

Case Report

Brainstem Pathology in Spasmodic Dysphonia

Kristina Simonyan, MD, PhD; Christy L. Ludlow, PhD; Alexander O. Vortmeyer, MD

Spasmodic dysphonia (SD) is a primary focal dystonia of unknown pathophysiology, characterized by involuntary spasms in the laryngeal muscles during speech production. We examined two rare cases of postmortem brainstem tissue from SD patients compared to four controls. In the SD patients, small clusters of inflammation were found in the reticular formation surrounding solitary tract, spinal trigeminal, and ambigular nuclei, inferior olive, and pyramids. Mild neuronal degeneration and depigmentation were observed in the substantia nigra and locus coeruleus. No abnormal protein accumulations and no demyelination or axonal degeneration were found. These neuropathological findings may provide insights into the pathophysiology of SD.

Key Words: Laryngeal dystonia, neuropathology, immunohistochemistry.

Laryngoscope, 120:121–124, 2010

INTRODUCTION

Spasmodic dysphonia (SD) is a primary focal dystonia, most frequently characterized by involuntary spasms in the closing muscles of the vocal folds during speech production. SD symptoms usually appear in mid-life, progress gradually, and remain chronic for life. The neuropathological bases of SD and other forms of primary focal dystonia are poorly understood, partly due to the rare availability of the postmortem tissue from these

From the Laryngeal and Speech Section, Medical Neurology Branch (K.S., C.L.L.), and the Surgical Neurology Branch (A.O.V.), National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, U.S.A.

Editor's Note: This Manuscript was accepted for publication July 9, 2009.

Presented at the American Academy of Otolaryngology–Head and Neck Surgery Annual Meeting, Los Angeles, California, U.S.A., September 25–28, 2005.

This work was supported by the Intramural Program of the National Institute of Neurological Disorders and Stroke, National Institutes of Health.

Send correspondence to Kristina Simonyan, MD, PhD, Laryngeal and Speech Section, Medical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, 10 Center Drive, Building 10, Room 5D38, Bethesda, MD 20814-1416. E-mail: simonyak@ninds.nih.gov

DOI: 10.1002/lary.20677

patients. To date, only a few studies have been conducted to examine the neuropathological abnormalities in a limited number of dystonic patients. Neuropathological case reports in patients with Meige's syndrome,^{1–3} and cranial and cervical dystonia,⁴ have found a mild neuronal loss in the substantia nigra, locus coeruleus, dorsal raphe nucleus, tectum, and dentate nucleus,^{1,3} and infrequent Lewy bodies in the substantia nigra,⁴ nucleus basalis of Meynert, and nucleus ambiguus.^{1,2} We recently found focal axonal degeneration and demyelination in the genu of the internal capsule, and clusters of mineral accumulations containing calcium, phosphorus, and iron, in the internal capsule, lentiform nucleus, and cerebellum in one SD patient.⁵ However, it remains unknown whether neuropathological changes are also present in the brainstem of SD patients, although several neurophysiological studies have pointed to brainstem abnormalities in these patients.^{6,7}

As a continuation of our previous study,⁵ in this case report we examined rarely available postmortem brainstem tissue from two SD patients compared to four controls to identify brainstem changes in SD patients, which may contribute to the pathophysiology of this disorder.

MATERIALS AND METHODS

Postmortem tissue of the brainstem was obtained from two SD patients (case 1: female, 85 years old; case 2: male, 65 years old; mean age, 70.5 years), and four controls (mean age, 68.5 years, age range 50–85 years, 3 females/1 male). Antemortem, both patients were diagnosed with adductor type of SD presented with moderate to severe voice breaks due to spasms in the closing muscles of the vocal folds during vowel production. Case 1 also had adductor vocal tremor during speech production. The onset of the disorder was at age of 56 and 36 years, respectively. Both patients were diagnosed and treated at the National Institute of Neurological Disorders and Stroke with botulinum toxin injections into their laryngeal muscles at regular intervals for several years. Clinical characteristics of patients and controls are summarized in Table I. The male patient's (case 2) neuropathological findings in the supramedullary brain regions were reported in a previous study.⁵ We did not have access to the supramedullary brain tissue from the female patient (case 1); only brainstem tissue of case 1 was available to us for neuropathological examination. The study was approved

TABLE I.
Clinical Characteristics.

Case	Gender	Diagnosis	Age at Onset (yr)	Age at Death (yr)	Cause of Death
1	F	ADSD, vocal tremor	56	85	Left temporal lobe infarct*
2	M	ADSD	36	65	Complications during cardiac bypass surgery
3	M	Control	N/A	50	Diffuse pulmonary hemorrhage
4	F	Control	N/A	58	Disseminated intravascular coagulation
5	F	Control	N/A	81	Respiratory failure due to small-cell lung cancer
6	F	Control	N/A	85	Dehydration with hypernatremia

*The left temporal lobe infarct had the most remote rostral location and, therefore, did not involve brain regions known to control voice and speech production. Hence, the cause of death in this patient should not have affected the findings of neuropathological changes in the brainstem.
ADSD = adductor spasmodic dysphonia; N/A = not available.

by the Institutional Review Board of the National Institute of Neurological Disorders and Stroke, National Institutes of Health.

Postmortem brainstem tissue from all subjects was fixed in 10% formalin solution, sectioned transversely into 6 blocks (1-cm thick) for gross examination and then embedded in paraffin. For histopathological examination, each block was sectioned into consequent 10 paraffin sections at 5 μ m and stained with hematoxylin and eosin, luxol fast blue/periodic acid Schiff, and Bielschowski silver stains to assess changes in cell morphology, myelin, and axonal course, respectively. Additionally, subsequent sections were stained with mouse monoclonal antibodies to human microglia/macrophage lineage (1:100, CD68, Clone KP1; Dako, Carpinteria, CA), human leukocyte/lymphocyte lineage (1:400, CD45, Leucocyte Common Antigen, Clone 2B11+PD7/26; Dako), alpha-synuclein (1:100; Oncogene Research Products, San Diego, CA), tau (1:1500, Clone Tau-2; Chemicon International Inc., Temecula, CA), and ubiquitin (1:100; Novocastra, Newcastle upon Tyne, UK). Primary antibodies were omitted for negative controls. For reaction product visualization, sections were incubated with a refined avidin-biotin kit (LSAB+ System-HRP; Dako). Stained sections were evaluated without prior knowledge of the subjects' identity (blinded) using light microscopy (Olympus BX51; Olympus Inc., Tokyo, Japan). Abnormalities were defined as mild, moderate, severe, or none. Mild abnormalities were defined as occasional yet definitive pathological changes, i.e., degeneration of single cells in the well-preserved neuroanatomical region. Moderate abnormalities were defined as significant alterations, encompassing 10% to 50% of the affected brain region. Severe abnormalities were defined as total or subtotal destruction of a specific neuroanatomical region of interest.

RESULTS

Gross neuropathological examination of the brainstem revealed no abnormalities in either patients or controls. Detailed microscopic examination was performed to investigate the multiple levels of the brainstem nuclei involved in the sensorimotor control of voice production. Normal morphology of the lower brainstem nuclei was identified in both patients and controls (Fig. 1A-I and 1B-I). No accumulations of abnormal proteins, such as tau, ubiquitin, or alpha-synuclein, and no signs of demyelination or axonal degeneration were found in either SD patients or controls. However, in both SD cases, small clusters of microglia/macrophages activation were found in the reticular formation surrounding the solitary tract and spinal trigeminal nuclei and in the pyramids (Fig. 1A-II and 1B-II). In SD

case 1, these clusters were also observed in the reticular formation around the nucleus ambiguus and in the inferior olive. Mild neuronal degeneration and depigmentation were observed in the pars compact of the substantia nigra and the locus coeruleus in both SD cases (Fig. 1C and 1D). These brainstem changes were predominant in SD case 1, present in SD case 2, and not found in any of the controls (Table II).

DISCUSSION

The major findings of the present case study are: 1) the absence of significant cellular morphology changes in the brainstem neuronal populations, 2) the presence of small clusters of microglia/macrophages activation in the reticular formation surrounding the lower brainstem nuclei involved in the control of laryngeal functions, and 3) mild neuronal degeneration and depigmentation in the substantia nigra and the locus coeruleus in SD patients compared to controls.

The findings of normal cell morphology and the absence of abnormal protein accumulations, such as tau, ubiquitin, and alpha-synuclein, in the brainstem nuclei of SD patients are in line with neuropathological reports in some other forms of primary focal dystonia.¹⁻⁴ In contrast, ubiquitin-positive perinuclear inclusion bodies were found in the midbrain reticular formation, periaqueductal gray, pedunculopontine, and cuneiform nuclei of patients with *DYT1* dystonia,⁸ suggesting the possibility of different neuropathological processes underlying the pathophysiology of focal and generalized primary dystonias.

The presence of small clusters of microglia/macrophages activation in the brainstem reticular formation of SD patients may represent a disorder-specific neuropathological process, because of their location in the vicinity of the nuclei responsible for sensory (solitary tract and spinal trigeminal nuclei) and motor (nucleus ambiguus) control of voice production. Upregulation of microglia/macrophages in the brain tissue is known to be associated with a large range of neurological pathologies, from trauma to autoimmune conditions. The presence of activated microglia as clusters of focal inflammation is usually a common finding in neurodegenerative disorders, such as Parkinson's and Alzheimer's diseases.⁹ However, to date, little knowledge is available about the molecular processes underlying microglial

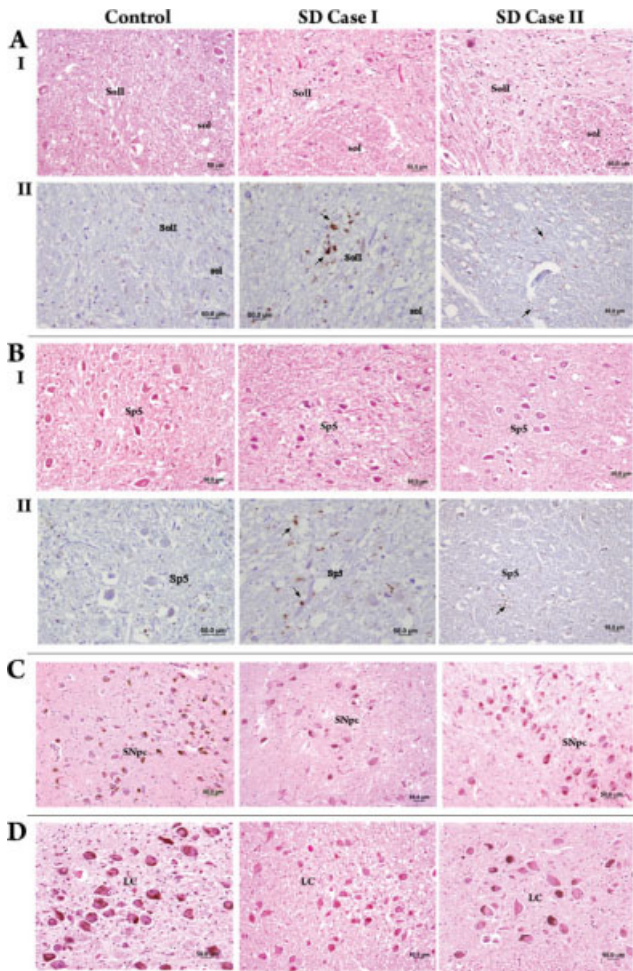


Fig. 1. Microphotographs of the brainstem regions in the controls and both spasmodic dysphonia cases show normal cell morphology in the interstitial part of the solitary tract nucleus (Soll) (A-I), and the spinal trigeminal nucleus (Sp5) (B-I) (hematoxylin and eosin [H&E] stain); clusters of microglial/macrophage activation (arrows) in the reticular formation surrounding the Soll (A-II) and Sp5 (B-II) (CD68/KP1 immunostain); and mild neuronal degeneration and depigmentation in the substantia nigra, pars compacta (C), and the locus coeruleus (D) (H&E stain), respectively.

activation and the exact biological consequences that may result from their upregulation within central nervous system tissue.¹⁰ In the present case, we suggest two possibilities for the presence of focal microglia/macrophages activation in the brainstem of SD patients. Based on the previous findings of neurophysiological brainstem abnormalities in SD,^{6,7} clusters of microglia/macrophages activation may represent loci of focal brainstem inflammation that alter sensorimotor processing at the level of the brainstem and modulate the ascending inputs to the supramedullary regions controlling voice and speech production in the SD patients. When compared to our previous findings of demyelination and axonal degeneration in the right genu of the internal capsule,⁵ the brainstem clusters of focal inflammation may represent an independent finding, both of which, in turn, may collectively contribute to the pathophysiology of SD.

TABLE II.
Brainstem Neuropathological Characteristics in SD.

ADSD Case	Cell Morphology	Neuronal Degeneration	Depigmentation	Demyelination	Axonal Degeneration	Microglia/Macrophage Activation	Leukocyte/Lymphocyte Activation	α -Synuclein Pathology	Tau Pathology	Ubiquitin Pathology
1	Normal	SNpc+++ , LC+	SNpc+, LC++	None	None	Sol+++ , Sp5+, NA+, RF+++ , IO+++ , Pyr+++	None	None	None	None
2	Normal	SNpc+, LC+	SNpc+, LC+	None	None	Sol+, Sp5+, RF+++ , Pyr+++	None	None	None	None

ADSD = adductor spasmodic dysphonia; SNpc = substantia nigra, pars compacta; + = mild; ++ = moderate; LC = locus coeruleus; Sol = solitary tract nucleus; Sp5 = spinal trigeminal nucleus; NA = nucleus ambiguus; RF = reticular formation; IO = inferior olive; Pyr = pyramids.

Alternatively, based on the location of microglia/macrophage clusters around the brainstem nuclei involved in the laryngeal control and the pyramids, their focal activation may reflect reactive changes surrounding the terminals of degenerative nerve fibers descending within the corticobulbar tract from the laryngeal motor cortex through the genu of the internal capsule to the brainstem nuclei¹¹ in these patients. The fact that we did not find axonal degeneration and demyelination in the brainstem of SD patients in the present study may be explained by very sparse distribution of these descending fibers in the brainstem, and by relative inferiority of conventional histological methods to stains for myelin and axonal course compared to more advanced immunohistochemical methods, such as using KP1 (CD68) marker. However, our finding of clusters of microglia/macrophage activation in the pyramids in both SD patients substantiates the recent observation of focal axonal degeneration and demyelination within the corticobulbar tract at the level of the genu of the internal capsule⁵ in the same male patient reported here, and suggests that reactive changes in the brainstem nuclei in SD patients may reflect the neuropathological abnormalities descending from the higher brain structures.

Other neuropathological findings in the brainstem of both SD patients included mild neuronal degeneration and depigmentation of the pars compacta of the substantia nigra and the locus coeruleus. Although these changes may be due to a normal aging process, they are also similar to those reported in patients with idiopathic cervical dystonia,³ Meige's syndrome,¹ and torsion dystonia.¹² Hence, the involvement of the substantia nigra and the locus coeruleus may represent a common neuropathogenic process in both early- and late-onset primary dystonias.

CONCLUSION

Subtle abnormalities in the brainstem of SD patients, such as clusters of microglia/macrophage activation in the reticular formation surrounding the lower brainstem nuclei and mild degeneration and depigmentation in the substantia nigra and the locus coeruleus, most likely represent disorder-specific abnormalities secondary to the changes in the supramedullary regions in this disorder. Future studies will require access to a larger number of postmortem brain specimens from SD patients for statistical analysis between patients and controls to elucidate the significance of subtle abnormalities between the groups and to identify the direct

relationships between the brainstem and supramedullary abnormalities in this disorder.

Acknowledgments

We are grateful to Viktoria Baker, Laboratory of Pathology, NCI for histology preparations, and to Dr. Mark Raffeld and Cynthia Harris, Molecular Pathology Section, NCI for immunohistochemistry preparations. Tissue specimens were obtained from the Human Brain and Spinal Fluid Resource Center, VAMC, Los Angeles, California, sponsored by NINDS/NIMH, National Multiple Sclerosis Society, VA Greater Los Angeles Healthcare System and Veterans Health Services and Research Administration, Department of Veterans Affairs.

BIBLIOGRAPHY

1. Kulisevsky J, Marti MJ, Ferrer I, Tolosa E. Meige syndrome: neuropathology of a case. *Mov Disord* 1988;3:170-175.
2. Mark MH, Sage JI, Dickson DW, et al. Meige syndrome in the spectrum of Lewy body disease. *Neurology* 1994;44:1432-1436.
3. Zweig RM, Hedreen JC. Brain stem pathology in cranial dystonia. *Adv Neurol* 1988;49:395-407.
4. Holton JL, Schneider SA, Ganesharajah T, et al. Neuropathology of primary adult-onset dystonia. *Neurology* 2008;70:695-699.
5. Simonyan K, Tovar-Moll F, Ostuni J, et al. Focal white matter changes in spasmodic dysphonia: a combined diffusion tensor imaging and neuropathological study. *Brain* 2008;131:447-459.
6. Deleyiannis FW, Gillespie M, Bielamowicz S, Yamashita T, Ludlow CL. Laryngeal long latency response conditioning in abductor spasmodic dysphonia. *Ann Otol Rhinol Laryngol* 1999;108:612-619.
7. Ludlow CL, Schulz GM, Yamashita T, Deleyiannis FW. Abnormalities in long latency responses to superior laryngeal nerve stimulation in adductor spasmodic dysphonia. *Ann Otol Rhinol Laryngol* 1995;104:928-935.
8. McNaught KS, Kapustin A, Jackson T, et al. Brainstem pathology in DYT1 primary torsion dystonia. *Ann Neurol* 2004;56:540-547.
9. McGeer PL, Itagaki S, Boyes BE, McGeer EG. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology* 1988;38:1285-1291.
10. Croisier E, Moran LB, Dexter DT, Pearce RK, Graeber MB. Microglial inflammation in the parkinsonian substantia nigra: relationship to alpha-synuclein deposition. *J Neuroinflammation* 2005;2:14.
11. Jurgens U. Neural pathways underlying vocal control. *Neurosci Biobehav Rev* 2002;26:235-258.
12. Zweig RM, Hedreen JC, Jankel WR, Casanova MF, Whitehouse PJ, Price DL. Pathology in brainstem regions of individuals with primary dystonia. *Neurology* 1988;38:702-706.