RESEARCH ARTICLE

Effect of Route of Preoperative Biopsy on Endoscopic Submucosal Dissection for Patients with Early Gastric Cancer

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Abstract

Objective: To observe and compare the effects of multi-patch biopsy under conventional white light imaging endoscopy (C-WLI) and precise targeted biopsy under magnifying narrow-band imaging endoscopy (M-NBI) on the endoscopic submucosal dissection (ESD) of early gastric cancers and intraepithelial neoplasias. Methods: According to the way of selecting biopsy specimens, patients were divided into C-WLI and M-NBI groups, 20 cases. The ESD operations of the 2 groups were compared quantitively. Results: The mean frequency of biopsy in M-NBI group was (1.00±0.00), obviously lower than in the C-WLI group (4.78±1.02) (P<0.01).The average total number of selected biopsy specimens was also fewer (1.45±0.12 and 7.82±2.22, respectively, P<0.01). There was no significant difference in the time of determining excision extension, marking time and the time of specimen excision of 2 groups during the ESD (P>0.05), whereas submucosal injection time, mucosal dissection time, stopping bleeding time, wound processing time in the M-NBI group were significantly shorter than in the C-WLI group (P<0.01). Conclusion: Precise targeted biopsy under M-NBI can obviously shorten the time of ESD operation, with small quantity of tissues but high pathological positive rate.

Keywords: Early gastric cancer- endoscopy - biopsy - magnifying narrow-band imaging - white light imaging

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Introduction

Pathological examination based on endoscopic biopsy is the golden standard of early discovery and early diagnosis of gastric cancer (Liu et al., 2013). At present, the main methods of pathological biopsy under the endoscopy include multi-times patch biopsy and precise targeted biopsy. Patch biopsy under conventional white light imaging endospcopy (C-WLI) was adopted generally for patients in our country for a long time, especially during endoscopic follow-up of precancerous lesions and diseases.

However, patients often suffer from multiple times of biopsy in multiple hospitals. With the application of magnifying narrow-band imaging endoscopy (M-NBI), precise targeted biopsy is realized for the morphologies of glandular tube and atypical vessels could be observed directly. Moreover, reducing the frequency of biopsy is of great significance to the process of endoscopic submucosal dissection (ESD) for gastric precancerous lesions and early gastric cancer. This study found that those 2 preoperative biopsy methods could influence the process of ESD directly and significantly.

Materials and Methods

General data

A total of 40 Patients performed with ESD due to early gastric cancer or intraepithelial neoplasias in sinuses ventriculi at Department of Gastroendoscope, Affiliated Hospital, Jiangnan University from Jan. 1st, 2011 to Dec. 31st, 3013 were selected and divided into C-WLI group and M-NBI group, 20 cases, respectively. Patients in C-WLI group were performed with multi-times patch biopsy under C-WLI, showing patients with moderate or severe erosive gastritis companied by intraepithelial neoplasia visiting more than 2 hospitals or more than twice stomachoscopy and patch biopsy, then performing with precise targeted biopsy under M-NBI. Patients in M-NBI group who were found suspicious lesions after the initial gastroscopic examination by C-WLI were sequentially confirmed by precise targeted biopsy under M-NBI and found glandular tube and atypical vessels.

There were 13 males and 7 females in C-WLI group, aged from 39 to 74 years with the mean age (53.8±9.5) years and 11 males and 9 females in M-NBI group, aged from 41 to 72 years with the mean age being (56.8±7.2)

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years. The average diameter of specimen in vitro by ESD was (4.72±1.52) cm in C-WLI group and (5.18±1.77) cm. Before ESD, patients were conducted to do ECG examination, X-ray, pulmonary function test, PT APTT, CEA, CA125, CA199, abdominal plain scan+contrast-enhanced CT and cardiopulmonary function evaluation before anesthesia. ESD was arranged under general anesthesia intubation in the operating room. Except from the frequency of biopsy, there was no significant difference in gender, age, ESD operation sites, the difficulty of the operation, gastric ESD surgeon and ways of anesthesia between 2 groups.

Experimental device

Endoscope: NBI host, OLYMPUS SCV-260SL ordinary endoscopy, OLYMPUSCF-H260AZI, magnifying endoscopy CV-260SL processor, CL-260SL endoscopic lighting system. This endoscope can magnify 80 times of ordinary pictures by regulating the joystick on the endoscopic control handle. 0.2% indigo carmine was used as colouring agent. Specification: MNT-DY-15, disposable biopsy forceps from Micro-Tech in Nanjing, Olympus NM-4L-1 injection needle, Olympus FD-1-1 hot biopsy forceps, Olympus HX-610-135L and HX-610-135 hemostatic clips, ERBE ICC 200 high frequency electric cutting device and APC 300 argon ion coagulator, Olympus Hook knife and insulation-tipped knife. Gastroscope front-end was added with transparent cap during the process of ESD.

Methods

C-WLI patch biopsy: In order to obtain accurate diagnosis, the general principles of gastric mucosa biopsy includes representative materials, large tissue in accurate cite, muscularis mucosa in depth with mucosal full-thickness exposed. The quantity of specimens: The number of specimen was taken according to the requirement and the operation was closely related to operating proficiency and experience of doctors so multipoint selection of tissues was needed, including 2 conventional tissues, 4 tissues in four directions surrounding the ulcer, multipoint selection of tissues in the top and pars basilaris in polypus; 5 tissues at least from suspicious malignancy. All tissues were fixed by 10% formalin, embedded into paraffin and 12 serial sections of each tissue were examined by microscopy after routine hematoxylin staining. Repeated biopsy was conducted during endoscopic follow-up of precancerous lesions of gastric cancer to make a definite diagnosis and avoid missed diagnosis. In C-WLI group, 20 patients were performed with repeated biopsy in many hospitals during follow-up before the operation of ESD. The quantity of selecting tissues was emphasized.

M-NBI one-time precise targeted biopsy: The diagnosis process of the early gastric cancer was changed after the introduction of the M-NBI system. Once lesions were found by C-WLI, the process was changed into NBI mode which could help gradually approach and magnify the lesions to observe shallow mucous membrane, blood vessels under mucous membrane and the microstructural changes of gastric mucosa glandular tube, consequently recognizing the early gastric cancer. The less but better selecting tissues was stressed for precise targeted biopsy in atypical micrangium.

The quantization of ESD operation (T: min): the distribution of ESD operation in sinuses ventriculi includes antetheca, paries posterior, greater curvature, lesser curvature and pylorus proparea. The time of the whole operation process were divided into the time of determining excision extension (T1), marking time (T2), submucosal injection time (T3), mucosal dissection time (T4), stopping bleeding time (T5), wound processing time (T6), the time of specimen excision (T7), the cumulative time of ESD operation (TZ). The determination of excision extension: The edge and depth of the lesion were needed to be confirmed through the edge of the lesion evaluated by M-NBI and the depth evaluated by lifting sign (+) of submucosal injection and endoscopic ultrasonography. Marking: the spot at every 2 mm away from the edge of the lesion was marked by APC. Submucosal injection: in the place 5 mm away from the marking spot was injected with submucosal injection including 3~5 mL indigo carmine, 1 mL epinephrine and 100 mL normal saline to make the lesion fully lifting, which was the committed step of success. The sequence of injection is the distal, bilateral and proximal part. Mucosal dissection: bush-hook oe dual knife was used to cut open mucous layer and submucosa along marking edge until complete cycle lesion was open, finally submucosa in basilar part of lesion was resected and separated until the whole lesion was separated completely. Large cluster of rete vasculosum was found by multi-times patch biopsy and if adhesion can cause the difficulty of resection, multiple submucosal injection was required. Stopping bleeding: electrocoagulation hemostasis and electric heating hemostatic forceps were used for stopping bleeding. Wound processing: the suspicious perforation was performed with endoscopic hemoclipping for quick sealing. In case of residual blood vessels, they were coagulated one by one to insure no bleeding of wound and Perforated back mirror. Specimen excision: Excised specimen was stretched, fixed by draw point and stained by pigment for determining pathologies of the head side, anal side, front wall and back wall of incisal edge.

Histological evaluation criterion

After the fixation of postoperative resected specimens, tissue was cut every 2 mm and the length of every section must be longer than that of the tumor between 2 adjacent slices and there was no cancer cell in the edge of every section. (1) Histological ompletely curative resection: there is no cancer cell found in the edge and basement of one-time monoblock resected specimen. (2) Possible

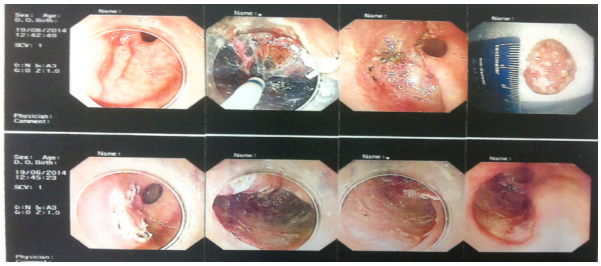

Figure 1. ESD Cases after 6 Times Gastroendoscopy and Biopsies

Table 1. Comparison of the Frequency of Preoperative Biopsy, Total Specimens, Coincidence Rate of Biopsy Pathology and ESD Postoperative Pathology between 2 Groups

Group	The mean frequency of biopsy	the average total number	Coincidence rate (%)
C-WLI group	4.78±1.02	7.82±1.22	65.00
M-NBI group	1.00±0.00	1.45±0.12	95.00
P value	*P*<0.01	*P*<0.01	*P*<0.05

Table 2. Quantitative Comparison of ESD Operation of 2 Kinds of Biopsy Methods

	Groups		*P* value
	C-WLI group	M-NBI group	
T1	3.82±1.45	3.21±0.35	*P*>0.05
T2	3.69±1.98	3.83±1.73	*P*>0.05
T3	6.52±4.80	3.52±3.45	*P*<0.05
T4	34.51±8.95	15.51±4.26	*P*<0.01
T5	9.37±5.27	1.19±0.38	*P*<0.01
T6	6.17±3.58	2.17±0.14	*P*<0.01
T7	3.52±0.58	3.12±0.14	*P*>0.05
TZ	67.6±3.80	32.6±1.49	*P*<0.01

curative resection: cancer cells might exist in the edge of section. (3) Histological non-curative resection: the above 2 points are outside the condition. The histological evaluation is not done due to piecemeal resection.

Results

Comparison of the frequency of preoperative biopsy, total specimens, coincidence rate of biopsy pathology and ESD postoperative pathology between 2 groups

The mean frequency of biopsy in M-NBI group was (1.00±0.00), decreased more obviously than that of C-WLI group (4.78±1.02) (*P*<0.01). M-NBI group (1.45±0.12) was decreased more obviously than C-WLI group (7.82±2.22) in the average total number of selecting biopsy specimens (*P*<0.01). The coincidence rates of biopsy pathology and ESD postoperative pathology of 2 groups were 95 % and 65%, respectively and there were significant difference (*P*<0.05). Of all 20 patients with gastric intraepithelial neoplasia during follow-up in C-WLI group, there were 45% (9/20) with early gastric cancer, 30% (6/20) with high-level neoplasia and 25% (5/20) with low-level neoplasia. Of 20 cases in M-NBI group, there were 40% (8/20), 50% (10/20) with high-level neoplasia and 10% (2/20) with low-level neoplasia. ESD postoperative pathological report showed 1 case with advanced gastric carcinoma, 1 with positive undercut incisal edge (+) and 2 performed with additional laparoscope radical operation. The incisal edge of the rest showed negative (-). The results of biopsy showed that multi-times patch biopsy of C-WLI group didn't increase the positive rate when compared with ESD postoperative pathology while M-BNI had high precise target, less frequency of biopsy and higher positive rate. See Table 1.

Quantitative Comparison of ESD Operation of 2 Kinds of Biopsy Methods

The average follow-up time of the patients was 10 months and there was no residue, reoccurred and ectopic focuses existing in local lesion. There was no significant difference in T1, T2 and T7 between C-WLI and M-BNI groups (*P*>0.05) and were significant differences in T3, T4, T5, T6 and TZ between 2 groups (*P*<0.01, or *P*<0.05). See Table 2.

Discussion

Pathological examinations in the process of endoscopic diagnosis and treatment of early gastric cancer include endoscopic mucosal biopsy and ESD operation of specimen incisal edge and undercut incisal edge. The conventional diagnostic process in China: For C-WLI, mucosal tissues were taken using biopsy forceps to conduct pathological diagnosis. The depth of resection is at the degree of mucosal muscularis with mucosal full-thickness exposed under gastroscope. Multiple patch biopsy is available for the correct diagnosis. Endoscopic mucosal biopsy includes multi-times patch biopsy and precise targeted biopsy. Zhang et al. (2011) found that the coincidence rate of gastric intraepithelial neoplasia or Early carcinoma preoperative biopsy and ESD postoperative pathology was up to 93.8% (183/195) with complete concordance rate of 50.8% (99/195), and the complete concordance rate of early cancer was 87.1% (27/31). There were 33.8% (66/195) patients were more aggravated in postoperative pathology than in preoperative biopsy pathology and 9.2% (18/195) patients were more alleviated in postoperative pathology than preoperative biopsy pathology. However, specificity of C-WLI is not high so false positive lesions are relatively big. During the diagnosis of C-WLI, follow-up patients with gastric intraepithelial neoplasia often experienced endoscopic biopsy for many times, even for patients who had the history of high frequency electric resection and EMR mucosal resection, repeated biopsy was easy to cause fiber adhesion and scar. The introduction of new type of special endoscopic technique have not only perfected the diagnosis system of existing early gastric cancer in our country but also made up for the shortness of multiple patch biopsies under C-WLI endoscopy. New type of special endoscopic technique can distinguish differentiated cancer from undifferentiated cancer based on the observation of glandular tube and the morphology of interstitial microvascular proliferation. Differentiated cancer cells were formed in glandular tube and proliferated in interstitial capillaries, whose density were higher than the capillaries in normal mucosa with different vascular diameter or irregular microvascular proliferation while the undifferentiated cancer cells were formed in muscularis mucosae, in which the capillaries and normal mucosal structures were damaged and partial capillaries showed wrinkle silk-like changes.

Precise targeted biopsy under M-NBI endoscopy can increase the early cancer detection rate with endoscopic mucosal biopsy (Guo et al., 2011; Li et al., 2014). In this study, lesion detected by C-WLI endoscopy was reexamined by M-NBI endoscopy for precise targeted biopsy and the results showed that the frequency of gastroscopy and tissue samples were relatively decreased

but the efficacy was obviously improved. Therefore, precise targeted biopsy is worth promoting. With the update of endoscopic equipment, new type of special optical endoscopy should become the conventional equipment of screening for gastric cancer. The results in this study suggested that the coincidence rate of endoscopic biopsy pathology and ESD postoperative pathology of C-WLI group and M-NBI group were 70% and 95%, respectively and there were significant difference (P<0.01). The sensitivity of M-NBI group was 60% (12/20), higher than the 40% (8/20) of C-WLI group but there was no significant difference (P>0.05). The specificity of M-NBI group was 90% (18/20), significantly higher than the 70% (14/20) in C-WLI group (P<0.01). The mean diagnostic time of M-NBI group were (30±8.30)s, evidently longer than that in C-WLI group. Diagnostic performance of C-WLI endoscopy combined with M-NBI endoscopy was superior to C-WLI endoscopy (P<0.01). In M-NBI group, because of high specificity, the amount of false positive lesions was relatively lower and the frequency of biopsy consequently reduced. In addition, C-WLI combined with M-NBI has very high performance in differentiating malignant tumor lesions from pitting benign lesions, even though there is no need of large tissues of histopathologic biopsy that the accurate diagnosis of early gastric cancer could still be realized. The microscopic structure is observed under M-NBI to further evaluate the features and predict the histopathological result. M-NBI, as the positioning means for lesions targeted biopsy and endoscopic treatment, helps realize real-time diagnosis of histology. M-NBI can avoid patients' repeated biopsy, reduce additional pathological examination fees and prevent excessive treatment. Therefore, precise target biopsy under M-NBI is worth promoting in the diagnostic process of early gastric cancer.

Reduced biopsy times and material samples and M-NBI accurate stereotactic biopsy can decrease ESD surgical difficulty so as to improve its technological promotion. ESD has the preferred surgical method for early gastric cancer in most countries and regions (Japan, Korea and Chinese Hongkong, etc.) (Zhang et al., 2011). It is also the optimal method of endoscopic therapy for early gastric cancer and precancerous lesion (4). ESD requires high endoscopic technique to determine the excised range and depth, nidus border marker, submucosal injection, submucosal dissection, intra-operative hemostasis as well as wound and isolated specimen management which should reach the negative oncocytes on edge and base incisal margin so as to promote the complete dissection rate of nidi and reduce the rates of severe complications such as uncontrollable massive hemorrhage and perforation, etc.. Biopsy method can not only affect the diagnosis of early gastric cancer but also impact the ESD surgical procedures as well as efficacy and risk evaluation (Liu et al., 2013). In this study, the block dissection rates with ESD in groups C and M were 92% and 100% while the complete dissection rates with ESD were 73.6% and 100%, respectively. However, there were no significant differences in determining the incisal range time, marking time and time to resolve the excised specimen (P>0.05), but the differences in time of submucosal injection, submucosal dissection, intra-operative hemostasis as well as wound and isolated specimen management were significant between two groups (P<0.001). Comprehensive analysis showed that the scars should be carefully dissected in patients who had incomplete bulge after submucosal injection when multiple biopsy depth had reached muscularis mucosae because of the scar adhesion after ulcer scar and repeated biopsy as well as close and rich submucosal vascular plexus (Zhou, et al., 2010). However, the subsequent surgical time, difficulty and risk for mucosal dissection were increased evidently, which leaded to decreased surgical safety. In group C, a patients who was diagnosed with erosive gastritis accompanied by low-degree intraepithelial neoplasia by gastroscope were given 6 times of gastroscope and biopsies in 3 years was diagnosed with high-degree intraepithelial neoplasia by ESD postoperative gastroscopic detection. Patient underwent the initial M-NBI accurate stereotactic biopsy had high positive sampling rate with less specimens and adhesion and 100% lifting sign rate due to submucosal injection. The longer the duration of liquid pad after submucosal injection, the easier the surgical operation, which could shorten the surgical duration, reduce intraoperative hemorrhage and surgical difficulty. Additionally, all nidi were one-timely dissected as monoblock.

During C-WLI follow-up, ESD postoperative pathology suggested that the positive incisal margins in patients with gastric intraepithelial neoplasia and even progressive gastric cancer, who should be highly concerned. ESD postoperative pathology demonstrated that the positive incisal margins included positive basement and surrounding incisal margins. Edge margin (+) was mainly caused by deficient diagnosis of nidi indigo carmine staining (Chen et al., 2012) and NBI-ME borders (Zhong et al., 2012), for which combined staining and NBI should be conducted to diagnose the borders. Positive base incisal margin include the nidi whose invasive depth was longer than the upper 1/3SM1 under mucosa, and 1 case of ESD postoperative pathology revealed progressive gastric cancer, then the gastroscope combined with laparoscope radical gastrectomy were performed for lymph node dissection in group C. In addition, multiple patch biopsies in group C could lead to submucosal adherence, which might trigger another severe problem whether the edge and base incisal margins were negative or not in causing the difficulty of ESD surgical dissection. As to 1 patient with positive base incisal margin in group C, local excision of the whole layer of gastric wall with ESD surgery or laparoscope should be additionally considered. It should be concerned that in the second application of ESD therapy, submucosal severe fibrosis and massive scar tissues could increase the surgical difficulty. The dissection depth should adhere and expose the muscularis propria due to improper lifting of lesions and small submocosal space so as to avoid the residue of base incisal marigin. The surgical rage should include residual nidi and normal mucosa 1.0 cm around. ESD could establish corresponding therapeutic protocols according to lesion locations, shapes, sizes and histological patterns, which could both ensure the radical

dissection of lesions and maximally maintain the normal tissues and their functions, and has strong pertinence in the individualized therapies (Zhou, et al., 2010).

In conclusion, this study introduced new endoscopic techniques, eg., special optical endoscopy enriches and completes the establishment of endoscopic diagnosis and minimally invasive therapy system of early gastric cancer, improves traditional diagnosis and treatment process of gastric cancer in China as well as the NBI-ME directional precise biopsy based on C-WLI and ESD dissection, and then significantly increase the efficiency of biopsy, reduce hemorrhage and adhesion during ESD surgeries, decrease operation difficulty and reduce the risk of hemorrhage and perforation, which could be conducted in rural hospitals in China.

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