92.2%). Three patients (5.9%) experienced tattooing-related adverse events. The visualization of marking with fluorescent clips was also good (94.1%) and there were no adverse effects related to clip marking and detection.

In this study, the lesions were detected as color changes of green in serosa by only naked eyes. ICG can be more sensitively detected using ICG fluorescence cameras. Further study using the ICG fluorescence camera will give more insight for the precise detection of tattooing lesions.

There were some limitations to this study. First, the present study was limited by its retrospective design. There were differences between the two groups in clinical characteristics (tumor location and operation type). Second, the period of study was different between the two groups. The ICG group had more recent cases than the India ink group. Thus, the endoscopists’ techniques may have differed between the two study periods. Third, there was no specific data of the leakage of tattooing agents in this study. However, this study had the strengths of including a relatively large number of patients and comparing a new approach with the conventional gold standard.

**CONCLUSION**

In conclusion, a direct injection method with ICG can be used as an alternative tattooing method for colorectal tumors when it is performed within 3 days before surgery.

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CONFLICT OF INTEREST
None.

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