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Observational Study

Could near focus endoscopy, narrow-band imaging and acetic acid improve the visualization microscopic features of stomach mucosa?

Kurtcehajic A *et al*. Endoscopic microanatomy of the gastric mucosa

Abstract

BACKGROUND

Conventional magnifying endoscopy with narrow-band imaging (NBI) observation of the gastric body mucosa, reporting dominant patterns in relation to regular arrangement of collecting venules (RAC), subepithelial capillary network (SECN) and gastric pits (GP).

AIM

To evaluate the possibilities of a new one - dual (near) focus, NBI mode, in the assessment of the microscopic features of gastric body mucosa, which we use instead of the conventional magnification.

METHODS

During 2021 and 2022, 68 patients underwent proximal gastrointestinal endoscopy using magnification endoscopic modalities, subsequently applying acetic acid (AA). The scope GIF-190HQ series NBI system, with dual focus capability, was used for the investigation of gastric mucosa. At the time of the endoscopy, the gastric body mucosa of all enrolled patients was photographed using the white light endoscopy (WLE), near focus (NF), NF-NBI, AA-NF and AA-NF-NBI modes.

RESULTS

From all patients, regarding the WLE, NF and NF-NBI endoscopic modes 204 images were classified in the same order into three groups. Regarding the AA-NF and AA-NF-NBI endoscopic modes, two images by the same patient in the couple were classified, in the same order. According to all three observers who completed the work independently, NF magnification was significantly superior to WLE ($P < 0.01$), and the NF-NBI mode was significantly superior to NF magnification ($P < 0.01$). After applying the AA, according to all three observers, AA-NF-NBI was significantly superior to AA-NF ($P < 0.01$). Interobserver kappa values for WLE were 0.609, 0.704 and 0.598, respectively, and in the case of NF magnification, they were 0.600, 0.721 and 0.637, respectively. For the NF-NBI mode, the values were 0.378, 0.471 and 0.553, respectively, for AA-NF, they were 0.453, 0.603 and 0.480, respectively and for AA-NF-NBI, they were 0.643, 0.506 and 0.354, respectively.

CONCLUSION
When investigating gastric mucosa at microscopic detail level, among the five endoscopic modalities which took part in this study, NF-NBI was the most powerful endoscopic mode for assessing RAC, SECN and GP. AA-NF-NBI was the most powerful endoscopic mode for analysing crypt opening and intervening part.

Key Words: Gastric mucosa; Endoscopic microanatomy; Magnifying endoscopy; Near focus; Narrow-band imaging; Acetic acid

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Core Tip: Narrow-band imaging have enabled the analysis of gastrointestinal mucosa at microscopic detail level. However, this technique gives a dark image and makes it impossible to identify the colour and structural microanatomy changes of the stomach mucosa, and it is necessary to combine it with the mechanical addict on the top of the scope (rubber) - conventional magnification. These additions improve the visualization of the gastric mucosa, but significantly complicate the procedure. We present a new endoscopic mode called "near focus" that achieves the same or better visualisation and does not require any additional accessories (rubbers).

INTRODUCTION

Currently, endoscopic platforms offer high-resolution images, image-enhanced endoscopy (IEE) techniques and magnification, allowing for the inspection of gastric mucosa at a more detailed level. Endoscopic microscopic features (microanatomy) of the gastric body mucosa are classified into the microvascular architecture, such as the regular arrangement of collecting venules (RAC) and the regular honeycomb subepithelial capillary network (SECN). The micro-surface structure is characterized by regular round gastric pits (GP), regular oval crypt opening (CO) and the intervening part (IP), which constitutes the space between the crypts. In gastric related diseases such as *Helicobacter pylori* (*H. pylori*) infection, intestinal metaplasia, and gastric atrophy the microanatomy has been structurally changed - absence of venules, irregularity of capillary network, enlarging pits and crypts[1-6].

In previous reports, white light endoscopy (WLE) has failed to assess endoscopic microanatomy. The need and wish to improve the differentiation of normal, inflammatory, and malignant lesions by gastrointestinal endoscopy has fuelled research to accelerate the development of novel types of video endoscopy systems, based on new optical technologies. Electronic chromoendoscopy *via* the most useful tool in the many clinical trials, namely, narrow-band imaging (NBI), has highlighted the vascular patterns of gastric mucosa[1,3,6-8]. Three studies focused on acetic acid (AA) which enhances and determines the pathology of the gastric lesions[9-11].

Aiming to assess the endoscopic microscopic features in more detail, there has been a need for magnification. Conventional magnifying endoscopy (ME) with NBI observation of the normal gastric mucosa has been described previously; studies reported dominant patterns with RAC, SECN and GP[2-4,6,7]. Recent, optical innovation relating to the dual focus function allows the endoscopist to select between a normal mode and a near focus (NF) mode. The NF mode is optimized for near-field observation with 45-fold magnification. Studies have reported that NF-NBI successfully replaced ME-NBI in the detection of pathological lesions in the oesophagus and pharynx, as well as the identification of celiac disease[12-15]. Recent studies reported the ability of the NF-NBI mode in the detection of early gastric cancer lesions[16,17]. Two recently published studies considered NF endoscopy in the evaluation of gastric atrophy, intestinal metaplasia, and *H. pylori* infection[18,19].

Conventional ME using a soft rubber at the top of the scope due to the demanding manipulation in stomach remaining to be finally replaced with the simple, more pragmatic, and novel endoscopic way of magnification. Therefore, in this study, we evaluate the possibilities of a new one - dual (near) focus, NBI mode, in the assessment of RAC, SECN, GP and even more CO in the gastric body (microscopic features of the mucosa), which we use instead of a conventional magnification.

The first aim of this study was to determine the clinical (endoscopic) usefulness of the NF-NBI mode in the observation of gastric microvascular architecture and micro-surface structure. Secondly, by applying the 1.5% AA, we compare the power of visualization of the AA-NF and AA-NF-NBI mode, when assessing micro-surface patterns containing CO and IP.

MATERIALS AND METHODS

Patients

Between September 2021 and May 2022, 68 patients underwent proximal gastrointestinal endoscopy using conventional WLE, NF magnification and the NF-NBI mode, with the subsequent application of AA. The patients consisted of 30 men and 38 women: The mean age was 38.5 years and the range, 25-65 years.

The study excluded patients who were *H. pylori* positive, either one serology or rapid urease test (RUT), those who had received anticoagulant therapy or drugs for chronic metabolic diseases (diabetes mellitus, hypothyroidism) and systemic inflammation disease, as well as nonsteroidal anti-inflammatory drugs and anxiolytics, and patients with chronic decompensated liver and kidney diseases. The study was approved by the ethical committee of the Blue Medical Group, and signed, well-informed consent was obtained from all participants.

Endoscopic modalities

The endoscopic video information system, EVIS EXERA III CLV-190, was used with an Olympus high-resolution endoscope, GIF-190HQ series NBI system, with dual focus capability for the investigation of the gastric mucosa. This scope allows to switch between two focus settings: “normal mode” and “near focus mode”. The “normal mode” or WLE suits normal observation at a distance of 5-100 mm and a 170° field of view, while the NF magnification of up to 70X allows close observation of the finest mucosal surfaces, at a distance of 2-6 mm. When switching to the NF mode at the simple touch of a button, the field of view will remain almost the same (160°).

NBI is based on the principle that depth of light penetration into tissues is directly proportional to the wavelength, which implies that the shorter the wavelength, the more superficial the penetration. The NBI resembles chromoendoscopy without dye, focusing on the capillaries.

AA *via* magnification enables vivid observation of the CO of the glandular epithelium, which has a deep brown appearance; the IP has a whitish appearance, because of reversible alterations in the molecular structure of the cellular proteins that are induced by the AA, and last from several seconds to several minutes.

Endoscopic procedure

Two hours before the procedure all patients took 80 mg of simethicone with a small amount of water, aiming to remove gastric mucus; the procedures were performed under the intravenous application of the propofol. An experienced gastroenterologist (Kurtcehajic A) performed all the procedures. The whole oesophagus, stomach and duodenum were examined to exclude obvious lesions with the conventional WLE, followed additionally by the manufacturer incorporated NF-NBI mode in the scope, by applying the 3 mL AA *via* a single catheter. The focus area was the anterior wall or greater curvature of the upper gastric body. Biopsies were taken from the antrum and corpus mucosa and RUT was performed to evaluate *H. pylori* infection.

Endoscopic patterns and scoring

At the time of the endoscopy, the gastric body mucosa of all enrolled patients was photographed using the WLE, NF, NF-NBI, AA-NF and AA-NF-NBI modes. Regarding the WLE, NF and NF-NBI endoscopic modalities, the regular/clear appearance of each mucosa microscopic feature, RAC, SECN, GP and CO scored 1 point; the unclear appearance of each scored a half point and the absence of each scored 0.

In relation to the WLE mode, the WLE-1a and WLE-2a patterns clearly show the RAC and score 1 point; WLE-2a presents a faded appearance of the RAC; the WLE-b pattern shows the RAC less clearly and scores a half point and the WLE-c pattern does not show the RAC and scores 0 (Figure 1).

With reference to the NF magnification, the NF-a pattern clearly shows the RAC, SECN and GP and scores 3 points. The NF-b pattern shows the RAC less clearly, but clearly shows the SECN and GP and scores 2.5 points. The NF-c pattern does not show the RAC, but clearly shows the SECN and GP and scores 2 points (Figure 2).

Regarding the NF-NBI endoscopic visualization, the NF-NBI-1a and NF-NBI-2a patterns clearly show the RAC, SECN, GP and CO and score 4 points; the 2a pattern has less distribution of the RAC and a slightly enlarged GP and CO than the 1a pattern. The NF-NBI-b pattern does not show the RAC, clearly shows the SECN and GP, shows the CO less clearly and scores 2.5 points (Figure 3).

According to the aforementioned scoring rules, one pattern could score the most points (4) (clear presence of all microscopic features), or the least number of points (0) (absence of all microscopic features). After enhancing the area of observation with AA, a pattern was suddenly visible on the AA-NF and AA-NF-NBI, containing CO and IP. On the AA-NF mode, the pattern shows small oval brown CO and light white IP. On the AA-NF-NBI mode, the pattern shows small oval black CO and dense white IP (Figure 4). The strong contrast between the CO regarding the shape/size and the IP on these two patterns within the same patient grades with 1point, medium contrast grades with a 0.5 point and low contrast grades with 0 points. Endoscopic patterns were observed and scored by three independent endoscopists.

Statistical analysis

The differences between the scoring of each endoscopic modality for all observers were compared using the Wilcoxon Matched Pairs Test. P values < 0.05 were considered significant. The interobserver diagnostic agreement was analysed with a kappa value. In theory, perfect disagreement has a kappa value of -1, and perfect agreement has a kappa value of +1. A value of 0 means an agreement by chance alone. As per the Landis and Koch scale, kappa values were graded as follows: 0.01-0.2 slight, 0.21-0.4 fair, 0.41-0.6 moderate, 0.61-0.8 substantial and 0.81-1.0 almost perfect. Cohen's suggested interpretation may be too lenient for health-related studies because it implies that a score as low as 0.41 might be acceptable (9, 14, 20). Data were analysed using SPSS 23 (IBM, United States).

RESULTS

Following the meeting criteria relating to long-term epigastric discomfort and non-specific abdominal pain, the study initially included 74 patients. During the endoscopy, four patients (three male, one female) did not undergo NF magnification, due to one patient having benign stenosis of the distal oesophagus, one having cancer of the cardia and two having pyloric stenosis. Two patients (one male, one female) were ruled out due to severe bile reflux.

Finally, 68 patients underwent proximal gastrointestinal endoscopy, using the WLE, NF and NF-NBI modes to analyse the microscopic features of gastric body mucosa. From all patients, 204 images were classified in the same order into three groups, in relation to the above endoscopic modality by endoscopist AK; they were observed separately and scored by two experienced endoscopists, JF (observer I) and PJ (observer II) and one inexperienced endoscopist, OZ (observer III).

Moreover, after applying AA in the area of observation, the CO and IP were suddenly visible. Regarding the AA-NF and AA-NF-NBI endoscopic modes, 136 images (couple by the patient) were classified in the same order. The contrast between the CO and IP in the same patient was observed and graded separately by the three observers. All observers had previously passed a live course regarding NF magnification and NBI chromoendoscopy mode with AA enhancing. The course was based on the 12 endoscopic patterns which would form part of this study; each pattern presented with 10 images.

The frequency and scoring for the WLE, NF and NF-NBI endoscopic modalities from the point of view of all three observers are shown in Table 1. According to the experienced observers (observer I and observer II), NF magnification was significantly superior to WLE ($P < 0.01$) and the NF-NBI mode was significantly superior to NF magnification ($P < 0.01$). Regarding the third inexperienced observer, NF magnification was significantly superior to WLE ($P < 0.01$) and the NF-NBI mode was significantly superior to the NF magnification ($P < 0.01$).

The frequency and scoring for the AA-NF and AA-NF-NBI endoscopic modalities from the point of view of all three observers are shown in Table 2. According to the experienced observers (observer I and observer II), AA-NF-NBI was significantly superior to AA-NF ($P < 0.01$). For the third inexperienced observer, AA-NF-NBI was significantly superior to AA-NF ($P < 0.01$).

The interobserver diagnostic agreement for all five endoscopic modalities was analysed with a kappa value. Interobserver kappa values for WLE were 0.609 for observer I and observer II, 0.704 for observer I and observer III and 0.598 for observer II and observer III. Interobserver kappa values for NF magnification were 0.600 for observer I and observer II, 0.721 for observer I and observer III and 0.637 for observer II and observer III. Interobserver kappa values for the NF-NBI mode were 0.378 for observer I and observer II, 0.471 for observer I and observer III and 0.553 for observer II and observer III. Interobserver kappa values for AA-NF were 0.453 for observer I and observer II, 0.603 for observer I and observer III and 0.480 for observer II and observer III. Interobserver kappa values for AA-NF-NBI were 0.643 for observer I and observer II, 0.506 for observer I and observer III and 0.354 for observer II and observer III.

DISCUSSION

According to the results of our research into the investigation of gastric mucosa at a detailed microscopic level, of the five endoscopic modalities used in this study, NF-NBI was the most powerful endoscopic mode for evaluating the RAC, SECN and GP, and AA-NF-NBI was the most powerful endoscopic mode for analysing the CO and IP. There have been many advances in endoscopic imaging technologies. Standard definition endoscopy produces image signals with a resolution of one to four hundred thousand pixels. High-resolution or high-definition (HD) endoscopy produces image signals with a resolution of up to a million pixels, which has the same effect as visualizing a surface at a 30- to 35-fold magnification. A novel IEE technique is electronic chromoendoscopy, which includes NBI, i-Scan and flexible spectral imaging colour enhancement. Over the last 15 years, NBI has been used most in clinical practice, however, it is too dark to identify the colour and structural mucosa changes in organs with a large lumen, such as the stomach, therefore, these should be combined with ME[1,6,8,14].

Over the last two decades, conventional ME was carried out with a soft rubber; before the procedure, soft black rubber was attached at the top of the scope. In this way, the area of interest was magnified, however the view for normal observation was reduced. Magnification with soft rubber requires skill and special training and for this reason, ME is not frequently used in Western countries[1-4,6].

Several studies using HD endoscopy, IEE, conventional magnifying, and histopathology have confirmed the normal appearance of gastric body mucosa with the RAC, SECN and GP[1-3,6-8,21-25]. In our study, we successfully replaced the conventional ME with the dual focus (NF magnification). Regarding our results, all three observers noted independently that the NF magnification showed more power of visualization than WLE ($P < 0.01$). NF magnification from the point of view of all three observers assessed the microscopic features of the gastric body mucosa, such as the RAC at 94%, 95% and 92%, respectively and the SECN and GP at 100%, in the same way as it was reported in studies powered by the conventional ME[4,22,23]. The diagnostic interobserver agreement for the NF magnification showed a "substantial" level.

A retrospective study from the United Kingdom demonstrated that NF magnification improved the diagnostic yield of upper gastrointestinal mucosal lesions, however, its usefulness for gastric lesions is questionable[15]. In our study, all three observers noted independently that the NF with the NBI showed significantly more power of visualization than the NF magnification ($P < 0.01$). In the NF-NBI mode, from the point of view all three observers, besides the RAC, SECN, GP and CO were clearly seen at levels of 95.5%, 97% and 92.6%, respectively. The diagnostic interobserver agreement in relation to the NF-NBI mode from the perspective of the experienced observers (one side) and the third inexperienced observer was at the "moderate" level. The diagnostic interobserver agreement among the experienced observers was at the "fair" level. The clinical explanation could be that NF-NBI "1a" and "1b" patterns were scored at the same and that the differences were qualitative (distribution of the RAC, size of GP and CO).

In the progression of gastric related diseases microscopic features of the stomach mucosa such as venules, capillary network, the shape and size of gastric fossa and recess have been structurally changed. For the first time, two studies used the NF-NBI mode in the stomach for the evaluation of a tumour lesion and its margin[16,17]. The role of the NF-NBI mode has been assessed recently for atrophic gastritis, according to the Kimura-Takemoto classification and intestinal metaplasia (tubular/granular GP pattern of the corpus)[18]. This study considered the shape of the GP without analysing the RAC, SECN and CO. In the absence of the previously verified gastric NF-NBI magnification pattern, it could not be appropriate definition of the pathology pattern.

In a recently published study by Fiuza *et al*[19], NF magnification was evaluated for the assessment of the mucosal surface pattern in *H. pylori* related gastritis. This study used the NF mode without NBI chromoendoscopy and was unable to consider the appearance of the CO.

Conventional ME requires more time, skills, and special endoscopic training; the mechanical addict on the top of the scope (rubber) and the view of visualization becomes less. On the other hand, NF magnification may be easily manipulated, there is no need for the mechanical attachment, the view of visualization remains normal and finally, in our study, NF magnification with NBI chromoendoscopy beside RAC, SECN and GP provided presence of CO.

The current era of NF magnification endoscopic technology may be contrasted with the research of Cho *et al*[25] who recently used conventional ME for the evaluation of *H. pylori* associated gastritis. The forthcoming studies *via* NF-NBI endoscopy aim to evaluate the presence/absence of RAC, the regularity/irregularity of SECN and the shape and size of the GP and CO in relation to the *H. pylori* infection, intestinal metaplasia, and atrophic gastritis, *etc.*

In the previous, aforementioned studies, the focus on the CO was less; a study by Kawamura *et al*[26] evaluated the role of conventional ME (without NBI) by analyzing the CO as part of *H. pylori* related gastritis. The whiteness of the CO was classified as the "white-edged dark spot" type, the "white" type and the "dense white pit" type.

Regarding our results, the NF-NBI mode assessed the presence of the CO as a black point, but to visualize the CO in more detail, enhancement was carried out using the AA. By applying the AA in the area of observation, we highlighted a unique pattern containing regular round brown CO, and whiteish IP in the normal gastric body mucosa. In the AA-NF-NBI mode, the CO suddenly became black, consequently there is a clear contrast between the CO and IP.

Our results, independently noted by all three observers, showed that AA-NF-NBI was superior to AA-NF, $P < 0.01$. Our results clearly push up the AA-NF-NBI mode in terms of analyzing the shape and size of the CO and IP. The diagnostic interobserver agreement for the AA-NF-NBI mode among the experienced observers was at a "substantial" level. The diagnostic interobserver agreement between the experienced observers (one side) and the third inexperienced observer was at the "moderate" level and "fair" level, respectively. An explanation for this could be that there was no question about the existence of the contrast, instead the question related to the grade of the contrast between the CO and IP.

One limitation of this study (as well as other studies related to this issue) is the relatively small number of patients included in the research. Additional research (preferably randomized trials or prospective collaborative studies) is required in order to improve the endoscopic investigation of gastric mucosa at a detailed microscopic level and to create the conditions for a better diagnosis and treatment of these diseases.

CONCLUSION

In this study, by investigating the microscopic details of gastric body mucosa among the five endoscopic modalities which took part in this study, NF-NBI was the most effective endoscopic mode for evaluating RAC, SECN and GP. AA-NF-NBI was the most effective endoscopic mode for analysing CO and IP. It provides a higher resolution observation tool for evaluating the relationship between the progress of gastric diseases and the existence of gastric venules, the regularity/irregularity of capillary network, the shape and size of GP and recess.

ARTICLE HIGHLIGHTS

Research background

Narrow-band imaging (NBI) is too dark to identify the colour and structural microanatomy of stomach mucosa due to large lumen, therefore, these should be combined with magnification.

Research motivation

Conventional magnification endoscopy using a soft rubber at the top of the scope due to the demanding manipulation in stomach remaining to be finally replaced with the simple, more pragmatic, and novel endoscopic way of magnification.

Research objectives

We evaluate the possibilities of a near focus (NF) magnification, NBI mode with acetic acid (AA), in the assessment of venules, capillary network, pits and crypts in the gastric body mucosa.

Research methods

The endoscopic video information system, EVIS EXERA III CLV-190, was used with an Olympus high-resolution endoscope, GIF-190HQ series NBI system, with dual focus capability for the investigation of the gastric mucosa. At the time of the endoscopy, the gastric body mucosa of all enrolled patients was photographed using the white light endoscopy (WLE), NF, NF-NBI, AA-NF and AA-NF-NBI modes.

Research results

From 68 patients, 204 images were classified in the same order into three groups (WLE, NF and NF-NBI). They were observed separately and scored by two experienced endoscopists and one inexperienced endoscopist. According to all three observers independently, NF magnification was significantly superior to WLE ($P < 0.01$), and the NF-NBI mode was significantly superior to NF magnification ($P < 0.01$). Interobserver kappa values for WLE were 0.609, 0.704 and 0.598, respectively, and in the case of NF magnification, they were 0.600, 0.721 and 0.637, respectively. For the NF-NBI mode, the values were 0.378, 0.471 and 0.553, respectively. For the endoscopic modalities with the AA, according to all three observers independently, AA-NF-NBI was significantly superior to AA-NF ($P < 0.01$). Interobserver kappa values for the AA-NF were 0.453, 0.603 and 0.480, respectively and for AA-NF-NBI, they were 0.643, 0.506 and 0.354, respectively.

Research conclusions

Among the five endoscopic modalities which took part in this study, NF-NBI was the most powerful endoscopic mode for assessing venules, capillary network, and gastric pits (GP). AA-NF-NBI was the most powerful endoscopic mode for analyzing crypts and space between crypts.

Research perspectives

The forthcoming studies *via* NF-NBI and AA-NF-NBI endoscopic modalities aim to evaluate the presence/absence of venules, the regularity/irregularity of capillary network and the shape and size of the GP and crypts in relation to the *Helicobacter pylori* infection, intestinal metaplasia, and atrophic gastritis, *etc.*

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