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SHORT COMMUNICATION

Efficacy of linked colour imaging in magnifying chromoendoscopy with crystal violet staining: a pilot study

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Abstract

Purpose Diagnosis of the depth of invasion is crucial in the endoscopic management of early colorectal cancer. Image-enhanced endoscopy (IEE) is a method for easily evaluating the depth of invasion. Linked colour imaging (LCI) is an IEE method that enables clearer identification of neoplastic lesions and mucosal inflammation. The aim of this experimental study was to explore the efficacy of LCI in vessel and pit pattern recognition when used in magnifying chromoendoscopy with crystal violet staining for superficial colorectal neoplasms.

Methods This was an experimental study. Colour difference (CD) values between the surrounding mucosa and vessels and pits were measured on white light (WLI), blue laser (BLI), and LCI images. The CD values of 10 neoplastic lesions were calculated and compared between WLI and the other techniques.

Results The CD value was 9.8 (interquartile range, 7.3–12.4) for WLI, 9.7 (6.7–13.4) for LCI, and 6.8 (5.1–9.3) for BLI. The CD value was statistically different between WLI and BLI but not between WLI and LCI. With regard to vessel description, the CD value was 7.5 (4.0–11.0) for WLI, 15.6 (11.6–23.9) for LCI, and 23.3 (15.8–30.4) for BLI.

Conclusions LCI provides more diagnostic information than other light modes. Further, it is superior to the other techniques in terms of vessel visibility and is comparable to them in terms of pit recognition. These unique features of LCI may lead to its use as an alternative to WLI and BLI for pit and vessel pattern evaluation in the future.

Keywords Magnifying endoscopy · Linked colour imaging · Colorectal neoplasm · Pit pattern · Vascular pattern

Introduction

Diagnosis of the depth of invasion is crucial in the endoscopic management of early colorectal cancer. Pit pattern analysis using crystal violet staining and magnification is recognized as the gold standard, with the highest diagnostic accuracy [1–3]. Image-enhanced endoscopy (IEE), such as blue laser imaging (BLI) or narrow-band imaging, is an alternative method for easy evaluation of the depth of invasion [4–7]. Linked colour imaging (LCI), which emphasizes colour changes in the mucosa, is the newest IEE method [8]. The key features of LCI are its improved contrast of haemoglobin and selective detection of mucosal microvessels.

IEE by white light (WLI), BLI, and LCI for observation of lesions after crystal violet staining has some unique features—namely, pits are most sharply demarcated in WLI, microvessels are clearest in BLI, and both pits and microvessels are clearly identifiable in LCI because of the optical characteristics of the system. In the present study, we aimed to objectively confirm the efficacy of LCI in vessel and pit pattern recognition when used in magnifying chromoendoscopy with crystal violet staining for superficial colorectal neoplasms.

Material and methods

Cases

Ten cases of colorectal tumours that had been observed by magnifying endoscopy (EC-L600ZP; Fujifilm Co., Tokyo, Japan) at the National Cancer Center Hospital, Tokyo, were

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examined in this study. All lesions were resected using an endoscopic or surgical method after observation under magnification for vessel pattern, surface pattern, and pit pattern with crystal violet staining. Histological specimens were assessed by a gastrointestinal pathologist. We reviewed all images and selected cases in which images had been taken under the same conditions using the three modes—WLI, BLI, and LCI. The histological diagnoses were tubular adenoma ($n = 2$), high-grade intraepithelial neoplasia ($n = 2$), adenocarcinoma ($n = 5$), and adenocarcinoma with tubulovillous adenoma ($n = 1$). All cases were evaluated by crystal violet staining, and the images were taken in each mode (WLI, BLI, and LCI) under the same conditions.

Setting of region of interest

We investigated the colour differences in a total of 10 cases. Each case comprised a set of three images of the same area taken in three light modes (WLI, BLI, and LCI) under identical conditions. In the three images, we chose multiple areas showing different pit or vessel patterns because of the presence of heterogeneous histological components. A single endoscopist selected five ROIs in each image, which were located at the same site in each of the three images of a set. A total of 110 ROIs from 22 different areas were selected for each modality (WLI, BLI, LCI), and the colour differences were calculated. ΔE was categorized into six categories on the basis of visibility: 0.0–0.5, trace; 0.5–1.5, slight; 1.5–3.0, noticeable; 3.0–6.0, appreciable; 6.0–12.0, much; ≥ 12.0, very much.

Analysis of colour differences

Colour analysis was performed using Image J (National Institutes of Health, Bethesda, MD). Colour differences between vessels and the surrounding mucosa and between pits and the surrounding mucosa were measured on WLI, BLI, and LCI images. To ensure accuracy, the region of interest (ROI) was selected under the following conditions: (1) the same area was selected as each ROI in the set of three images; (2) domains with excess brightness, darkness, or particular halation were excluded; and (3) each ROI was square-shaped with 3 × 3 pixels.

Colour differences (ΔE) were calculated using the International Commission on Illumination (CIE) 1976 (L∗, a∗, b∗) colour space [9]. ΔE was calculated using the procedures in steps (1) to (3): (1) Five vessels and pits were selected, and ROIs (3 × 3 pixels) for the vessels and pits were determined. The second and third ROIs for the surrounding mucosa were placed next to the first ROI (Fig. 1). (2) The red-green-blue mean value of 3 × 3 pixels in each ROI was measured using Image J. The L∗a∗b∗ values were calculated from the red-green-blue mean values of 3 × 3 pixels in each ROI. (3) ΔE between the first ROI and second ROI (ΔE1–2) and between the first ROI and third ROI (ΔE1–3) was calculated using the following formula: ΔE = √(ΔL*) 2 + (Δa*) 2 + (Δb*) 2. The average of ΔE1–2 and ΔE1–3 was defined as the colour difference (ΔE) for the vessel or pit. ΔE is expressed according to the evaluation criterion of the National Bureau of Standards (NBS) unit for colour difference. ΔE was converted to NBS units using the following formula [10]: NBS units = ΔE × 0.92.

Statistics

All data for continuous variables were expressed as median and interquartile range. All tests were two-tailed, and probability (p) values < 0.05 were considered statistically significant.

Results

Regarding pit description, the colour difference value was 9.8 (7.3–12.4) for WLI, 9.7 (6.7–13.4) for LCI, and 6.8 (5.1–9.3) for BLI. Given that the standard method for pit observation is WLI, we compared WLI with the other modalities. There was a statistically significant difference in the colour difference value between WLI and BLI but not between WLI and LCI. With regard to vessels, the colour difference value was 7.5 (4.0–11.0) in WLI, 15.6 (11.6–23.9) in LCI, and 23.3 (15.8–30.4) in BLI. We compared the colour difference value between BLI and the other modalities because BLI is thought to be the standard for interpretation of vessel patterns. The colour difference value for BLI was statistically significantly higher than that for the other modalities. Considering the correlation between ΔE and visibility in NBS, the description of pits with WLI and LCI was categorized as "much (6.0–12.0)," and that with BLI was categorized as "much" or "appreciable (3.0–6.0)." The description of vessels with BLI was categorized as "very much (> 12.0)," that with LCI as "very much" or "much," and that with WLI as "much" or "appreciable."

Discussion

The results of this study support our hypothesis that LCI after crystal violet staining provides more diagnostic information on simultaneous staining. In general, pit pattern analysis is performed using WLI, but evaluation of the vascular pattern has been difficult. On the other hand, whereas the vascular pattern has been evaluated using BLI, pit pattern analysis using BLI is almost impossible. LCI enables acquisition of information on the vascular pattern and pit pattern simultaneously. Hence, LCI may be advantageous in cases wherein there is less crystal violet staining. Such cases might be misdiagnosed as a type VN pit using the definitions of pit pattern analysis, especially by endoscopists who have less experience with observation under

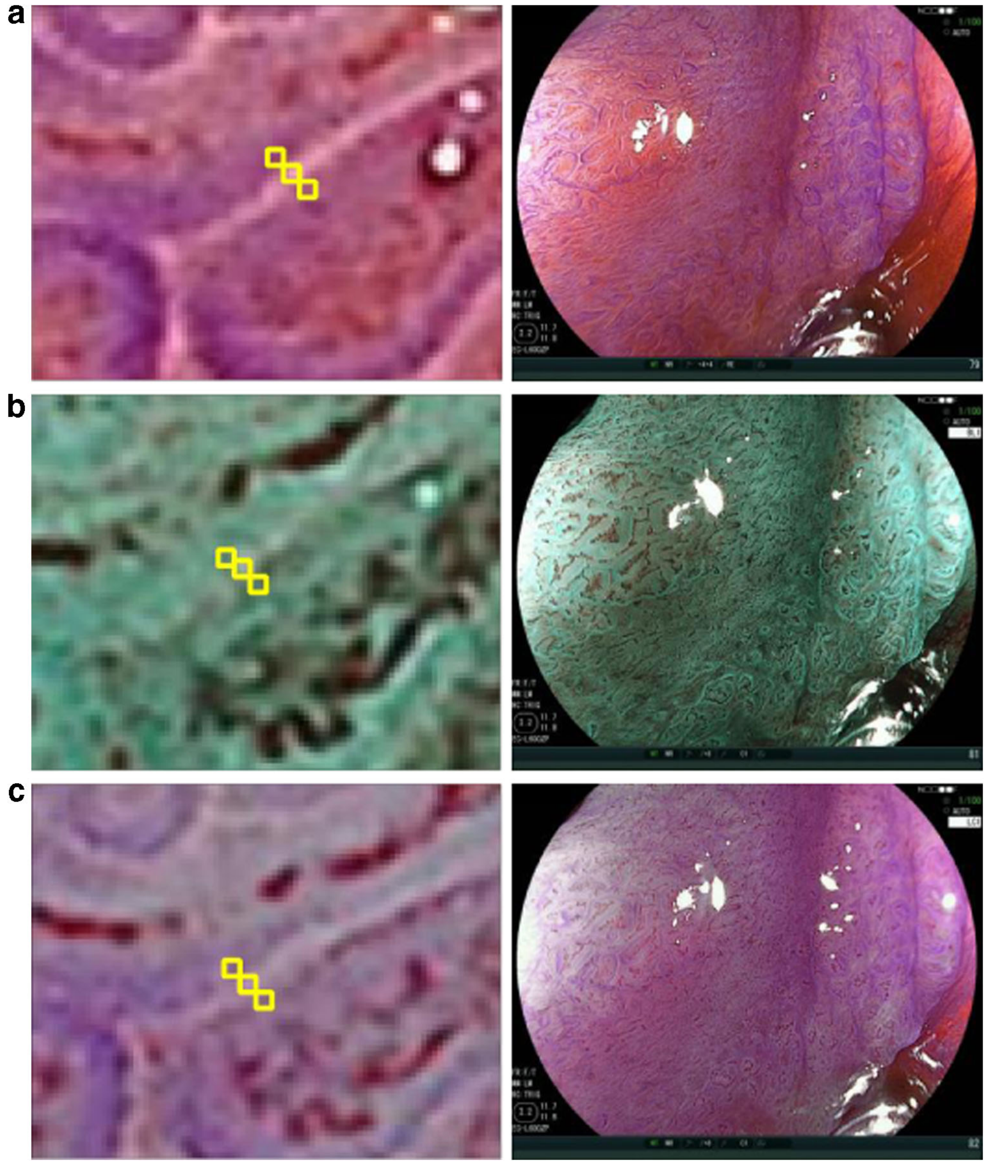

Fig. 1 Representative still images of the spots that were captured for calculation of the colour difference of the vessel, pit, and surrounding mucosa. A pit on **a** WLI, **b** BLI, and **c** LCI images

magnification. A type VN pit is strongly suggestive of submucosal deep invasive cancer, and the treatment strategy is surgery. Diagnosis of invasive cancer is straightforward when the area exhibits less staining accompanied by avascular or loose vessel finding. In cases in which the area exhibits less staining and clear vascularity, there is a possibility of intramucosal cancer or shallow submucosal invasion, and endoscopic treatment should be considered.

However, LCI also has a disadvantage. LCI was inferior to WLI for measuring the colour difference value, particularly in cases that showed type VI pits with high irregularity. LCI seems to exhibit better colour contrast and visibility than WLI. Considering the optical characteristics of LCI, the technique could be enhanced by improving image data processing.

There are several limitations to this study. First, the sample images were collected in a single institution. As endoscopists could freely adjust the structure and colour of the images depending on their preference, we cannot confirm the robustness of the study results. Second, we used a limited number of still images, and ROIs were set by only three endoscopists, although they all had sufficient experience in endoscopic observation under magnification.

LCI might provide more diagnostic information than other light modes. Further, it is superior to the other techniques in terms of vessel visibility and is comparable to them in terms of pit recognition. These unique features of LCI may lead to its use as an alternative to WCI and BCI for pit and vessel pattern evaluation in the future.

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Compliance with ethical standards

The study was conducted in accordance with the guidelines of our institutional review board, which approved this study. The need for informed consent from the participants for this study was waived. However, all patients provided written informed consent for the colonoscopic and endoscopic treatments.

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