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Immunohistochemical Localization of D-β-Aspartic Acid and Periostin in Vocal Fold Polyps

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Long-term voice abuse or sudden vocal fold microvascular disruption may lead to injury and subsequent repair/remodeling in the vocal fold mucosa. Periostin is known to be involved in airway remodeling and also in various otolaryngological diseases. D-β-aspartic acid is the major isomer of D-aspartic acid found in elderly tissue. In this study we investigated the expression and the role of D-β-aspartic acid and periostin in the formation of vocal fold polyps. The expression patterns of D-β-aspartic acid and periostin in 36 surgical specimens of vocal fold polyps from 36 patients were investigated immunohistochemically. In the epithelium of vocal polyps, D-β-aspartic acid was expressed in all cases. Expression of D-β-aspartic acid was detected in 25 samples obtained from patients with vocal fold polyps stroma. Expression of periostin was detected in 28 samples obtained from patients with vocal fold polyps. Two patterns of D-β-aspartic acid expression were observed in vocal fold polyps stroma: positive type and negative type. The following four patterns of periostin expression were observed in vocal fold polyps: negative type, superficial type, infiltrative type, and diffuse type. An association was observed between D-β-aspartic acid expression patterns and periostin expression patterns. From these findings we speculate that periostin and D-β-aspartic acid participate in certain pathological changes in vocal fold polyps, such as extracellular matrix accumulation, local fibrosis, and the formation and development of vocal fold polyps.

Keywords: D-β-aspartic acid; periostin; vocal fold polyp
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Introduction

Vocal fold polyps are the most common benign lesions of the vocal folds (Kumai 2019). The presenting symptoms of voice disorders, such as polypoid degeneration, granuloma of the larynx, and vocal fold polyps, include hoarseness and throat discomfort, which not only decrease the quality of life but also impede communication in daily life (Martins et al. 2011). Chronic trauma to the vocal folds from bacteria, viral pathogens, gastric acid reflux, and prolonged voice abuse might induce inflammation and tissue repair, contributing to the pathophysiology of vocal fold polyps (White 2019). Vocal polyps are characterized by the frequent complications of vasodilation, thrombus formation, hemorrhage, edema, and fibrin deposition within the polyp. There is evidence that the location of vocal fold fibroblasts in the lamina propria and their ability to respond to external stimuli by producing inflammatory molecules

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might be involved in the pathophysiology of vocal fold polyps (Naunheim and Carroll 2017; White 2019). Recent studies indicate that periostin is a regulator of fibrosis and collagen deposits, and it has been recognized for its important role in airway remodeling and formation of nasal polyps in chronic rhinosinusitis (Ishida et al. 2012; Sato et al. 2023). Similarly, there are indications that sudden vocal-fold microvascular disruption or prolonged voice abuse may lead to injury and subsequent periostin-mediated repair/remodeling in the vocal fold mucosa. Recent studies have found the following four patterns of periostin expression in vocal fold polyps: negative, superficial, infiltrative, and diffuse. An association was observed between periostin expression patterns and the histological subtypes of vocal fold polyps (Tateda et al. 2022).

All proteins in the body are composed of L-amino acids. But recently it has been reported that ultraviolet (UV) light and aging cause an increase in D-amino acids, which should not be present, in some organs (Helfman and Bada 1976; Masters et al. 1977; Shapira and Chou 1987; Powell et al. 1992; Fujii et al. 1994, 1999, 2002; Ritz-Timme et al. 2003; Kaji et al. 2006, 2007, 2015). The immunohistochemical localization of D-*β*-aspartic acid in various parts of the body is now a major subject of research. UV irradiation promotes the racemization of amino acids, and proteins containing D-*β*-aspartic acid are now being found in certain tissues exposed to UV light, including pinguecula (Kaji et al. 2006), cataracts (Masters et al. 1977), and actinic keratosis of the skin (Kaji et al. 2015). Therefore, D-*β*-aspartic acid is also considered a useful indicator of UV irradiation history. D-*β*-aspartic acid has also been found in proteins in the following human tissues in the elderly: ligaments (Ritz-Timme et al. 2003), brain (Shapira and Chou 1987), lens (Fujii et al. 1999; Kaji et al. 2007), aorta (Powell et al. 1992), skin (Fujii et al. 2002), and teeth (Helfman and Bada 1976). The presence of D-*β*-aspartic acid in various age groups of an organism is thought to be due to racemization of L-*β*-aspartic acid in

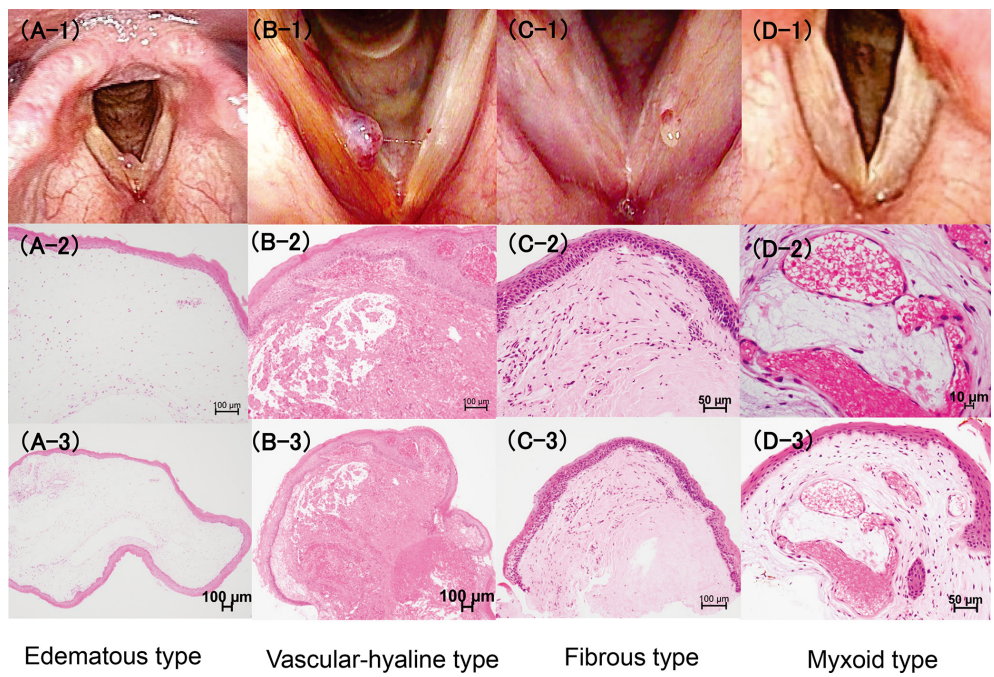

Fig. 1. Laryngeal findings, hematoxylin and eosin (HE) staining in vocal fold polyps.
In case A, A-1 (endoscopic photo), A2-3 (HE staining). (A-1) A 36-year-old Japanese male with a white polyp on the right vocal fold. (A2-3) This case is a typical edematous type. Submucosal accumulation of pale blue- to pink-stained material admixed with a sparsely cellular and variably vascularized stroma is observed (HE staining, original magnification × 100 and × 40, respectively).
In case B, B-1 (endoscopic photo), B2-3 (HE staining). (B-1) A 35-year-old Japanese male with red polyps on the right vocal fold. (B2-3) This case is a typical vascular-hyaline type. Dense eosinophilic submucosal deposition of fibrin material in close proximity to a vascular space is observed (HE staining, original magnification × 100 and × 40 respectively).
In case C, C-1 (endoscopic photo), C2-3 (HE staining). (C-1) A 48-year-old Japanese male with a white polyp on the left vocal fold. (C2-3) This case is a typical fibrous type. Moderately cellular submucosa consisting of uniform oval to spindle-shaped cells associated with a varying amount of fibrous tissue deposition is observed (HE staining, original magnification × 200 and × 100, respectively).
In case D, D-1 (endoscopic photo), D2-3 (HE staining). (D-1) A 50-year-old Japanese female with a white polyp on the left vocal fold. (D2-3) This case is a typical myxoid type. Markedly prominent dilated vascular spaces with or without associated hemorrhage and a combination of the above subtypes in a single lesion is observed (HE staining, original magnification × 400 and × 200, respectively).

proteins (Kaji et al. 2007). It is well known that aspartic acid has the highest rate of racemization of amino acids. Therefore, the presence of D-β-aspartic acid in proteins of living organisms is considered to be a useful marker of aging (Fujii et al. 1999). However, D-β-aspartic acid expression in vocal fold polyps has not been studied. Therefore, the purpose of this study was to investigate the immunohistochemical localization of D-β-aspartic acid and periostin in vocal fold polyps.

Materials and Methods

Subjects

The patient group consisted of 36 subjects with vocal fold polyps, aged between 26 to 77 years (mean age 51.6 ± 14.1 years), who did not respond to at least three months of conservative therapy, necessitating surgical removal of the vocal fold polyp(s). All the patients underwent endolaryngeal microsurgery at the Division of Otolaryngology, Tohoku Medical and Pharmaceutical University Hospital between August 2016 and November 2019. All 36 patients had single lesions. Only cases with typical clinical and histological findings were included in the patient group. Clinically, vocal fold polyps were considered as nodular lesions of various sizes that were localized on the free margin or upper surface of the vocal fold. They appeared visually to be single lesions with smooth and regular surfaces that were grayish red to dark red in color with distinct margins surrounded by normal tissue of the vocal fold. Small blood vessels appearing as hemorrhages sometimes were seen through the thin epithelial layer. Histopathological diagnosis of the vocal fold polyps was made at the Division of Pathology, Tohoku Medical and Pharmaceutical University Hospital. The study was approved by the Ethics Review Committees at Tohoku Medical University Hospital (approval number 2016-2-058) and Tohoku University Graduate School of Medicine (approval number 2017-1-321), and the requirement for informed consent was waived due to the opt-out policy adopted in the study.

Immunohistochemistry to detect Dβ-aspartic acid

Four-micrometer sections were taken from paraffin-embedded tissue blocks, and deparaffinized and rehydrated. They were then heated (Autoclave, 121°C) in Antigen Retrieval Solution (pH 9.0) for five minutes for antigen retrieval. Endogenous peroxidase activity was blocked with H_2O_2 3% in absolute methanol at room temperature for 10 minutes. All sections were preincubated in Normal Goat Serum (ImmPRESS HRP REAGENT Kit Anti-Rat: MP-7444; Vector Laboratories, Newark, CA, USA) at room temperature for 20 minutes to block nonspecific background staining. The sections were then treated with a polyclonal antibody (diluted 1:800) against D-aspartic acid (AB-T047; Advanced Targeting Systems, Carlsbad, CA, USA), kept at 4°C overnight. The sections were then incubated with a secondary antibody (Anti-Rat HRP Reagent; ImmPRESS HRP Reagent Kit Anti-Rat: MP-7444; Vector Laboratories) for 30 minutes at room temperature and finally incubated with Lipuid diaminobenzidine (DAB) + Substrate Chromogen System (cat No. K3468; Dako, Glostrup, Denmark) and counterstained with hematoxylin.

Immunohistochemistry to detect periostin

Four-μm sections were taken from paraffin-embedded tissue blocks. Deparaffinized tissue sections were rehydrated in a graded series of alcohols. Endogenous peroxidase activity was blocked with H_2O_2 3% in absolute methanol at room temperature for 10 minutes. All sections were preincubated in Protein Block Serum-Free (Cat No. X0909; Dako) at room temperature for 20 minutes to block nonspecific background staining. The sections were then treated with a polyclonal antibody (diluted 1:2,730) against polyclonal anti-periostin antibody, kept at 4°C overnight.

The sections were then incubated with a secondary antibody (EnVision+ Dual Link System-HRP (Cat No. K4063, Dako) for 60 minutes at room temperature and finally incubated with Lipuid diaminobenzidine (DAB)+ Substrate Chromogen System (cat No. K3468, Dako) and

Table 1. D-β-aspartic acid expression patterns and histological subtypes of vocal cord polyps.

Vocal fold polyp stroma D-β-aspartic acid	Histological subtypes of vocal cord polyps				*P*-value
	Edematous type (n = 13)	Vascular-hyaline type (n = 11)	Fibrous type (n = 10)	Myxoid type (n = 2)	
Positive type (n = 25)	7	10	6	2	< 0.17
Negative type (n = 11)	6	1	4	0	

Table 2. D-β-aspartic acid expression and periostin expression patterns of vocal cord polyps.

Vocal fold polyp stroma D-β-aspartic acid	Periostin expression patterns				*P*-value
	Negative type (n = 8)	Superficial type (n = 11)	Infiltrate type (n = 13)	Diffuse type (n = 4)	
Positive type (n = 25)	4	6	13	2	< 0.01
Negative type (n = 11)	4	5	0	2	

counterstained with hematoxylin.

Assessment of slides

Immunostained sections were assessed at × 40, × 100 and × 200 magnification under a light microscope with an eyepiece reticle. Cell counts were expressed as means per high-power field (0.202 mm^2). At least two sections were immunostained, and more than five areas were evaluated via the reticle.

Statistics

Variables were compared by Wilcoxon test and Fisher's exact test using the statistical software SAS 9.4; SAS Institute Inc. (Cary, NC, USA). A P-value of less than 0.05 was considered to be statistically significant.

Results

Histological subtypes of vocal fold polyps

The vocal fold polyps were categorized into the following four histological subtypes: edematous (Fig. 1A1-3), vascular-hyaline (Fig. 1B1-3), fibrous (Fig. 1C1-3), and myxoid (Fig. 1D1-3). The edematous type in Fig. 1A2-3 is characterized by submucosal accumulation of pale blue- to pink-stained material admixed with a sparsely cellular and variably vascularized stroma. The vascular-hyaline type in Fig. 1B2-3 is characterized by dense eosinophilic submucosal deposition of fibrin material, often in close proximity to a vascular space. The fibrous type in Fig. 1C2-3 is charac-

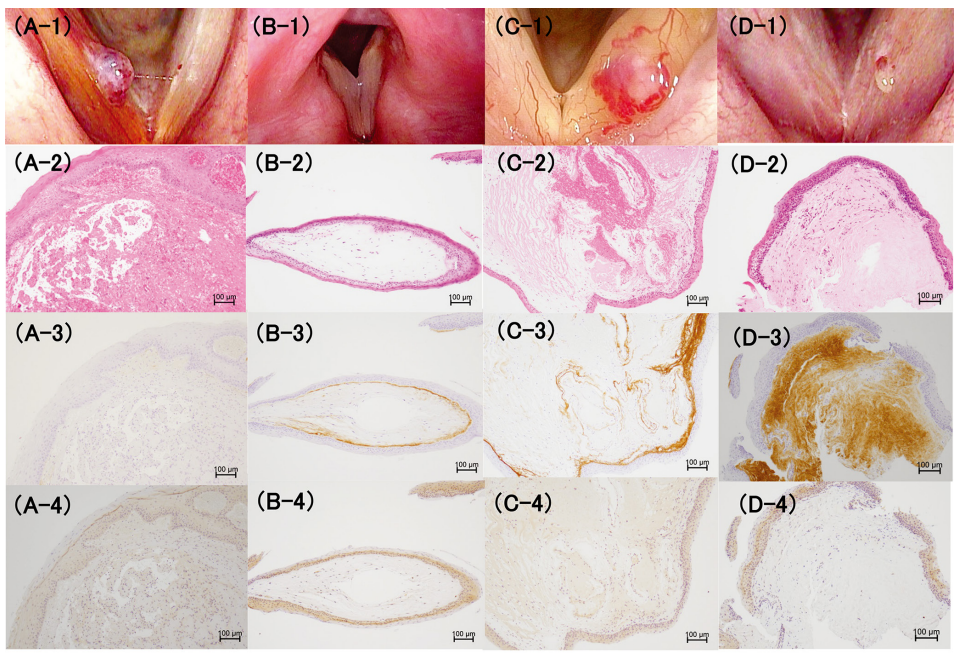

Fig. 2. Immunohistochemical staining patterns of periostin in vocal fold polyps were categorized into the following four types: negative type (n = 8), superficial type (n = 11), infiltrative type (n = 13), and diffuse type (n = 4) (Original magnification ×100).

In case A, A-1 (endoscopic photo), A-2 (HE staining), A3-4 (immunostaining). (A-1) A 35-year-old Japanese male with a red polyp on the right vocal fold. (A-2) Dense eosinophilic submucosal deposition of fibrin material in close proximity to a vascular space is observed. The diagnosis of vascular-hyaline type was made (HE staining). (A-3) Periostin is not observed: negative type (immunostaining). (A-4) D-*β*-aspartic acid is observed in the epithelium and stroma.

In case B, B-1 (endoscopic photo), B-2 (HE staining), B3-4 (immunostaining). (B-1) A 39-year-old Japanese female with a white polyp on the right vocal fold. (B-2) Submucosal accumulation of pale blue- to pink-stained material admixed with a sparsely cellular and variably vascularized stroma is observed, and the diagnosis of edematous type was made (HE staining). (B-3) Periostin is detected only in the subepithelial layers between the basement membrane and the stromal tissue; superficial type (immunostaining). (B-4) D-*β*-aspartic acid is observed in the epithelium but not in the stroma.

In case C, C-1 (endoscopic photo), C-2 (HE staining), C3-4 (immunostaining). (C-1) A 68-year-old Japanese female with red polyps on the left vocal fold. (C-2) Dense eosinophilic submucosal deposition of fibrin material in close proximity to a vascular space is observed, and the diagnosis of vascular-hyaline type was made (HE staining). (C-3) Periostin is observed from the basement membrane to the lamina propria in varying degrees, and the diagnosis of infiltrative type was made (immunostaining). (C-4) D-*β*-aspartic acid is observed in the epithelium and stroma.

In case D, D-1 (endoscopic photo), D-2 (HE staining), D3-4 (immunostaining). (D-1) A 48-year-old Japanese male with a white polyp on the left vocal fold. (D-2) Moderately cellular submucosa consisting of uniform oval to spindle-shaped cells associated with a varying amount of fibrous tissue deposition is observed, and the diagnosis of fibrous type was made (HE staining). (D-3) Periostin is expressed throughout the lamina propria starting from just below the basement membrane, and the diagnosis of diffuse type was made (immunostaining). (D-4) D-*β*-aspartic acid is observed in the epithelium but not the stroma.

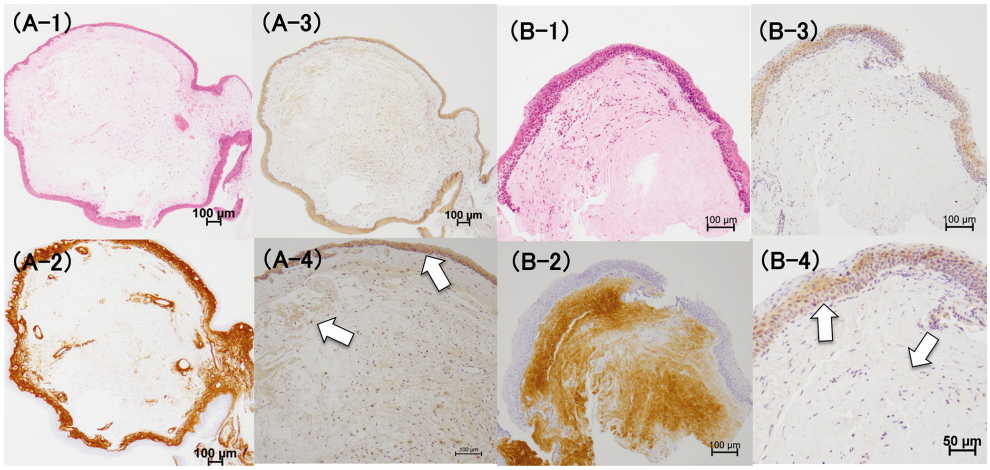

Fig. 3. Immunohistochemical staining patterns of D-β-aspartic acid in vocal fold polyps stroma were categorized into two types: positive type (n = 25) and negative type (n = 11) (Original magnification × 200, × 100 and × 40, respectively).
In case A, A-1 (HE staining), A2-4 (immunostaining). A 67-year-old Japanese female. (A-1) Dense eosinophilic submucosal deposition of fibrin material in close proximity to a vascular space is observed, and the diagnosis of vascular-hyaline type was made (HE staining). (A-2) Immunohistochemical staining for periostin in vocal fold polyps. Periostin is observed from the basement membrane to the lamina propria in varying degrees, and the diagnosis of infiltrative type was made (immunostaining). (A3-4) Immunohistochemical staining for D-β-aspartic acid in vocal fold polyps. D-β-aspartic acid is observed in the epithelium (arrow) and stroma (arrow) (immunostaining).
In case B, B-1 (HE staining), B2-4 (immunostaining). A 48-year-old Japanese male. (B-1) Moderately cellular submucosa consisting of uniform oval to spindle-shaped cells associated with a varying amount of fibrous tissue deposition is observed, and the diagnosis of fibrous type was made (HE staining). (B-2) Immunohistochemical staining for periostin in vocal fold polyps. Periostin is expressed throughout the lamina propria starting from just below the basement membrane, and the diagnosis of diffuse type was made (immunostaining). (B3-4) Immunohistochemical staining for D-β-aspartic acid in vocal fold polyps. D-β-aspartic acid is observed in the epithelium (arrow) but not the stroma (arrow) (immunostaining).

terized by moderately cellular submucosa consisting of uniform oval to spindle-shaped cells associated with a varying amount of fibrous tissue deposition. The myxoid type in Fig. 1D2-3 is marked by prominent dilated vascular spaces with or without associated hemorrhage and a combination of the above subtypes in a single lesion. Edematous, vascular-hyaline, and fibrous types were the most common of the four types (Table 1).

Expression of periostin in vocal fold polyps

The expression of periostin was investigated in 36 samples of vocal fold polyps from 36 patients (Table 2). It was detected in 28 (77.7%) samples obtained from patients with vocal fold polyps. The following four patterns of periostin expression were observed in the vocal fold polyps: negative (Fig. 2A1-4), superficial (Fig. 2B1-4), infiltrative (Fig. 2C1-4), and diffuse (Fig. 2D1-4). In the superficial pattern of periostin expression, periostin was detected only in the subepithelial layers between the basement membrane and the stromal tissue (Fig. 2B-3). In the diffuse pattern, periostin was expressed throughout the lamina propria starting from just below the basement membrane (Fig. 2D-3), and in the infiltrative pattern, periostin was observed from the basement membrane to the lamina propria in varying degrees (Fig. 2C-3).

Expression of D-β-aspartic acid in vocal fold polyps

The expression of D-β-aspartic acid was investigated in the same 36 samples (Tables 1 and 2). In all 36 cases the epithelium was stained (100%). In the vocal polyp stroma, D-β-aspartic acid expression was detected in 25 samples (69.4%). The following two patterns of D-β-aspartic acid expression were observed in vocal fold polyps stroma: positive type (Fig. 3A1-4) and negative type (Fig. 3B1-4).

Expression of D-β-aspartic acid and clinic histological factors

An association was observed between D-β-aspartic acid expression patterns and periostin expression patterns of vocal fold polyps stroma (Table 2).

The infiltrative pattern was significantly dominant in D-β-aspartic acid positive types of vocal fold polyps stroma (Table 2).

No relationship between the D-β-aspartic acid expression pattern and the histological subtype was observed (Table 1).

No relationship between the D-β-aspartic acid expression pattern and clinical characteristics, including comorbidities of smoking, intubation, or periods of illness, was observed (Table 3).

Expression of periostin and clinic histological factors

No relationship between the periostin expression pattern and clinical characteristics, including comorbidities of

Table 3. Clinical characteristics and expression of D-*β*-aspartic acid (n = 36).

Characteristics of factor	Total (n = 36)	Vocal fold polyps stroma		*P*-value
		D-*β*-aspartic acid Positive (n = 25)	D-*β*-aspartic acid Negative (n = 11)	
Sex				0.78
Male, n (%)	18 (50%)	13 (40%)	5 (10%)	
Female, n (%)	18 (50%)	12 (30%)	6 (20%)	
Mean age (years)				
Mean ± SD	51.6 ± 14.1	52.6 ± 14.6	49.4 ± 12.6	0.51
Period of illness (month)				
Mean ± SD	29.0 ± 89.0	38.0 ± 107.4	9.0 ± 5.5	0.33
Polyps color				1
White, n (%)	24 (70%)	17 (50%)	7 (20%)	
Red, n (%)	12 (30%)	9 (30%)	3 (8%)	
Tabacco, n (%)	23 (60%)	17 (50%)	6 (20%)	1
Alcohol, n (%)	22 (60%)	14 (40%)	8 (20%)	0.47
Intubation, n (%)	3 (8%)	2 (6%)	1 (3%)	1

Table 4. Clinical characteristics and expression of periostin in vocal fold polyps (n = 36).

Characteristics of factor	Total (n = 36)	Periostin					*P*-value
		Negative type (n = 8)	Positive total (n = 28)	Superficial type (n = 11)	Infiltrate type (n = 13)	Diffuse type (n = 4)	
Sex							0.2
Male, n (%)	18 (50%)	4 (11%)	14 (38.8%)	4 (11%)	6 (20%)	4 (11%)	
Female, n (%)	18 (50%)	4 (11%)	14 (38.8%)	7 (20%)	7 (20%)	0 (0%)	
Mean age (years)							
Mean ± SD	51.6 ± 14.1	45.1 ± 12.3	53.4 ± 14.2	48.0 ± 11.9	56.7 ± 15.7	58.3 ± 6.9	0.17
Period of illness (month)							
Mean ± SD	29.0 ± 89.0	13.5 ± 22.2	33.4 ± 101.6	12.0 ± 13.3	60.2 ± 141.2	5.5 ± 4.0	0.9
Polyp color							0.35
White, n (%)	24 (70%)	3 (8%)	21(58%)	7 (20%)	9 (30%)	5 (10%)	
Red, n (%)	12 (30%)	4 (11%)	8 (22%)	4 (11%)	4 (11%)	0 (0%)	
Tabacco, n (%)	23 (60%)	4 (11%)	19 (53%)	7 (20%)	9 (30%)	3 (8%)	0.8
Alcohol, n (%)	22 (60%)	4 (11%)	18 (50%)	9 (30%)	6 (20%)	3 (8%)	0.28
Intubation, n (%)	3 (8%)	0 (0%)	3 (8%)	2 (6%)	1 (3%)	0 (0%)	0.72

smoking, intubation, or periods of illness, was observed (Table 4).

Discussion

Amino acids contain one or more asymmetric tetrahedral carbon atoms, which make D-enantiomeric and L-enantiomeric structures.

The chemical and physical properties of L-amino acids and D-amino acids are the same. However, their optical characteristics are different (Kaji et al. 2007). Fujii, Kaji and their colleagues reported D-aspartic acid formation to be accompanied by isomerization from natural *α*-aspartic acid to abnormal *β*-aspartic acid via a succinimide (Fujii et al. 1999; Kaji et al. 2007). This leads to the formation of the following four isomers: normal L-*α*-aspartic acid, biologically uncommon L-*β*-aspartic acid, D-*α*-aspartic acid, and D-*β*-aspartic acid in proteins. D-*β*-aspartic acid is normally rare but is the major isomer found in elderly tissues (Fujii et al. 1994).

The presence of D-*β*-aspartic acid is thought to be the result of racemization of L-*β*-aspartic acid in proteins (Kaji et al. 2007). D-*β*-aspartic acid in the proteins of living creatures is considered to be a novel marker of damage caused by aging (Fujii et al.1999; Kaji et al. 2007). UV irradiation of the skin is closely related to the formation of D-*β*-Asp in the elastic fibers of the skin (Miura et al. 2004).

In this study, D-*β*-aspartic acid expression was detected in all the vocal fold polyps epithelium, despite the laryngeal cavity not having been exposed to UV irradiation. These results indicate that the racemization of L-amino

acids may occur for reasons other than aging or UV irradiation.

Two patterns of D-β-aspartic acid expression were observed in all histological subtypes of vocal fold polyps stroma: positive and negative. Various levels and patterns of periostin expression were observed in all histological subtypes of vocal fold polyps.

Microscopic examination of the vocal polyps revealed vascular dilation, hemorrhage, capillary proliferation, fibrin exudation, and fibrosis.

The findings differed among the vascular-hyaline, edematous, myxoid, and fibrotic types of vocal polyps. It can be suggested that the disease etiology of vocal fold polyps is caused by the injury of vessels from either long-term voice abuse or sudden vocal fold microvascular disruption due to phonotrauma (abrupt movements of the vocal fold membrane during phonation).

The latter case injures the vessels and causes hemorrhage, leakage of fibrin, thrombosis, proliferation of capillaries, hyalinization, and fibrosis. This pathology can also be caused by aggravated tension of the vocal folds or by other factors (such as smoking, alcohol, infection, dust, or endocrine dysfunction), which affect the mucosa of the vocal folds, thus damaging the walls of blood vessels (Tateda et al. 2022).

The etiology of vocal polyps is thought to be the repeated degeneration of blood vessels in the vicinity of the free vocal folds, which run parallel to the vocal fold margin, due to voice abuse causing repeated disruption of these vessels. The essence of vocal fold vibration is said to be a traveling wave phenomenon, i.e., a mucous membrane wave whose wave head is a mucous membrane ridge that moves continuously from the inner lower surface of the vocal folds to the upper outer surface.

In this study, all cases showed D-β-aspartic acid expression in the vocal polyp epithelium, suggesting that the traveling wave phenomenon may be the reason for the racemization of L-amino acids in the vocal polyp epithelium. The expression of D-aspartic acid in the vocal polyp stroma was observed in 25 cases (69.4%), suggesting that the effect of racemization of D-aspartic acid spills over into the vocal polyp stroma as the disease progresses. The expression of D-β-aspartic acid appeared to be low at the sites where periostin was expressed. During the process of vocal polyp formation, local circulatory disturbance, hematoma/edema, fibrosis, and angiogenesis are expected to occur. We suggest that periostin was involved in fibrosis in the vocal polyps, and D-β-aspartic acid was involved in local circulatory disturbances and hematoma/edema.

All of the cases we studied showed D-β-aspartic acid expression in the epithelium of the vocal polyps. It is possible that the first step may be associated with the expression of D-β-aspartic acid in the epithelium of vocal polyps, which may then affect the expression of D-β-aspartic acid in the stroma of vocal polyps.

Our study has several limitations. First, the number of patients with laryngeal polyps recruited in this study was relatively small because the samples were collected from only a single center. Second, we have not determined which protein contains D-β-aspartic acid in laryngeal polyps and sourrounding normal tissues. Identifying this protein may lead to elucidation of the pathogenesis of laryngeal polyps. Further research with many more samples is required to reveal the mechanism of expression of D-β-aspartic acid in laryngeal polyps. Third, the relationship between so-called "voice abuse" and the rate or D-β-aspartic acid in vocal cord tissues has not be examined in the present study.

In conclusion, our results indicate that overexpression of D-β-aspartic acid and periostin is likely involved in the pathogenesis of vocal fold polyps, and we speculate that D-β-aspartic acid and periostin could be a novel biomarker for, and a therapeutic target in, vocal fold polyps.

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Conflict of Interest

The authors declare no Conflict of Interest.

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