Histopathological Characteristics of Sulcus Vocalis
Ahmet Volkan Sunter, Ozgur Yigit, Gulben Erdem Huq, Zeynep Alkan, Ismail Kocak and Yalcin Buyuk
Otolaryngology -- Head and Neck Surgery 2011 145: 264 originally published online 22 April 2011
DOI: 10.1177/0194599811404639

The online version of this article can be found at:
http://oto.sagepub.com/content/145/2/264

Published by:
SAGE
http://www.sagepublications.com

On behalf of:
AMERICAN ACADEMY OF
OTOLARYNGOLOGY--
HEAD AND NECK SURGERY
F O U N D A T I O N
American Academy of Otolaryngology- Head and Neck Surgery

Additional services and information for Otolaryngology -- Head and Neck Surgery can be found at:

Email Alerts: http://oto.sagepub.com/cgi/alerts
Subscriptions: http://oto.sagepub.com/subscriptions
Reprints: http://www.sagepub.com/journalsReprints.nav
Permissions: http://www.sagepub.com/journalsPermissions.nav
Histopathological Characteristics of Sulcus Vocalis

Ahmet Volkan Sunter, MD1, Ozgur Yigit, MD1, Gulben Erdem Huq, MD2, Zeynep Alkan, MD1, Ismail Kocak, MD3, and Yalcın Buyuk, MD4

No sponsorships or competing interests have been disclosed for this article.

Abstract

Objective. To describe the frequency of sulcus vocalis in a population and to establish its histopathological properties.

Study Design. A cadaver study.

Setting. Istanbul Training and Research Hospital and Ministry of Justice National Forensic Institute.

Subjects and Methods. One hundred (21 women, 79 men) fresh cadaver larynges were examined. Two hundred vocal folds were coronally sectioned and examined by light microscopy. Sulcus types, depths, degree of fibrosis in Reinke space, basement membrane thickness, and perisulcus degeneration were determined.

Results. Sulcus vocalis was found in 39 of 100 cases (39%), with a male preponderance of 41.8% compared with women at 28.6%. Twenty-one (54%) cases had unilateral and 18 (46%) cases had bilateral sulcus. Among 200 vocal folds, 57 had sulcus (28%). A total of 21 sulcus cases were type 1 (37%), 18 were type 2 (31.5%), and the remaining 18 cases were type 3 (31.5%). Of the 39 (30.7%) sulcus cases, 12 were confirmed by histopathological examination, although they looked morphologically normal following macroscopic evaluation. Vocal ligament involvement was found in 14 cases with type 2 (78%) and in 15 cases with type 3 sulcus (83%).

Conclusion. Sulcus vocalis is a common pathology in the population and can be observed at all ages. The accuracy of diagnosis seems to depend on the measurement of the sulcus depth and the histological characteristics. For this reason, tools and strategies should be established in living subjects for effective management of this challenging disorder.

Keywords

sulcus vocalis, vocal fold, histopathology

Received May 26, 2010; revised February 22, 2011; accepted March 2, 2011.

Sulcus vocalis can be defined as a groove on the medial surface of the human vocal folds and may have unilateral or bilateral involvement. This condition produces an inhomogeneous defect in the cover layer, causing incomplete vibratory glottic closure, asymmetry, and tension. This results in mild to severe dysphonia, characterized by a high pitch and a breathy and diplophonic quality. Ford et al1 divided sulcus disorders of the vocal folds into 3 groups according to clinical and histopathological features. In type 1, epithelial invagination is limited to the lamina propria; however, because this does not cause vibratory defects, normal voice quality is retained (referred to as being in physiological ranges). Type 2, also designated as “sulcus vergeture” by Bouchayer et al,2 characteristically describes an epithelial invagination along the vocal fold length that causes vibratory disturbances and dysphonia. Type 3 is the true sulcus vocalis (pocket type) and represents an epithelial invagination that may penetrate into the vocal ligament and/or vocalis muscle layers, thereby causing serious dysphonia. Type 2 and type 3 are accepted as pathological types.1

The etiology of this condition has not yet been clearly defined. Whether sulcus vocalis is congenital or acquired is also debated. The incidence of sulcus vocalis in adults varies between 0.4% and 48% in the literature.3,4 Ishii et al5 found a sulcus incidence of 2.5% in 200 autopsy larynges, Shin6 found an incidence of 0.4% in 1200 postmortem larynges, and Hsiung et al6 found a rate of 36%. The reason for this wide...
interval in incidence is the difficulty of diagnosing sulcus vocalis and the possibility that type 1 cases might be easily overlooked. For accurate diagnosis in living subjects, examination of the vocal fold under general anesthesia, by palpating or suction, would seem to be essential. Therefore, postmortem pathological studies have significant importance in determining the incidence in the general population. This cadaver study was planned as a way to determine the incidence of sulcus vocalis in our general population by categorizing its histological characteristics.

**Methods**

The study was conducted on 100 cadavers within 24 hours of death; these were considered fresh cadavers for the study. Laryngeal specimens were obtained during routine autopsy. The larynges were removed and the vocal folds were first examined macroscopically and then sectioned for histopathologic examination. During removal, the laryngeal framework was preserved from the hyoid to tracheal rings for standard configuration and length of vocal folds. Those cases with a history of intubation or laryngeal surgery, laryngeal cancer, burns, or drowning; those with blunt or penetrating trauma on the neck area according to their medical reports; and those with operation marks on the neck were excluded from the study. The study was approved by the Ethics Committee of the Directorate of Forensic Medicine Institution of the Turkish Ministry of Justice.

Two hundred vocal folds (from 21 women and 79 men) from larynges were examined. Ages of cases varied from 1 to 87 years (mean age, 41.7 ± 20.3 years). Each larynx was split in half by vertically incising the posterior cricoid lamina at the midpoint to expose the endolaryngeal structures. Macroscopic examinations were done by an experienced clinician using the naked eye, under good illumination, and without any magnification. A middle ear elevator was used and the tissue was palpated gently over the epithelium of the membranous vocal fold to locate any grooving, stiff areas, or sulcus openings. Vocal folds and ventricular bands were removed after elevating the inner thyroid perichondrium. The specimens were fixed in neutral formaldehyde solution with 10% buffer. After fixation, vocal folds were first cut coronally at the midline through their full thickness and then sliced 3 to 4 mm anteriorly and posteriorly along the membranous fold. Sliced tissues were placed into tracking tapes and embedded into paraaffin blocks. After 16 hours, the blocks were sectioned at 3- to 5-µm thickness with a microtome and stained with hematoxylin-eosin (HE) stain for photomicroscopic examination. Fibrosis was evaluated using Masson-trichrome stain to show collagen, and periodic acid shift (PAS) was used to define the basement membrane. All specimens were examined by the same pathologist (G.E.H.). For immunohistochemical staining of laminin and collagen IV, sectioned samples were placed on positively charged slides (Menzel-Gläser SuperFrost Ultra Plus; Menzel-Gläser, Braunschweig, Germany) and put into a Bond-maX (Leica Microsystems, Wetzlar, Germany) automatic immunohistochemical device. Bond Polymer Refine Detection Kits (DS9800; Leica), collagen IV (CIV 22; DAKO Corp, Carpinteria, California), and laminin (NeoMarkers, Fremont, California) were used in the device. For histochemical staining, Masson-trichrome, PAS, and Verhoeff–van Gieson elastin stains were used. The depth of sulcus, thickness of the basement membrane around the sulcus, level of fibrosis, vascularization, inflammation, degree of degeneration, status of the epithelium, and vocal ligament and muscle involvement were noted during histopathological examination.

Depth of sulcus was measured from the level of the epithelial surface to the maximum distance in micrometers where the sulcus reaches within the superficial lamina propria. Basement membrane evaluation was conducted subjectively, according to the presence of basement membrane staining at the bottom of sulcus. The basement membrane is not usually easy to observe in normal vocal folds. In pathologic cases, the basement membrane becomes apparent as a band just under the epithelium. Therefore, apparent cases were accepted as positive for thickening. Fibrosis and vascularization were also evaluated as normal or increased, according to the abundance of collagen fibers under the sulcus. The quality of sulcus epithelium was classified in 3 groups as normal, hyperplastic, or atrophic. The degenerated tissue at the vicinity of the sulcus epithelium was evaluated by the presence of edema and by disoriented collagen fibers mixed with myxoid stroma. Vocal ligament and muscle involvement was evaluated as proximity and invasion of the sulcus epithelium toward these structures, and these were noted as existing or not existing. Microscopic evaluations and measurements were performed using a trinocular microscope (Olympus BX51TF; Olympus Corp, Tokyo, Japan) and a linear micrometer (Eyepiece Graticule, Olympus Corp).

**Statistical Analysis**

The SPSS 13.0 (SPSS, Inc, an IBM Company, Chicago, Illinois) program was used for statistical analysis. Average and standard deviation methods were used in data evaluation. One-way analysis of variance (ANOVA), χ², and Spearman correlation tests were used for group comparisons. The value of $P < .05$ was accepted as statistically significant.

**Results**

Sulcus deformity was found in 39 of 100 cases (39%) and in 57 of 200 vocal folds (28.5%). In total, 21 of 57 sulcus cases were physiological (type 1; 37%), 18 cases had vergeture type (type 2) sulcus (31.5%), and the remaining 18 cases were pocket-type sulcus vocalis (type 3; 31.5%) (Figures 1-3).

Pathological sulcus vocalis rate (type 2 and type 3) was 23% in 100 cases. Age distributions in cases with sulcus varied between 15 and 80 years (mean, 41.29 ± 15.17). For cases younger than 15 years ($n = 9$), no sulcus was detected. A unilateral sulcus occurred in 21 of 39 larynges (54%), and bilateral sulci were found in 18 (46%). Men accounted for 41.8% and women for 28.6% of the cases of sulcus vocalis (Table 1).

No correlation was noted between age, gender, and the sulcus types ($r = 0.003; P = .984$).

Average depths of sulci were $540 \pm 138$ µm in type 1, $836 \pm 320$ µm in type 2, and $1084 \pm 653$ µm in type 3.
Vocal ligament involvement was not found in physiological sulcus cases. A total of 14 type 2 cases (78%) and 15 type 3 cases (83%) had vocal ligament involvement.

Vocal muscle involvement was also not observed in physiological sulcus cases. Only 1 case of type 2 vergeture (5%) and 7 cases of type 3 (39%) sulcus vocalis were found.

Degeneration of the perisulcus lamina propria was detected in 24% of the physiological sulcus cases; it was present in 61% of the cases with vergeture and in 72% of the type 3 sulcus cases. A positive correlation was noted between the type of sulcus and the degeneration rate ($P = .006$).

Thickened basement membrane was detected in 10% in type 1, 22% in type 2, and 44% in type 3 sulci and was significantly correlated with the type of sulcus ($P = .04$).

Increased fibrotic tissue was present in 19% in type 1, 44% in type 2, and 55% in type 3 sulci. However, no statistically significant difference was found between the sulcus types ($P = .054$).

Vascularization rates were 14% for type 1, 11% for type 2, and 33% for type 3 sulci. These differences between groups were not statistically significant.

Inflammatory infiltration at the perisulcus region was high. Type 2 had 77% and type 3 had 83% infiltration; however, the

| Table 1. Types and Properties of Sulci |

<table>
<thead>
<tr>
<th>Type</th>
<th>No. (%) of Cases</th>
<th>No. (%) of Vocal Folds</th>
<th>Age, y, Mean ± SD</th>
<th>Gender, Male/Female, No. (%)</th>
<th>Depth, µm, Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16 (16)</td>
<td>21 (37)</td>
<td>40.37 ± 10.83</td>
<td>11 (14)/5 (24)</td>
<td>540 ± 138</td>
</tr>
<tr>
<td>2</td>
<td>10 (10)</td>
<td>18 (31.5)</td>
<td>44.10 ± 17.95</td>
<td>9 (11)/1 (5)</td>
<td>836 ± 320</td>
</tr>
<tr>
<td>3</td>
<td>13 (13)</td>
<td>18 (31.5)</td>
<td>41.76 ± 16.78</td>
<td>13 (16)/—</td>
<td>1084 ± 653</td>
</tr>
<tr>
<td>Normal</td>
<td>61 (61)</td>
<td>143 (71.5)</td>
<td>41.65 ± 23.39</td>
<td>46 (58)/15 (71)</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>39/100 (39)</td>
<td>57/200 (28.5)</td>
<td>41.29 ± 15.17</td>
<td>Male: 33/79 (41.8) female: 6/21 (28.6)</td>
<td>805 ± 127</td>
</tr>
</tbody>
</table>

Figure 1. Type 1 sulcus vocalis. Minimal indentation of epithelium through the superficial lamina propria (hematoxylin-eosin [HE] ×10). In the right lower corner, the epithelium has normal thickness, an apparent basement membrane, and a high vascularity (arrows) (HE ×100).

Figure 2. Type 2 sulcus vocalis. Loss of the superficial layer of the lamina propria is seen (hematoxylin-eosin [HE] ×10). Epithelium is atrophic, and its integrity is impaired in one region (right lower corner). The basement of the sulcus is located at a deeper level than the muscle fibers (big arrow), and the epithelium of the sulcus is positioned with a cluster of muscle fibers (small arrow) (HE ×100).
difference between these 2 groups was not statistically significant (Table 2).

In total, 12 (6%) cases that were missed by macroscopic examination were subsequently diagnosed after histopathologic examination. Of these, 10 were type 1, and the remaining 2 cases had type 2 sulcus. One case was found with 2 type 1 sulci on a single fold, parallel to each other. One case with type 2 sulcus had an intracordal cyst on the other fold.

Discussion
Sulcus vocalis remains an unresolved problem in laryngology and is gaining importance among most voice surgeons. Several vocal fold pathologies, including malignancies, and their relationships with sulcus disease are now being actively researched.

A varied sulcus incidence has been reported in several studies. In early studies, reported rates of sulcus vocalis disorder were low because of methodological differences that favored macroscopic evaluation. However, later studies, such as done by Hsiung et al, with microscopic study in 1-mm sections found a sulcus vocalis and pathological sulcus rates of 36% and 14%, respectively. The incidence of sulcus vocalis in our study was 39%, with a pathological sulcus vocalis rate of 23%, which resembled the results of the study by Hsiung et al.

These rates are considerably high for any disease. Therefore, exact diagnosis and classification are essential for treatment, follow-up, and prevention of concomitant pathologies where the incidence may be higher. Nakayama et al found sulcus vocalis at the contralateral fold at a rate of 48% in cases operated on for laryngeal cancer. Pathological examination showed that inflammation and irritation in pathogenesis played a significant role in the increase in fibrosis and vascularization in tissues adjacent to the sulcus.

The disorder has a higher incidence in males, at about 1.46 times the rate in females. In a clinical study, the ratio was 2.58 times higher in males than in females. Evidence of sulcus has been presented in pediatric and adolescent cases; however, in our study, the occurrence of sulcus was only observed after 15 years of age. We were unable to detect sulcus in pediatric cases, in contradiction to the study by the same authors. A larger population study may obviate the pediatric rates of sulcus vocalis and promote new evidence to understand its etiopathogenesis.

Of the different sulcus types, physiological sulcus is the most frequently encountered type. Current literature defines physiological sulcus as asymptomatic; however, no data have yet been provided to define its vocal characteristics and to compare these to normal voice quality. Therefore, whether type 1 sulcus cases have a voice quality within the acceptable perceptual limits of the general population is still an unknown issue.

The depth of the sulcus epithelium may determine the type of the sulcus and hence the severity of symptoms. Therefore, treatment requirements may tend to increase. Current strategies are mostly surgical. The surgical options may change

Table 2. Histopathologic Characteristics of Sulci

<table>
<thead>
<tr>
<th></th>
<th>Type 1 (n = 21), No. (%)</th>
<th>Type 2 (n = 18), No. (%)</th>
<th>Type 3 (n = 18), No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vocal ligament involvement</td>
<td>0/21 (0)</td>
<td>14/18 (78)</td>
<td>15/18 (83)</td>
</tr>
<tr>
<td>Vocal muscle involvement</td>
<td>0/21 (0)</td>
<td>1/18 (5)</td>
<td>7/18 (39)</td>
</tr>
<tr>
<td>Increased inflammation</td>
<td>11/21 (52)</td>
<td>14/18 (77)</td>
<td>15/18 (83)</td>
</tr>
<tr>
<td>Increased vascularization</td>
<td>3/21 (14)</td>
<td>2/18 (11)</td>
<td>6/18 (33)</td>
</tr>
<tr>
<td>Increased degeneration</td>
<td>5/21 (23)</td>
<td>11/18 (61)</td>
<td>13/18 (72)</td>
</tr>
<tr>
<td>Increased basement membrane thickness</td>
<td>2/21 (10)</td>
<td>4/18 (22)</td>
<td>8/18 (44)</td>
</tr>
<tr>
<td>Increased fibrosis</td>
<td>4/21 (19)</td>
<td>8/18 (44)</td>
<td>10/18 (55)</td>
</tr>
</tbody>
</table>

Figure 3. Type 3 sulcus vocalis. (A) Basement membrane thickening is shown with periodic acid shift (PAS) staining (PAS ×100). (B) Basement membrane thickening (arrow) and increased vascularization are seen in the same area with immunohistochemical staining of collagen IV (×100). (C) Fibrosis and collagen fiber increase (arrow) visualized with Masson-trichrome stain (×100). (D) Basement membrane thickening, hyalinization, and prominent myxoid degeneration around the lamina propria are seen with hematoxylin-eosin (HE) staining below the hyperplastic epithelium (×400).
from restoring the layered structure to complicated framework procedures to alleviate problems with the voice and relieve symptomatology. In pathological sulcus cases, the degree of chronic inflammatory processes that result in fibrosis is high. The thickened basement membrane and perisulcus degeneration, which increases with the type, may make these procedures more challenging.

Diagnosis of sulcus vocalis in the clinical setting is also challenging. Patients may not be aware of the problem until a secondary pathology arises because symptomatology varies from normal vocal quality to severe dysphonia. Also, the pathology can be missed even with gross observation with palpation. Missed cases are also possible with indirect and even with endoscopic laryngoscopy; however, no study has yet compared the techniques available to provide a better diagnosis of this specific disorder with satisfactory sensitivity and specificity. During suspension laryngoscopy, the surgeon can miss a vocal fold with sulcus if a meticulous examination that includes palpation, suction, or supravital staining is not also performed.

Sulcus vocalis is located at the squamous epithelium of the vocal folds. The deep layers beyond the sulcus show thinning of the lamina propria, dense and thick fibrotic bands due to collagen formation, and thickening of the lamina reticularis layer of the basement membrane. The most distinct differences in pathological examination are the depth of the sulcus. The area of the sulcus epithelium, its depth, and its exposure to the laryngeal lumen might produce a drainage problem for epithelial debris inside the sulcus, which could be the reason for sulcus cysts. At present, no data yet exist that describe the behavior of epithelial regeneration, migration, or desquamation of vocal folds under pathological conditions. However, the experience of most laryngologists is that type 3 sulcus cases have a higher probability of producing these types of cystic formations. Basement membrane thickness and findings of high vascularity and inflammation might also be related to this process, as might intrinsic mechanical trauma during phonation.

Some electron microscopy studies have shown that abnormal collagen formation decreases the number of elastic fibers in the vocal ligament, resulting in distorted and reduced fibrils in the macula flava. Therefore, the sulcus disorder cannot be regarded solely as a disorder of the cover because it also affects the ligament layer. In the etiology, although recurrent infections or trauma have been postulated, developmental problems of the vocal ligaments and their integration with the overlying cover layer should also be investigated further. In recent studies, chronic irritation, inflammation, and aging had an essential role in sulcus formation. In our study, increased fibrosis was observed in the area adjacent to the sulcus. This may be due to contact near the ligament, which is high in collagen type I–producing fibrocytes. These cells are induced either primarily or secondarily by microfractures in the perisulcus medium, with persistent inflammatory processes secondary to intrinsic mechanical trauma or intrasulcus infections.

The etiology of sulcus is not known, and therefore prevention does not seem possible. The differences in findings in multiple studies should prompt researchers to seek ethnic, environmental, or genetic factors that may play a role in etiology. In vivo intraoperative histological examination is currently possible with technologies such as optical coherence tomography. A new classification or auxiliary classification seems suitable according to symptomatology or depth measurements.

This study revisits the incidence and histopathological characteristics of the sulcus disorder of the vocal folds. Sulcus disorder was found to be a frequent disorder in the general population, especially among adults. Although the etiology and treatment strategies have not yet been well established, the micromorphometric structure and measurements reported here may provide useful data for further studies in the pathogenesis, symptomatology, and treatment of this disorder.

Author Contributions
Ahmet Volkan Sunter, conception, design, data collection and analysis, manuscript preparation and review; Ozgur Yigit, manuscript preparation and review; Gulben Erdem Huq, conception, design, data collection and analysis; Zeynep Alkan, manuscript preparation and review; Ismail Kocak, manuscript review; Yalcin Buyuk, data collection and analysis.

Disclosures
Competing interests: None.
Sponsorships: None.
Funding source: None.

References

