Stretching Causes Extensive Changes of Gastric Submucosa: Is It Acceptable to Define 500 μ m as the Safe Margin?

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Background/Aims: Endoscopic mucosal resection can cure early gastric cancer. The risk of lymphatic metastasis is related to the depth of submucosal invasion by the mucosal malignancy, with a resection depth of 500 μ m generally accepted as a safe cut-off. However, excessive thinning induced by stretching of the resected tissue sometimes preventing a precise diagnosis. We studied the effects of stretching on different layers and sites of gastric tissue. **Methods:** Porcine stomachs were cut into 2.0×2.0 cm pieces, and pieces from body were stretched to 2.5, 3.0, and 3.5 cm. Pieces from the cardia, body, and antrum were also stretched to 3.0 cm. The thickness of each layer was measured and analyzed statistically. **Results:** Whole gastric wall and submucosal layers showed gradual thinning, with stretching to 3.5 cm tearing the tissues and resulting in imperfect extension. The submucosa was thinner in body tissue than in cardia and antrum tissues. Stretching to 3.0 cm induced a consistent decrease in submucosal thickness (30-70%). The change in thickness varied widely between individual samples. **Conclusions:** A resection margin of 500 μ m might be insufficient for the complete removal of malignancy. Moreover, the thickness of the submucosal layer differs with the gastric site and between individuals. Future studies are needed to confirm the findings in human tissue. (*Gut and Liver* 2008;2:199-204)

Key Words: Stomach neoplasms; Gastric mucosa; Gastroscopy; Submucosa; Stretching

INTRODUCTION

Gastric cancer is the fourth most common malignant disorder worldwide.¹ It still has the most common morbidity and mortality among various types of malignancies in East Asia and the most common malignancy in South Korea.² Gastric malignancies are more and more diagnosed on an earlier stage, and endoscopic mucosal resection (EMR) has been used for completely removing gastrointestinal tumors such as adenocarcinoma without distant metastasis, realizing an en bloc resection.

If the removed early-stage gastric malignancy is pathologically confirmed to invade into submucosal layer, risk of lymphatic spread is rendered to be higher.^{3,4} Researchers therefore divided this layer into two or three equal thickness and depicted as sm1, sm2, sm3 from the luminal side to stratify invasion of primary mucosal lesion into submucosa; this enabled physicians to estimate the probability of lymphatic spread and to differentiate an appropriate management. Accordingly, the Endoscopic Classification Review Group (ECRG) suggested a cutoff value of sm1, or 500 μ m for early gastric cancer, warranting free of distant or regional spread after endoscopic removal.^{5,6}

Some researchers, however, criticize this guideline inadequate for practical application. Thickness of tissue may vary depending on the manual force given for stretching before tissue fixation, and not the whole layer but partial layer including mucosa and superficial submucosa are taken after EMR.⁷ In our view, submucosal thickness still has reason to be evaluated as a component of intact

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thickness, although the tissue retrieved after EMR is partial rather than a full layer of gut. This premise originates from an idea to validate the previously suggested cut-off value of 500 μm by the ECRG. This also comes from a concept of investigating the nature of gut as a full layer rather than a partial one after artificial stretching. Furthermore, simple one-step stretching attempted in the previously cited report⁷ would not prove any correlation of the extent of stretching and subsequent thinning of submucosal layer.

To verify the usefulness of the guideline suggested for early gastric cancer of 500 μm depth, authors examined the relationship between thickness of full layer and stretching degree using porcine stomach pieces. We also studied whether the difference of sites in stomach influences the thickness of submucosa, either in intact form or after stretching. Additionally, authors evaluated if there is any variation of wall thickness among experimental animals.

MATERIALS AND METHODS

This study utilized porcine stomach: experimental procedures were performed in accordance with the National Institute of Health Guidelines for the humane handling of animals. Gastric pieces of pigs of a similar weight were taken and immersed into normal saline to minimize any tissue damage or denaturalization. After cut into an identical size, gastric tissues were fixed on a cork board with a different length of edge. Each tissue was processed for light microscopy, and photographs were taken after inspection under magnification. Computer program was used to measure the thickness of each layer in gastric tissue.

1. Tissue change after stepwise stretching

Fresh porcine stomach was obtained and the body (corpus) portion of a full thickness was cut out in a size of 2.0×2.0 cm of four pieces, and one piece was fixed on a cork board with pins without stretching. Rest of the pieces were also fixed, with each edges stretched into 2.5 cm, 3.0 cm, and 3.5 cm, respectively. After fixation, tis-

sues were immersed into 1% formalin solution for approximately 24 hours; gastric pieces were then processed for light microscopic examination, staining with ordinary hematoxylin and eosin, and Masson's trichrome stain. This experiment was repeated four times.

2. Tissue change after uniform stretching according to their sites

Two pieces of stomach were obtained in a size of 2.0×2.0 cm from cardia, body, and antrum, respectively. Each pair of porcine stomach tissue was treated as follows: one was fixed to the cork board with pin without any stretching, and the other was stretched into 3.0 cm of edge (equivalent of 150% lengthening). These pieces were also processed for light microscopy with same staining procedures. This experiment was performed four times repeatedly.

3. Microscopic inspection and measurement of gastric wall

An expert pathologist (J.H.L.) evaluated pathologic slides, checking general appearance and any damage of stained porcine tissues with a conventional light microscope (BX 41, Olympus, Tokyo, Japan). Slides stained with Masson's trichrome were also examined, with photographs taken with a mounted digital camera (DP 70, Olympus, Tokyo, Japan) on a magnifying power of ×12.5 or ×40. Thickness of each layer contained in gastric wall was measured from digital photographs using a special computer program, Image-Pro Plus[®] V 5.1.2.59 (Media Cybernetics, Silver Spring, Maryland, USA).

4. Data analysis

Measured thickness was evaluated for 1) change of thickness of each layer, 2) change of thickness according to site of porcine stomach, and 3) any observable differentiation among individuals. Data were handled with a statistical computer program, SPSS ver. 10.0 (SPSS Inc. Headquarters, Chicago, IL, USA), describing in mean± standard deviation (SD). Non-parametric test was used for statistical analysis (Wilcoxon's signed rank test).

Table 1. Thickness of Porcine Stomach according to Site

Sites	Whole layer (μm)		Submucosal layer (μm)	
	Mean±1 SD	Range	Mean±1 SD	Range
Cardia	3,012.55±1,307.93	1,763.90-4,480.00	973.80±664.42	305.7-1,740.0
Body	4,054.13±1,963.08	2,440.70-6,591.50	442.70±223.72	206.7-638.1
Antrum	8,910.05±4,076.21	4,766.20-14,323.00	1,215.1±495.1	568.4-1,740.0

SD, standard deviation.

Fig. 1. Thickness of the entire gastric layer (A) and submucosal layer (B) according to gastric site. The gastric wall was thicker at the antrum than at the cardiac and body wall, and the submucosal layers became thinner in the following order: antrum>cardia>body.

Fig. 2. Stepwise stretching of porcine gastric pieces from the body. Partial tearing occurred on the mucosal surface when it was stretched to 3.5 cm (175% of the original length of its edges).

RESULTS

1. Thickness of porcine stomach without stretching

Thickness of gastric tissue from eight animals differed according to the obtained site of stomach, with the tissue from antrum being the thickest, subsequently followed by body and cardia (Table 1, Fig. 1A). In contrast, the thickness of submucosal layer showed a descending order of antrum, cardia, and body (Table 1, Fig. 1B).

2. Gastric wall thickness according to the extent of stretching

Stretching the gastric piece up to 3.5 cm (175%) induced some tearing on mucosa and submucosa with sub-maximal lengthening; some injurious tears were seen even by a naked eye on the site of pinning (Fig. 2). On measuring thickness of each layers after stretching, mucosal and submucosal layers showed limited thinning, comparable to muscularis propria (Fig. 3). Referring to this result, subsequent experiments were performed by stretching gastric tissues to a fixed length of 3.0 cm.

Fig. 3. Measurements in each layer of porcine gastric pieces for stepwise stretching (mean values in μm , $n=2$). The muscular layer stretched more than the mucosa and submucosa as tissues were stretched.

3. Thickness of submucosal layer according to its site

Gastric pieces uniformly stretched to 3.0 cm revealed an order of whole-layer thickness, coinciding with the results

Table 2. Thickness Change of the Submucosal Layer: before and after Stretching

	Before stretching	After stretching	Changes (%, Mean $\pm$ 1 SD)
Cardia	973.80 $\pm$ 664.42 μ m (305.70-1,740.00 μ m)	403.88 $\pm$ 129.98 μ m (236.70-539.60 μ m)	42.54 $\pm$ 35.23
Body	442.70 $\pm$ 223.71 μ m (206.70-638.10 μ m)	309.43 $\pm$ 167.44 μ m (125.60-461.60 μ m)	31.52 $\pm$ 5.44
Antrum	1,215.10 $\pm$ 495.14 μ m (568.40-1,740.00 μ m)	795.93 $\pm$ 514.99 μ m (401.50-1,549.00 μ m)	38.43 $\pm$ 19.21

Fig. 4. Microscopy findings of porcine gastric pieces at each site, before and after stretching to 150% (from 2.0 to 3.0 cm). **Fig. 5.** Thickness changes according to site, before and after stretching. The submucosal layer thinned by 30-70%.

of non-stretched gastric specimens. Thickness of submucosal layers after stretching to 3.0 cm were measured as cardia 236.70-539.60 μ m (403.88 $\pm$ 129.98 μ m), body 125.60-461.60 μ m (309.43 $\pm$ 167.44 μ m), and antrum 401.50-1,549.00 μ m (795.93 $\pm$ 514.99 μ m), respectively, showing thinning of submucosal layers on a percentage of cardia 42.54 $\pm$ 35.23%, body 31.52 $\pm$ 5.44%, and antrum 38.43 $\pm$ 19.21% (Table 2, Fig. 4, 5). Significant difference was verified on statistical evaluation (Wilcoxon's signed rank test, $p < 0.05$). The thickness of stretched sub-

mucosal layer also showed an order identical to the thickness of non-stretched pieces.

DISCUSSION

Our experimental study shows that gastric wall thinning is significant with injurious tissue damage on mucosal and submucosal layer after excessive (i.e., 175% in our study) stretching. Porcine gastric tissues we used consist of an entire layer including the thick and less elastic muscular layer, and this characteristic limited the specimen from full stretching and pinning. On the contrary, tissues obtained after EMR or ESD usually consist of whole mucosa and partial submucosa, with a tendency to extend much easily than full thickness intestinal tissue. Stretching this to a maximal extent may result in even more extended piece with a significantly large size.

Several studies suggested unique physical properties of gastrointestinal tract.^{8,9} Submucosal layer mainly consists of collagen and elastic fiber bundle, and collagen fiber bundles can endure great mechanical impact. Submucosa carries deformation strength 3-4 times larger than muscular layer, while muscular layer has a maximal strength of 3-4 times compared with submucosa. Therefore, mechanical duration of digestive tract depends mainly on submucosa and muscular layer, with relatively low contribution from mucosa and serosa. During our first ex-

periment of stepwise stretching, tissues with an edge of 2.0 cm were torn when stretched up to 3.5 cm. This experience led the authors to step to the next experiment stretching the same size of tissue only up to 3.0 cm.

The present study also revealed different thickness of submucosal layer according to their original sites of stomach. By stretching the piece to 150% of its initial size, thickness of submucosal layer showed various degree of thinning in a range of 3 to 70%. Tissues from cardia, which was previously clarified by Fujishiro *et al.*¹⁰ to be the most similar layer to human gastric submucosa from a viewpoint of structure, revealed submucosal shrinking up to 70% from its original thickness. This result derives to a postulation of individual variation even from an identical organ of an identical species. Somewhat wide range of thinning seen in our study also emphasizes the importance of non-stretching tissue handling of the specimen obtained after EMR.

EMR was developed some 20 years ago, gradually replacing surgical management for early gastric cancer.¹¹⁻¹³ Introduction of endoscopic submucosal dissection (ESD) permitted endoscopists to remove mucosal lesions previously not regarded as an indication.^{10,11,14} ESD has a superiority over EMR by removing most of the submucosal layer, and this enables better diagnosis and prediction for distant metastasis. ESD can perform a safe resection as deep as 1,500 μ m of submucosal layer, whereas EMR only removes the superficial aspect of the same layer, showing the technical limitation of EMR.¹⁵

Deep invasion of mucosal cancer into the submucosal layer heightens the possibility of distant metastasis.^{3,4,16} In other words, submucosal invasion less than 500 μ m has a low probability of lymphatic spread, securing perfection on endoscopic removal.^{5,6} This cutoff value, however, stems from a study utilizing gastric cancer specimen from surgical resections instead of endoscopic removal, such as EMR or ESD. Moreover, this value originated from empirical data collected from a group of researchers without no obvious conception about stretching of surgical specimen.³ It is commonly known that surgical specimen is stretched before fixation in various strengths.

Five hundred μ m as a cutoff value therefore may have limitation for determining probability of distant metastasis. Endoscopists must give their best to fix the tissue similar to its original size and orientation on handling. To maintain the original size of specimen close to the pre-resected one, manual stretching force should be properly carried out while fixing it to a flat board. A group of pathologists adopted a recommendation concerning handling and management of tissues procured after EMR and ESD, addressing the importance of stretching specimen

and fixation into a flat board to prevent the tissue resection margin from rolling into the cut surface.¹⁷ On the other hand, it may be reasonable to measure the lesion according to the margins depicted by marking on mucosal surface with several kinds of measuring devices such as a pliable ruler applicable via the working channel of fiberscope.

In conclusion, a cutoff value of 500 μ m may be improper to regard sm1 as a malignancy-invading depth. Meticulous handling and pinning of excised tissue will minimize any damage, and eventually facilitate a better pathological diagnosis of submucosal infiltration. Further investigation is required, employing human surgical specimens for studying submucosal invasion of mucosal cancer.

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