Predictors for Submucosal Fibrosis in Patients With Superficial Squamous Esophageal Neoplasia Undergoing Endoscopic Submucosal Dissection

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INTRODUCTION: **Submucosal fibrosis greatly hinders the success of endoscopic submucosal dissection (ESD). This study determined ESD outcomes in patients with esophageal submucosal fibrosis and further explored the predictors.**

METHODS: **We retrospectively analyzed 163 patients with superficial squamous esophageal neoplasia. The degree of submucosal fibrosis was classified as follows: F0, none; F1, mild; and F2, severe. ESD outcomes as a function of the degree of submucosal fibrosis and biopsy were determined. The potential predictors of submucosal fibrosis were analyzed.**

RESULTS: **En bloc resection, R0 resection, and procedure time were significantly different between the F0-F2 groups ($P = 0.009$, $P = 0.002$, and $P < 0.001$, respectively). Perforation and immediate bleeding rates of F2 were significantly higher than the F0/F1 groups ($P < 0.001$ and $P < 0.001$, respectively). However, the nonbiopsy group vs the biopsy group and the delayed ESD group (postbiopsy >21 days) vs the early ESD group (postbiopsy ≤21 days) showed no statistical differences regarding the en bloc resection, R0 resection, and ESD complications (all $P > 0.05$). Further analysis indicated that it was not the biopsy history and delayed ESD (both $P > 0.05$), rather submucosal invasion vs intramucosal tumor (odds ratio = 4.534, $P = 0.003$) and current smoker vs nonsmoker (odds ratio = 2.145, $P = 0.043$) were independent risk factors for endoscopic submucosal fibrosis.**

DISCUSSION: **Esophageal submucosal fibrosis was shown to be closely related to unsatisfactory ESD outcomes. Biopsy history and delayed ESD had no adverse effect on submucosal fibrosis and ESD outcomes. Submucosal invasion and current cigarette smoking were predictors of submucosal fibrosis.**

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INTRODUCTION

Superficial squamous esophageal neoplasia (SSEN) is defined as squamous cell carcinoma confined to the mucosa or submucosa whether there is lymph node metastasis (1). At present, endoscopic submucosal dissection (ESD) is the first choice for SSEN without lymph node metastasis (2). Unlike the former endoscopic mucosal resection, ESD has the advantage of en bloc resection, which allows precise histologic assessment of the resected specimen, avoids residual disease, and reduces local recurrence (3,4); however, ESD is more likely to lead to severed complications, such as perforation and massive bleeding (5,6).

Novice endoscopists, large tumor size, deep submucosal infiltration, and submucosal fibrosis are believed to be important contributing factors for difficult ESD (7–11). Moreover, submucosal fibrosis seems to significantly hinder the success of ESD (7,12,13). Previous studies have reported that severe submucosal fibrosis leads to difficult separation of the submucosa and muscularis propria during colorectal ESD, which results in a higher incidence of perforation and incomplete resection, and a longer procedure time (8,14). Preoperative biopsy is believed to be an important cause of colorectal submucosal fibrosis (15,16); however, very few studies have focused on the relationship between the degree of submucosal fibrosis in SSEN and outcomes of ESD (12). In addition, whether a biopsy has a promoting effect on submucosal fibrosis in SSEN, and thus, results in a higher incidence of ESD complications, is still inconclusive. Therefore, we

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conducted this research to determine the relationship between biopsy, submucosal fibrosis, and esophageal ESD outcomes and further explore the predictive factors of submucosal fibrosis in patients with SSEN.

METHODS

Patients

This retrospective study was based on the medical records of patients with SSEN who underwent ESD at Nanfang Hospital (Guangzhou, China) from January 2014 to January 2019. A total of 182 patients with 186 lesions were identified. Among these patients, 19 were excluded because of the following factors: (1) esophageal squamous high-grade intraepithelial neoplasia with basaloid carcinoma underneath the submucosa (1 case), (2) a history of radiotherapy because of malignancy (4 cases), (3) patients in whom the specific time of initial biopsy could not be determined (7 cases), (4) patients with a history of previous endoscopic treatment for esophagus-related diseases (5 cases), and (5) insufficient endoscopic data to determine the degree of submucosal fibrosis (2 cases). A total of 163 patients (median age, 59.5 years; age range, 37–86 years; male: female = 101:62) containing 167 lesions were included in our study. Written informed consent was obtained from all patients after preoperative interviews. This study was approved by the Institutional Review Board of Nanfang Hospital.

ESD procedure

ESD was performed with the patient under general anesthesia. A single-channel endoscope with a water-jet function (GIF-Q260J

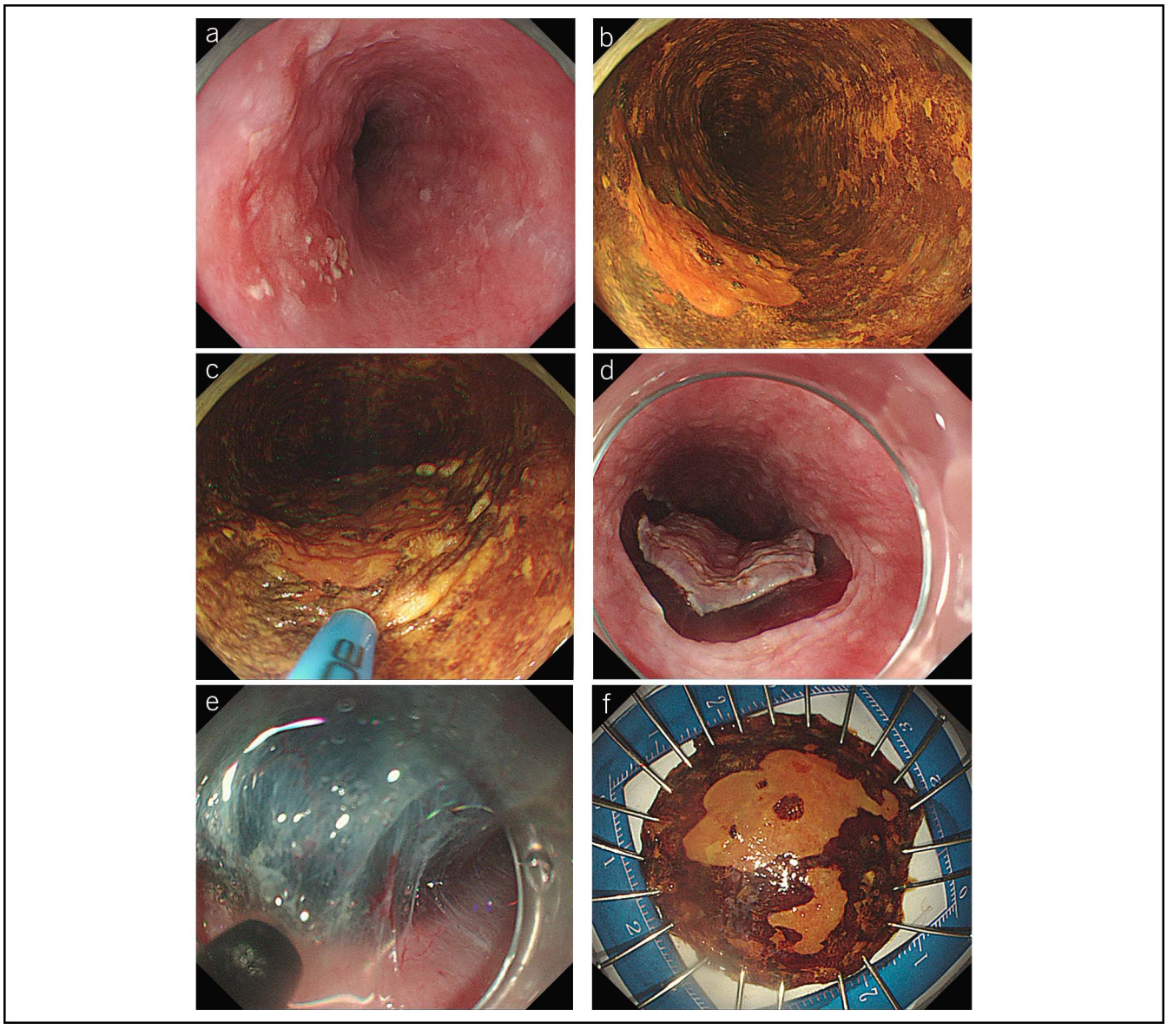

Figure 1. Endoscopic submucosal dissection of an esophageal squamous cell carcinoma. (**a**) A red and rough lesion in the middle thoracic esophagus on conventional white-light endoscopy. (**b**) Chromoendoscopy with Lugol dye solution to demarcate the lesion. (**c**) Marking the margin of the lesion using argon plasma coagulation. (**d**) Circumferential mucosal incision of the lesion. (**e**) Submucosal dissection from the oral side. (**f**) En bloc resected specimen presenting iodine-free areas.

and GIF-HQ290J; Olympus, Tokyo, Japan) was used during the procedures. A transparent cap (ND-201-11802) was mounted on the tip of the endoscope to provide a constant view. Two percent Lugol iodine solution was sprayed to delineate the lesion boundary. Subsequently, marking dots were placed outside the margin of the lesion using argon plasma coagulation. A mixture of 10% glycerol solution, 0.125% hyaluronic acid solution, and diluted epinephrine (1:200,000) was injected into the submucosa of the lesion. A circumferential mucosal incision of the lesion was then performed using a hook knife (KD-620LR; Olympus) or insulated-tip knife (KD-611L; Olympus). The hook knife, insulated-tip knife, or a hybrid knife (ERBE, Tübingen, Germany) was used to carefully dissect the submucosal tissue from the muscularis propria from the oral to anal side, thereby completing an en bloc resection of the entire lesion (Figure 1).

Histopathologic assessment

The resected specimens were fixed in formalin and sectioned at 2-mm intervals. The histologic types, depth of invasion, margins of the lesion, and macroscopic type were evaluated in each slice by experienced pathologists based on the Japanese classification of esophageal cancer (17,18). Whether the highly dysplastic tumor cells encroached through the muscularis mucosa and had submucosal infiltration, lesions were divided into intramucosal and invasive cancer. Unplanned piecemeal resection was regarded as resection of a target lesion into 2 or more pieces. ESD failure referred to the forced termination of the endoscopic treatment before completion because of technical difficulties and severe complications, and R0 resection, namely complete resection, was defined as an en bloc resection with no residual tumor in the lateral and vertical margins based on histologic evaluation (18).

Definitions

The degree of submucosal fibrosis was classified into 3 grades based on the findings of the submucosal layer after a hyaluronic acid mixture injection (Figure 2), as follows: F0 (no fibrosis), which presented as a transparent layer; F1 (mild fibrosis), which appeared as a white web-like structure in the submucosal layer; and F2 (severe fibrosis), which was characterized as a white muscular structure without a transparent layer in the submucosal layer (14). The degree of fibrosis was assessed independently by 2 endoscopic physicians and were further discussed with endoscopists who performed the ESD if discrepancies in identification existed. Procedure time was defined as the period from the insertion of the gastroscope to complete removal of the entire lesion, including wound surface hemostasis. Intraoperative adverse events included bleeding, perforation, and muscle injury. Intraoperative bleeding was defined as spurting or massive oozing of blood during the ESD procedure. Perforation was diagnosed when visualized by endoscopy or mediastinal emphysema was observed on radiography or chest CT scan. Muscle injury was defined as a muscularis propria defect, but no perforation, which was caused by thermal injury and was observed endoscopically.

Statistical analysis

All analyses were carried out using IBM SPSS Statistics software (version 22.0; IBM, New York, NY). Continuous variables are summarized by the median ± SD. Categorical variables are expressed as frequencies. The Student *t* test and Kruskal-Wallis tests were used for comparison of continuous variables, as indicated. A χ^2 test or Fisher exact tests were used to compare

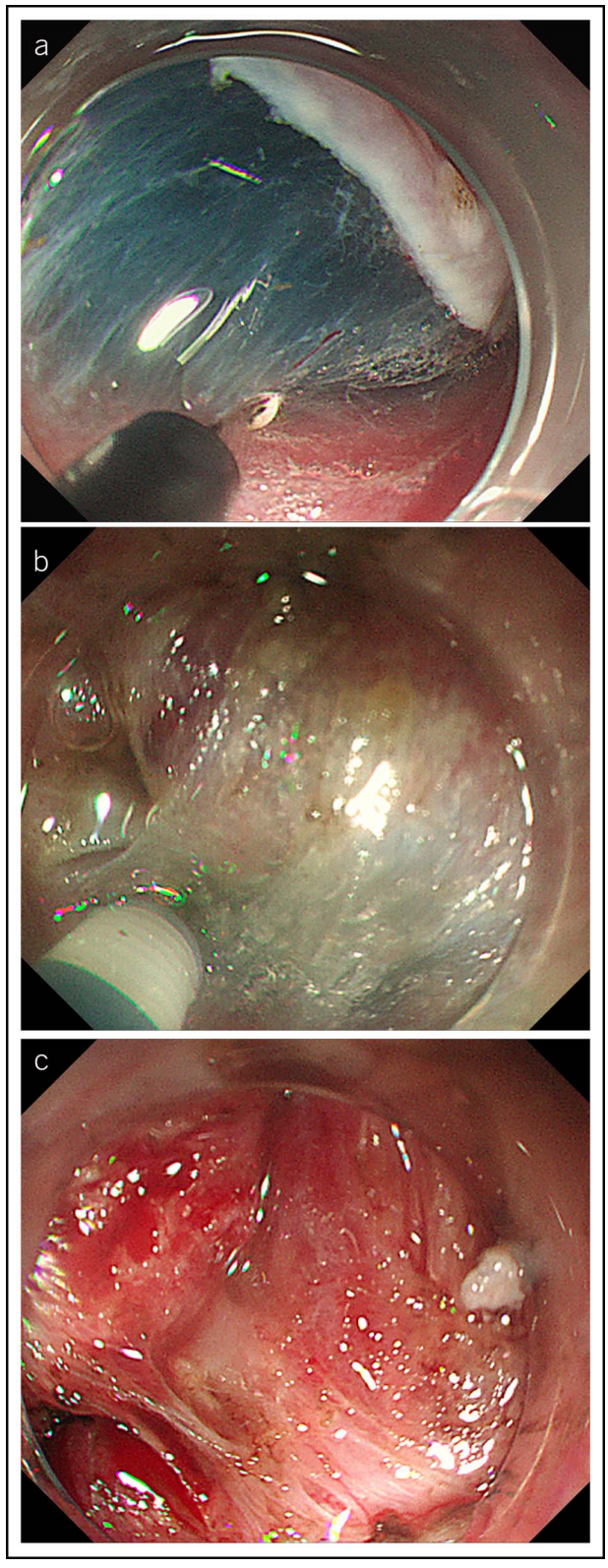

Figure 2. Degree of fibrosis in the submucosal layers in superficial esophageal squamous neoplasia. (**a**) F0, no fibrosis, which presented as a transparent layer. (**b**) F1, mild fibrosis, which appeared as a white web-like structure in the submucosal layer. (**c**) F2, severe fibrosis, which was characterized as a white muscular structure without a transparent layer in the submucosal layer.

Table 1. Baseline characteristics of included patients

Variables	Value (n = 163 patients/167 lesions)
Age (yr)	
Mean	59.3 ± 8.9
Range	37–86
Sex (male/female)	101 (62.0)/62 (38.0)
Smoking status	
Current smoker	46 (28.2)
Nonsmoker/past smoker	117 (71.8)
Drinking status	
Current drinker	38 (23.3)
Nondrinker/past drinker	125 (76.7)
Hospitalization period	
≤14 d	132 (81.0)
>14 d	31 (19.0)
Combined the symptoms of GRED	
Yes	32 (19.6)
No	131 (80.4)
Iodine staining history	
Yes	140 (83.8)
No	27 (16.2)
Biopsy history	
None	8 (4.8)
Performed 1 time	116 (69.5)
Performed ≥2 times	43 (25.7)
No. of biopsies	
None	8 (4.8)
1 or 2	127 (76.0)
More than 2	32 (19.2)
Length of tumor (mm)	
≤20	56 (33.5)
>20	111 (66.5)
Circumferential extension	
<1/4	56 (33.5)
1/4–2/4	56 (33.5)
2/4–3/4	34 (20.4)
≥3/4	21 (12.6)
Location	
Upper third	9 (5.4)
Middle third	121 (72.5)
Lower third	37 (22.2)
Macroscopic type	
Depressed	135 (80.8)
Nondepressed	32 (19.2)
Submucosal fibrosis	
F0	109 (65.3)
F1	43 (25.7)
F2	15 (9.0)
Depth of invasion	
Intramucosa	150 (89.8)
Submucosa	17 (10.2)

GRED, gastroesophageal reflux disease.

categorical variables, as indicated. A multiple logistic regression analysis was used to determine independent predictors of submucosal fibrosis. Interobserver agreement of submucosal fibrosis was analyzed by Cohen kappa. A P value <0.05 was considered statistically significant.

RESULTS

Clinicopathologic features of patients enrolled

A total of 163 patients, including 101 men (62.0%) and 62 women (38.0%), were enrolled in this study. The patients had a mean age of 59.3 ± 8.9 years (range, 37–86 years). As shown in Table 1, 46 patients (28.2%) were current smokers (≥5 cigarettes per day) and 38 (23.3%) had a history of alcohol consumption (≥50 g liquor per day). The average hospital stay was 11.8 ± 3.7 days. Less than 20% of patients (31 [19.0%]) were hospitalized for >2 weeks. Thirty-two patients (19.6%) combined the symptoms of gastroesophageal reflux disease (GRED), namely acid reflux and/or heartburn. Iodine staining were performed in 140 lesions (83.8%).

Greater than 90% of lesions had been biopsied. Only 8 lesions (4.8%) had not been biopsied preoperatively. Except for the above 8 lesions, 127 lesions (76.0%) had 1 or 2 biopsy tissues and the remaining 32 lesions (19.2%) had more than 2 biopsy tissues. The maximum number of biopsies from 1 lesion was 6. Based on the assessment of endoscopic submucosal findings, there were 109 cases (65.3%) shown to be F0 (no fibrosis), 43 (25.7%) shown to be F1 (mild fibrosis), and 15 (9.0%) shown to be F2 (severe fibrosis). The weight kappa coefficient was 0.792 (95% CI: 0.705–0.879, $P < 0.001$) between the 2 independent endoscopists, showing substantial agreement. Pathologic evaluation demonstrated that 150 lesions (89.8%) had intramucosal carcinoma, whereas 17 (10.2%) had submucosal infiltration.

Submucosal fibrosis and ESD outcomes

ESD outcomes associated with the degree of endoscopic submucosal fibrosis are shown in Table 2. The mean procedure time for all lesions was 93.31 ± 7.15 minutes (range, 26–540 minutes). The more severe the submucosal fibrosis, the longer the time it took to complete the treatment. The average procedure time for the F0, F2, and F3 groups showed significant differences between the 3 groups ($H = 17.499$, $P < 0.001$). The 3 groups showed no statistical differences in the mean duration of hospitalization ($H = 5.305$, $P = 0.07$), but there was increased tendency with more severe submucosal fibrosis. The duration of hospitalization was longest in the F2 group, and nearly 2 days longer than the F0 group (13.5 ± 2.8 days vs 11.6 ± 3.6 days). Regarding the ESD treatment effect, the overall R0 resection rate was 89.2% (149/167)

Table 2. Outcomes of ESD according to the degree of endoscopic submucosal fibrosis

Variables	Submucosal fibrosis			*P* Value
	F0 (n = 109)	F1 (n = 43)	F2 (n = 15)	
Procedure time (min)	75.5 ± 42.0	92.2 ± 46.6	241.1 ± 174.3	<0.001[a]
Hospitalization period				
Hospitalization (d)	11.6 ± 3.6	11.9 ± 4.4	13.5 ± 2.8	0.07[a]
ESD result				
En bloc resection	107 (98.2)	42 (97.7)	12 (80.0)	0.009[b]
Piecemeal resection	2 (1.8)	1 (2.3)	1 (6.7)	
ESD failure	0 (0)	0 (0)	2 (13.3)	
R0 resection				
Yes	102 (93.6)	38 (88.4)	9 (60.0)	0.002[b]
No	7 (6.4)	5 (11.6)	6 (40.0)	
Muscle injury				
Yes	8 (7.3)	8 (18.6)	3 (20.0)	0.059[b]
No	101 (92.7)	35 (81.4)	12 (80.0)	
Perforation				
Yes	1 (0.9)	2 (4.7)	7 (46.7)	<0.001[b]
No	108 (99.1)	41 (95.3)	8 (53.3)	
Immediate bleeding				
Yes	3 (2.8)	1 (2.3)	7 (46.7)	<0.001[b]
No	106 (97.2)	42 (97.7)	8 (53.3)	

[a]Kruskal-Wallis test.
[b]Fisher exact test.
ESD, endoscopic submucosal dissection.

and the en bloc resection rate was 96.4% (161/167). The more severe the submucosal fibrosis, the lower the en bloc and R0 resection rates, which had significant differences between the 3 groups ($P = 0.009$ and $P = 0.002$, respectively). In the F2 group, the en bloc and R0 resection rates were much lower than the F1 and F0 groups (80.0% vs 97.7% vs 98.2%, and 60.0% vs 88.4% vs 93.6%, respectively). In addition, the only 2 cases (1.20% [2/167]) of failed ESD occurred in the F2 group.

Regarding intraoperative complications, the overall muscle injury, perforation, and immediate bleeding rates were 11.4% (19/167), 6.0% (10/167), and 6.6% (11/167), respectively. Compared with the average, the above complication rates for lesions accompanied by F2 fibrosis were far higher (20.0% [3/15], 46.7% [7/15], and 46.7% [7/15], respectively). Perforation and immediate bleeding rates among the 3 groups had statistically significant differences ($P < 0.001$ and $P < 0.001$, respectively). The muscle injury rate had no significant differences between the 3 groups (7.3% vs 18.6% vs 20.0%, $P = 0.059$).

Relationship between biopsy and ESD outcomes

Previous studies demonstrated that biopsy history and delayed ESD after initial biopsy were independent predictive factors for endoscopic submucosal fibrosis (12,15). To test the hypothesis that biopsy is closely related to a higher risk of endoscopic submucosal fibrosis and thus results in more complications of ESD, we first investigated the relationship between biopsy and ESD outcomes. As shown in Table 3, we divided all the included lesions into nonbiopsy and biopsy groups, and delayed (postbiopsy >21 days) and early ESD groups (postbiopsy ≤21 days). The 2 above groups had no significant differences in invasion depth, length, and circumferential extension of lesions, suggesting good comparability. The ESD results, R0 resection rate, and ESD complications were not significantly different between the nonbiopsy and biopsy groups (all $P > 0.05$). Biopsy history had no effect on ESD outcomes. In addition, the timing of ESD after initial biopsy had no correlation with esophageal ESD outcomes. There were no significant differences between the delayed and early ESD groups regarding the ESD results, R0 resection rates, and incidences of muscle injury, perforation, and immediate bleeding (all $P > 0.05$). The severity of submucosal fibrosis showed no relationship with biopsy history or the timing of ESD. No significant differences were identified in the degree of submucosal fibrosis between nonbiopsy and biopsy groups ($P = 0.729$) or between delayed and early ESD groups ($P = 0.160$).

Clinicopathologic factors related to the degree of submucosal fibrosis

As shown in Table 4, to further validate whether the biopsy history is a predictor for submucosal fibrosis, as has been reported elsewhere (12,15), and investigate the other relevant clinicopathologic risk factors, we compared the nonsubmucosal (F0, n = 109) and submucosal fibrosis groups (F1/F2, n = 58). Univariate analysis

Table 3. Relationship between biopsy and outcomes of ESD

Variable	Non-biopsy group (n = 8)	Biopsy group (n = 159)		P_1	P_2
		Postbiopsy ≤21 d (n = 69)	Postbiopsy >21 d (n = 90)		
Depth of invasion					
mucosa	8 (100)	59 (85.5)	77 (85.6)	0.601[a]	0.993
submucosa	0 (0)	10 (14.5)	13 (14.4)		
Length of tumor					
≤20 mm	3 (37.5)	21 (30.4)	32 (35.6)	1.000[a]	0.497
>20 mm	5 (62.5)	48 (69.6)	58 (64.4)		
Circumferential extension					
<1/2	7 (87.5)	41 (59.4)	64 (71.1)	0.634[a]	0.173
1/2–3/4	1 (12.5)	19 (27.5)	14 (15.6)		
≥3/4	0	9 (13.0)	12 (13.3)		
ESD results					
En bloc	8 (100)	65 (94.2)	88 (97.8)	1.000[a]	0.446[a]
Piecemeal	0 (0)	3 (4.3)	1 (1.1)		
ESD failure	0 (0)	1 (1.4)	1 (1.1)		
R0 resection					
Yes	7 (87.5)	60 (87.0)	82 (91.1)	1.000[a]	0.401
No	1 (12.5)	9 (13.0)	8 (8.9)		
Muscle injury					
Yes	1 (12.5)	8 (11.6)	10 (11.1)	1.000[a]	0.924
No	7 (87.5)	61 (88.4)	80 (88.9)		
Perforation					
Yes	0 (0)	7 (10.1)	3 (3.3)	1.000[a]	0.103[a]
No	8 (100)	62 (89.9)	87 (96.7)		
Immediate bleeding					
Yes	1 (12.5)	2 (2.9)	8 (8.9)	0.427[a]	0.189[a]
No	7 (87.5)	67 (97.1)	82 (91.1)		
Submucosal fibrosis					
F0	7 (87.5)	50 (72.5)	52 (57.8)	0.729[a]	0.160
F1	1 (12.5)	14 (20.3)	28 (31.1)		
F2	0 (0)	5 (7.2)	10 (11.1)		

[a]Fisher exact test, P_1: nonbiopsy groups vs biopsy groups, P_2: postbiopsy≤21 days vs postbiopsy >21 days.
ESD, endoscopic submucosal dissection.

demonstrated that the 2 groups did not differ significantly regarding average age, gender ratio, alcohol consumption, tumor location, circumferential extension, iodine staining history, and length of lesions (all $P > 0.05$), which suggested good comparability. No significant difference was identified in the symptoms of GRED between the 2 groups ($P = 0.212$). There were no significant differences in macroscopic type and lesion erosions or ulcers ($P > 0.05$). Regarding preoperative biopsy, the 2 groups showed no significant differences in the time biopsies obtained ($P = 0.287$) and the number of biopsy tissues ($P = 0.460$). What is more, the severity of submucosal fibrosis showed no connected with the timing of ESD after initial biopsy, which displayed no significant differences between the 2 groups (66.7 % vs 51.0%, $P = 0.056$), whereas current smoking and submucosal invasion were shown to be significant factors ($P = 0.023$ and $P = 0.001$, respectively).

It has been previously reported that biopsy history and delay ESD are important risk factors for submucosal fibrosis (15), and thus, we included biopsy history, delay ESD, and other significant factors in a multivariate analysis (Table 5). Multivariate logistic regression analysis demonstrated that submucosal invasion vs intramucosal tumor (odds ratio [OR] = 4.534, $P = 0.003$) and current smoking vs nonsmoker (OR = 2.145, $P = 0.043$) were independent risk factors for endoscopic submucosal fibrosis. Neither biopsy history nor delayed ESD > 21 days from initial biopsy was an independent predictor of submucosal fibrosis (both $P > 0.05$).

Table 4. Submucosal fibrosis in relation to clinicopathologic factors

Factor	Non-submucosal fibrosis (F0, n = 109)	Submucosal fibrosis (F1/F2, n = 58)	*P* Value
Age (yr)	61.7 ± 10.8	59.9 ± 5.4	0.736
Sex			
Male	66 (61.7)	35 (62.5)	0.919
Female	41 (38.3)	21 (37.5)	
Current smoker			
Yes	24 (22.4)	22 (39.3)	0.023
No	83 (77.6)	34 (60.7)	
Current drinker			
Yes	21 (19.6)	17 (30.4)	0.124
No	86 (80.4)	39 (69.6)	
Combined the symptoms of GRED			
Yes	18 (16.8)	14 (25.0)	0.212
No	89 (83.2)	42 (75.0)	
Iodine staining history			
Yes	89 (81.7)	51 (87.9)	0.294
No	20 (18.3)	7 (12.1)	
Length of tumor (mm)			
≤20	40 (36.7)	16 (27.6)	0.235
>20	69 (63.3)	42 (72.4)	
Circumferential extension			
<1/2	79 (72.5)	33 (56.9)	0.087
1/2–3/4	20 (18.3)	14 (24.1)	
≥3/4	10 (9.2)	11 (19.0)	
Location			
Upper esophagus	5 (4.6)	4 (6.9)	0.773[a]
Middle esophagus	79 (72.5)	42 (72.4)	
Lower esophagus	25 (22.9)	12 (20.7)	
Time of ESD from initial biopsy[b]			
≤21 d	50 (49.0)	19 (33.3)	0.056
>21 d	52 (51.0)	38 (66.7)	
Biopsy history			
None	7 (6.4)	1 (1.7)	0.287[a]
Performed 1 time	77 (70.6)	39 (67.2)	
Performed ≥2 times	25 (22.9)	18 (31.0)	
No. of biopsies			
None	7 (6.4)	1 (1.7)	0.460[a]
1 or 2	81 (74.3)	46 (79.3)	
More than 2	21 (19.3)	11 (19.0)	
Macroscopic type			
Depressed	21 (19.3)	12 (20.7)	0.826
Nondepressed	88 (80.7)	46 (79.3)	
Depth of invasion			
Mucosa	101 (92.7)	43 (74.1)	0.001
Submucosa	8 (7.3)	15 (25.9)	
Lesion ulcer or erosion			
Yes	17 (15.6)	14 (24.1)	0.176
No	92 (84.4)	44 (75.9)	

[a]Fisher exact test.
[b]Excluding 8 nonbiopsied lesions.
ESD, endoscopic submucosal dissection; GRED, gastroesophageal reflux disease.

DISCUSSION

ESD is the preferred treatment for SSEN, which has a special advantage for en bloc resection, thus, enabling accurate histologic assessment (2,19); however, submucosal fibrosis is considered to be a significant factor for technical difficulty and poor ESD outcomes. It has been reported that early gastric cancer accompanied by severe submucosal fibrosis significantly reduces the rate of complete en bloc resection by ESD and is associated with a higher likelihood of complications (13,20). Similarly, for colorectal ESD, submucosal fibrosis is an important factor related to incomplete resection and perforation that does not result in significant improvements even when performed by an experienced operator (7,14). Regarding the risk factors for submucosal fibrosis, it has been reported that biopsy history is an important factor that leads to a nonlifting sign in colorectal cancer (21). Prolonging the period between the biopsy and endoscopic mucosal resection has a positive correlation with the nonlifting sign. However, little attention has been paid to the influence of submucosal fibrosis on SSEN. Indeed, this is a rare report to identify the relationship between submucosal fibrosis and incidence during esophageal ESD with the largest study sample (167 lesions).

We found that submucosal fibrosis is not only associated with long procedure time and an increasing trend of average hospital stay but also is an important risk factor for esophageal ESD complications. Compared with the F0-1 groups, the F2 group presented with a lower en bloc resection (only 80%) and a far higher rate of perforation and immediate bleeding (46.7% and 46.7%, respectively). The complications were mainly attributed to technical difficulties, which increased with the severity of submucosal fibrosis. When compared with the incidence of ESD with other sites, the perforation rate of the F2

Table 5. Multivariate logistic regression analysis of factors predictive for submucosal fibrosis

Factor	Statue	OR	95% CI	*P* Value
Depth of invasion	Mucosa	1		
	Submucosa	4.534	1.700–12.090	0.003
Current smoker	Yes	1		
	No	2.145	1.023–4.499	0.043
Time of ESD from initial biopsy	≤21 d	1		
	>21 d	1.956	0.943–4.057	0.072
Biopsy history	Performed ≤1 time	1		
	Performed >1 time	1.498	0.675–3.328	0.321
Lesion ulcer or erosion	No	1		
	Yes	1.509	0.636–3.581	0.351

ESD, endoscopic submucosal dissection.

group in esophageal ESD was far higher than colorectal ESD in other reports (range 7.5%–19.4%) (7,8,22). This finding may be because esophageal ESD is more technically difficult than gastric or colorectal ESD because of the narrow lumen and continuous movements with respirations and cardiac activity (23,24).It was a challenge to maintain a certain depth of resection in a narrow lumen during esophageal ESD with submucosal fibrosis. In the case of shallow submucosal resection to avoid perforation, there was an increased risk of residual tumor; however, when we attempted to resect submucosal tissue cleanly from the muscularis propria, there was a higher risk of perforation because of the lack of demarcation between fibrotic submucosal tissue and the muscularis propria. Therefore, preoperative evaluation of the degree of submucosal fibrosis accompanying adequate preoperative preparation, such as the use of carbon dioxide insufflation, endotracheal intubation, and a more experienced operator, is of great significance to reduce the incidence of complications.

Thus far, iodine staining combined with biopsy are still considered the gold standard for the preoperative diagnosis of SSEN (25,26). Regarding whether biopsy history and delay ESD had a promoting effect on submucosal fibrosis and thus resulted in more complications of ESD, there was no final conclusion. Fukunaga et al. (15) reported that preoperative biopsy sampling for a colorectal laterally spreading tumor might cause severe submucosal fibrosis but had no adverse effect on ESD outcomes. Huh et al. (12) demonstrated that delayed esophageal ESD is an independent risk factor for submucosal fibrosis, which led to more complications, and thus recommended immediate ESD after biopsy. Unlike the abovementioned studies, we found that routine preoperative biopsy and delayed ESD (postbiopsy >21 days) did not promote esophageal submucosal fibrosis, and there was no relationship with ESD treatment outcomes. Our study has a large sample size. The adopted evaluation criteria for endoscopic fibrosis have been shown to be objective and reliable in previous studies (8,12,13,15). Furthermore, by comparing the ESD complications between biopsy and nonbiopsy groups and early ESD and delay ESD groups, we found that biopsy history and delay ESD had no relationship with ESD outcomes. Abovementioned results were consistent with the conclusion that ESD time were no independent risk factor for esophageal submucosal fibrosis. Therefore, we think that our results are plausible.

For the abovementioned different conclusions, we assumed the following possible causes. First, the results may be due to different pathologic histology. The esophagus is covered by nonkeratinized epithelium and has a thick muscularis mucosa. The standard biopsy forceps can only acquire the mucosa at a superficial depth. Even with a deep biopsy, there is a very small invasion of the submucosa. Second, epithelial damage by biopsy usually causes tiny areas of iatrogenic acute inflammation. Rather than acute inflammatory reactions, pathogenic fibrosis typical results from chronic inflammatory reactions (27). Third, the degree of submucosal fibrosis is to some extent related to the area of the mucosal defect. The greater the area of the mucosal defect, the more severe the submucosal inflammatory response and the greater the degree of submucosal fibrosis and esophageal stenosis. The diameter of the opening standard biopsy forceps is approximately 6 mm. Therefore, unless multiple biopsies were obtained simultaneously, the mucosa defect resulting from the biopsy were small in size. It has been reported that the proliferation of the epithelium has started from the periphery to cover the defect epithelium just 7 days after the mucosal damage, and by 14 days, the new epithelium could reached a range of 11.7–13.5 mm (28), suggesting that the defect epithelium caused by standard biopsy would be quickly covered by proliferating epithelial cells. The continuous layer of epithelial cells protected the submucosa from mechanical and chemical injuries or infections, and thus could not cause the chronic inflammation that leads to the process of fibrosis (29). Based on the above understanding, endoscopic transplantation of autologous oral mucosal epithelial cell sheets has become one of the therapies to prevent esophageal stenosis caused by extend defect of esophageal mucosa (30). Therefore, we have good reasons to believe that previous biopsies more than 21 days before ESD had little relationship with esophageal submucosal fibrosis. Endoscopists do not need to deliberately advance the ESD date within 21 days from the initial biopsy in an effort to prevent submucosal fibrosis.

To further identity the potential factors for submucosal fibrosis during esophageal ESD, we compared the clinicopathologic factors

between the nonfibrosis and fibrosis groups. Multivariate analysis showed that the depth of invasion is an independent risk factor (OR = 4.534, $P = 0.003$), which is similar to the study conducted by Kim et al. (7). It is known that tumors with submucosal invasion are commonly accompanied by extensive inflammatory cell infiltration and interstitial fibrosis (31). Huh et al. (12) demonstrated that endoscopic submucosal fibrosis had a tendency to be related to the depth of invasion but with no statistical significance, which may be largely because of the small sample size. Moreover, we found that smoking is significantly associated with submucosal fibrosis (OR = 2.145, $P = 0.043$). Smokers have a higher risk of gastroesophageal reflux, which is a chronic inflammatory state (32,33). Chronic inflammation often triggers fibrosis (34). In addition, it has been reported that cigarette smoke augments renal inflammation, oxidant radicals, and thus results in renal fibrosis (35). Smoking is a major high-risk factor for pulmonary fibrosis, resulting from accumulation of inflammatory cells, fibroblast hyperplasia, and scar formation (36). Therefore, it is reasonable to assume that the exposure of cigarette smoke extracts can also augment extensive inflammatory cell infiltration of SSEN, and thus exacerbate submucosal interstitial fibrosis. Further studies with a focus on the underlying mechanism to test our conclusions are needed.

This study had some limitations. First, the study was a single-center respective study; however, the baseline characteristics and incidence of ESD outcomes were similar to other center studies (37,38). Therefore, we believed that the samples in our studies were representative. Second, we did not evaluate the relationship between endoscopic and histologic classification of submucosal fibrosis because of a shortage of adequate tissue specimens. However, to complement this limitation, the degree of fibrosis was assessed independently by 2 endoscopic physicians and showed a good consistency (kappa = 0.792). Finally, other potential risk factors for submucosal fibrosis, such as the GRED and the expertise of the endoscopist, were not included in the present study. A large prospective study is expected to further investigate the abovementioned factors.

In conclusion, we found that esophageal submucosal fibrosis is closely related with unsatisfactory ESD outcomes. Routine preoperative biopsy and the time of ESD from initial biopsy had no adverse effects on submucosal fibrosis and ESD outcomes; however, submucosal invasion and current cigarette smoking were independent risk factors for submucosal fibrosis. For such lesions, more adequate preoperative preparation is needed to overcome technically difficult ESD caused by potential submucosal fibrosis.

CONFLICTS OF INTEREST

Guarantor of the article: No.

Specific author contributions: All authors have contributed to the content of the manuscript. X.Z. was involved in study design, analysis and interpretation of data, and drafting of the manuscript. X.M and Z.C. were in involved study design and interpretation of data. J.W. was involved in acquisition and analysis of data. J.W., K.Q., S.W., and G.D. were involved in critical revision of the manuscript for important intellectual content and material support. Y.B. was in involved in study concept, acquisition of data, and study supervision. All authors were involved in the modification of the article.

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Study Highlights

WHAT IS KNOWN

- ✓ Submucosal fibrosis greatly hinders the success of ESD.
- ✓ Biopsy history is closely related with colorectal submucosal fibrosis.

WHAT IS NEW HERE

- ✓ Biopsy history and delayed ESD had no adverse effect on esophageal submucosal fibrosis and ESD outcomes.
- ✓ Submucosal invasion and current cigarette smoking were predictors of esophageal submucosal fibrosis.

TRANSLATIONAL IMPACT

- ✓ Endoscopists do not need to deliberately advance the ESD date within 21 days from the initial biopsy for fear of submucosal fibrosis.
- ✓ For current cigarette smokers or lesions of submucosal invasion, more adequate preparation is needed to overcome technically difficult ESD caused by potential submucosal fibrosis.

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