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The Effect of Electronic Nicotine Delivery Systems on the Vocal Folds

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THE EFFECT OF ELECTRONIC NICOTINE DELIVERY SYSTEMS ON THE VOCAL FOLDS

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THE EFFECT OF ELECTRONIC NICOTINE DELIVERY SYSTEMS ON THE VOCAL FOLDS

HILARY SAMPLE

ABSTRACT

Electronic Nicotine Delivery Systems (ENDS) are non-combustible tobacco products that are rapidly gaining in popularity worldwide. ENDS are marketed as safer alternatives to cigarettes; however, very little research is available to support or deny these claims. ENDS aerosol is inhaled over the vocal folds and into the lungs consistent with cigarette smoke. The larynx is among the primary locations affected by smoking; therefore, it is necessary to evaluate the effect, if any, of ENDS on the larynx.

The goal of the present study was to evaluate the laryngeal appearance and function of seven ENDS users as compared to four cigarette smokers. A control group of six nonsmokers was included in this study. Vocal tasks were assessed through acoustic analysis and videostroboscopy. No significant relationship was found between ENDS use and abnormal acoustic measures; however, data compared to norms for age and gender revealed a pattern of raised shimmer percent among ENDS users and smokers. Videostroboscopic analysis revealed significant relationships between ENDS use and abnormal mucosal wave, free edge, phase closure, vocal fold varices and vocal fold edema, as well as a marginally significant relationship between ENDS use and abnormal phase symmetry. These results indicate that ENDS use may have a significant effect on laryngeal structures and their function.

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CHAPTER I

INTRODUCTION

Electronic Nicotine Delivery Systems (ENDS), also termed electronic cigarettes, e-cigarettes, and e-cig, are non-combustible tobacco products that are rapidly gaining in popularity since their introduction into the global market in 2004. ENDS are hand-held battery-powered devices that heat a solution, termed “e-liquid” to generate an aerosol, which is then inhaled. ENDS were designed to substitute cigarettes by providing a similar physical and sensory experience to that of smoking (Asgharian, Price, Rostami & Pithawala, 2018; Chapman & Wu, 2014). ENDS is perceived as a safer alternative to cigarettes (Chapman & Wu, 2014); however, very few studies exist on the effects of ENDS use, and limited research is available to either prove or disprove these assertions.

Following a puff, ENDS aerosol is delivered over the vocal folds and into the lungs. The remaining aerosol is exhaled back over the vocal folds, through the oral cavity, and into the environment (Cheng, 2014). The aerosol travels through the respiratory tract in the same manner as conventional cigarettes. The larynx, which is located in the upper respiratory tract, has been examined histologically, acoustically, and perceptually to determine health effects after exposure to cigarette smoke. Researchers have found that the larynx, especially the true vocal folds, is the main site affected by inhalation of cigarette smoke (Auerbach, Hammond, & Garfinkel, 1970; Kelleher, Siegmund, & Chan, 2014).

Numerous studies have been conducted that describe anatomical and physiological changes in the vocal folds and larynges of smokers that occurs as a result of smoking (Myerson, 1964; Auerbach et al., 1970; Awan & Morrow, 2007; Banjara, Mungutwar, Singh, & Gupta, 2011; Kelleher, Siegmund, & Chan, 2014; Öğüt & Kiliç, 2013; Stanciu & Bennett, 1972). It is reasonable to believe the same anatomical structures most impacted by cigarette smoke exposure may also be impacted by ENDS aerosol exposure. However, due to the recent availability of ENDS on the United States market, research is extremely limited on the effects of long-term chronic ENDS exposure on the larynx. Therefore, evaluation of ENDS users' laryngeal anatomy and physiology is necessary to assess the effects, if any, of ENDS on the human larynx.

ENDS Devices

ENDS devices have three basic parts: a rechargeable lithium battery, an atomizer, and a reservoir, cartridge or pod, which contains e-liquid. Activation of the heating coil within the atomizer transforms the e-liquid into nicotine aerosol (Kaisar, Prasad, Liles & Cucullo, 2016). ENDS devices may have either an airflow sensor or a manual trigger that activates a heating coil. The heating coil raises the temperature of the atomizer, which produces an aerosol from the e-liquid. The aerosol is then puffed through a mouthpiece connected to the e-liquid cartridge (Figure 1.2). There are two types of ENDS: open and closed systems. These are also referred to as open and closed tank respectively. The cartridge or tank is refillable in open system devices, and the cartridge is disposable and prefilled with e-liquid in closed system devices.

The design, technical characteristics and operation of ENDS are rapidly evolving. Technical characteristics include the maximum battery output, temperature of the heat coil, and the ability to modify the device. Advances in technical characteristics over time differentiate devices into subtypes or “generations”. The four main subtypes of ENDS devices include: disposable cigalike, rechargeable cigalike, rechargeable vaporizers, and rechargeable pod systems (Glasser et al., 2017).

Disposable Cigalikes. Disposable cigalikes are single-use first-generation devices that closely resemble cigarettes, complete with a light emitting diode (LED) at the tip that is activated when a user inhales (Figure 1.1). These electronic cigarettes are neither rechargeable nor refillable. Disposable cigalikes are discarded in their entirety when e-liquid is depleted. The low voltage battery is activated by an airflow sensor when the user inhales, which then activates the heating coil, resulting in aerosol production.

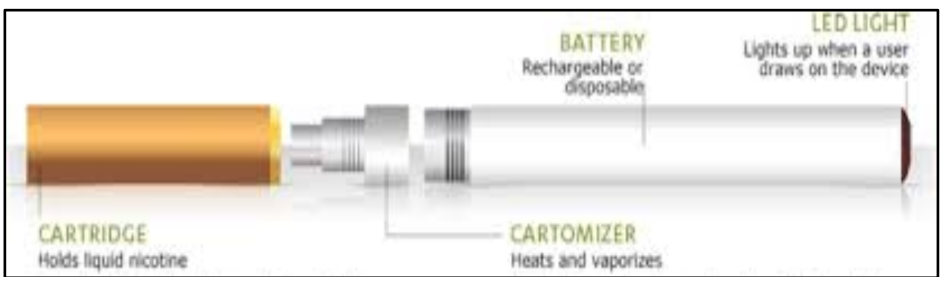


Figure: 1.1 Disposable/rechargeable cigalike components
(From Choosing the Right Type of Electronic Cigarette (Vape). (2014, March 1). Retrieved January 13, 2019, from Vaporferver website: https://vaporferver.com/electronic-cigarette-info/choosing-the-right-type-of-electronic-cigarette/)

Rechargeable cigalikes. Rechargeable cigalikes closely resemble disposable cigalikes but include a rechargeable battery (Figure 1.1). Rechargeable cigalikes are second generation devices that have two options: manual trigger or airflow sensor. With an airflow sensor, otherwise termed “automatic draw”, the battery is activated by inhalation and requires a disposable cartomizer. A cartomizer is a combined unit for both the atomizer and pre-filled cartridge. The battery for manual trigger, also termed “manual draw”, is activated by pressing a button.

Rechargeable vaporizers. Rechargeable vaporizers are third-generation devices that do not resemble cigarettes and include “vape pens” (Figure 1.2), “mods” and “box mods” (Figure 1.3). These rechargeable devices are open tank systems. They are larger than cigalikes with modifiable, higher voltage batteries and more advanced atomizers. With refillable reservoirs, users have the option to customize e-liquid. These devices are either variable voltage or temperature controlled. Variable voltage devices have modifiable batteries, which allow the user to modify the device voltage and temperature of the coil. Increasing the temperature of the coil alters the density and “hit” of the aerosol, creating a similar physical sensation to cigarette smoke inhalation.

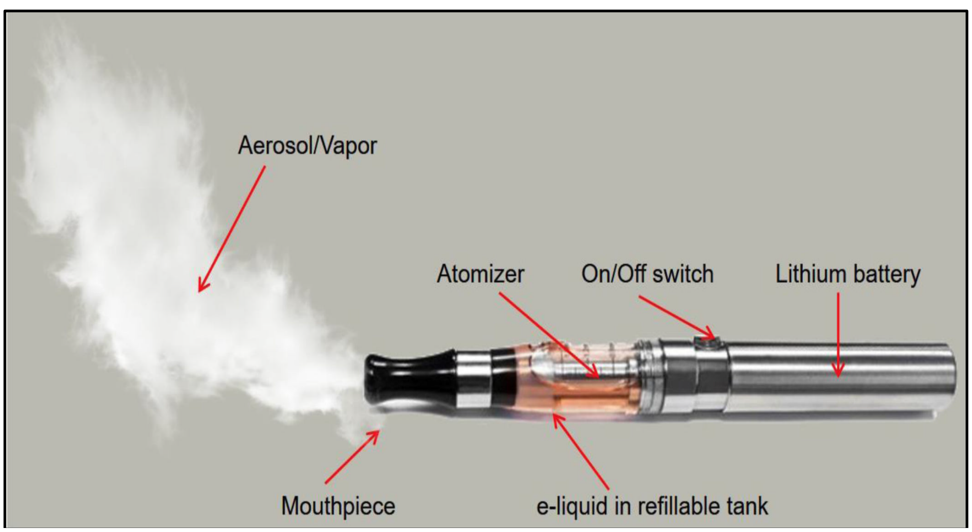


Figure 1.2 Systematic illustration of rechargeable vape pen
(*From* Kaisar et al., 2016)

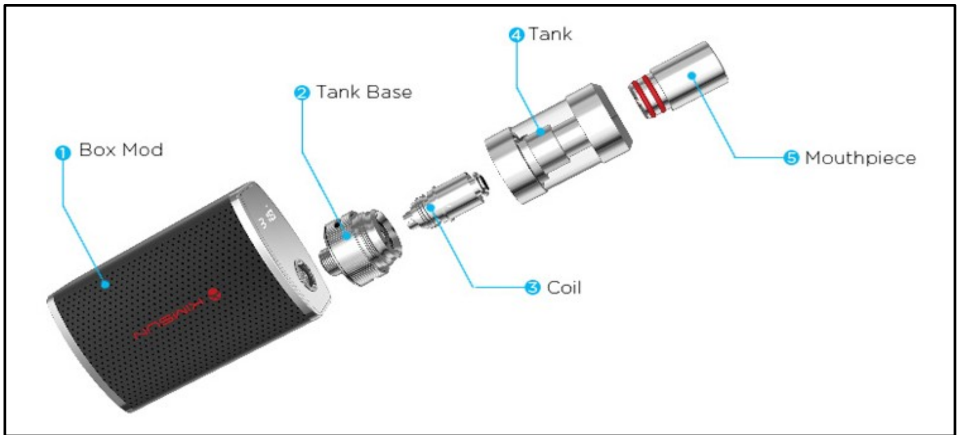


Figure 1.3 schematic illustration of components of a “box mod” rechargeable vaporizer
E-Cig 101 - Informative and Always Tuition Free! (n.d.). Retrieved January 13, 2019, from https://www.nicotinenirvana.com/ecig-101-s/12.htm

Rechargeable pod systems. Rechargeable pod systems are current generation devices that come in a multitude of designs (Figure 1.4). These devices may be user-modifiable, are rechargeable, and are either open tank or close tank systems. Devices may be variable voltage or temperature controlled. Temperature controlled models allow the user to program a fixed temperature into the microchip for a consistent aerosol output. Rechargeable pods include brands like “Suorin Drop”, “myblu”, and “Juul”.

Juul has become its own category of vape devices and users, especially within the adolescent and 18-25-year old young adult population, who now replace the verb “vaping” with “juuling” (Huang et al., 2018). The voltage of these devices is controlled application, meaning that users cannot modify voltage output. The amount of nicotine per e-liquid pod is approximately equivalent to one pack of cigarettes or 200 puffs (Kee, 2018; Huang et al., 2018; Dawkins, Kimber, Puwanesarasa & Soar, 2015).

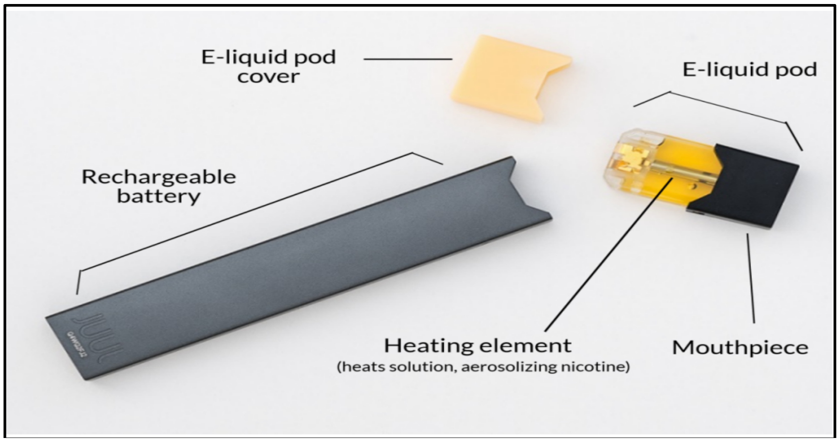


Figure 1.4 Rechargeable pod schematic illustration of juul components
from King County - E-cigarettes and vaping products (n.d.). Retrieved January 13, 2019, from https://www.kingcounty.gov/~/media/depts/health/tobacco-vapor/images/juul-diagram-carousel.ashx

ENDS Regulation and Standardization

The U.S. Food and Drug Administration (FDA) has authority to regulate tobacco products, and in 2016, extended that regulatory authority to ENDS products; however, the ENDS industry has an extended deadline of 2022 to comply with new FDA guidelines (US Food and Drug Administration, 2016; Zare, Nemati & Zheng, 2018). Just as the FDA heavily regulates cigarettes, including flavor, nicotine levels, and the number of cigarettes per pack, it will potentially regulate ENDS attributes, including but not limited to flavors, nicotine strength, and e-liquid size and contents. At the present time, ENDS products are not standardized and are not yet required to conform to regulatory guidelines. Due to the lack of regulation and standardization of ENDS devices, e-liquids and devices with the same name and the same listed ingredients have shown inconsistencies in contents, including nicotine, when examined by researchers (Etter, Zather, & Svensson, 2013; Famele et al., 2015; Goniewicz et al., 2015; Goniewicz, Hajek, & McRobbie, 2014; Goniewicz, et al., 2014; Hahn et al, 2014; Lisko et al, 2016; Kaisar et al., 2016).

ENDS Toxicity

ENDS e-liquid contains nicotine, a carrier liquid, such as vegetable glycerin and propylene glycol, and any number of flavoring agents that make up thousands of available flavors (Zhu, Sun, Bonnevie, Cummins, Gamst, Yin & Lee, 2014; Soussy, El-Hellani, Baalbaki, Salman, Shihadeh & Saliba, 2016). Research on the chemical composition of ENDS e-liquid and aerosol has revealed substances that are known to be harmful in cigarette smoke (Kosmider et al, 2014; Fowels & Dybing, 2003). Additionally, toxicity of ENDS aerosol has been shown to increase relative to increased sweetener concentration and increased heat due to high battery voltage output (Kosmider et al, 2014).

Toxins contained in e-liquids and aerosol. Research on the chemical composition of ENDS has found heavy metals, carcinogens and teratogenic agents, including carbonyl compounds, aldehydes, volatile organic compounds, and tobacco-specific nitrosamines present in ENDS e-liquid and aerosol (Farsalinos et al., 2013; Tierney, Karpinski, Brown, Luo & Pankow, 2016; Kaisar et al., 2016). The majority of ENDS contain vegetable glycerin and propylene glycol as carrier liquids. These substances are '*generally recognized as safe*' (GRAS) by the Flavor Extracts Manufacturer's Association (FEMA); however, this perception refers primarily to the effect of ingestion on the digestive tract. Chronic exposure of aerosolized forms of these substances may affect the airway differently. This may include reducing the consistency of fluid in the tissue or exposing the tissue of the airway to harmful toxins formed during the heating process (Callahan-Lyon, 2014; Kosmider, Sobczak, Fik, Knysak, Zaciera, Kurek, Goniewicz, 2014).

Propylene glycol and vegetable glycerin both thermally degrade to toxic carbonyl compounds (Laino, Tuma, Moor, Martin, Stolz & Curioni, 2012; Kosmider et al., 2014; IARC, 2012). Acetone and aldehydes, including formaldehyde, benzaldehyde, and acrolein, among others, found within ENDS aerosol are formed during the heating process (Kosmider et al., 2014). Acetone is a known mucous membrane irritant (Buron, Hacquemand, Pourie, & Brand, 2009), and aldehydes and heavy metals have been shown to have cytotoxic effects on epithelium (Rowell & Taran, 2015). Formaldehyde is classified as a Group 1 human carcinogen by the International Agency for Research of Cancer, and acetaldehyde is classified as Group 2B, possibly carcinogenic to humans (IARC, 2012). Acrolein has been found to cause irritation in the nasal cavity and damage to pleural lining (U.S. EPA, 2003). Exposure to carbonyls causes mouth and throat irritation (Bullen et al, 2010), which are among the most highly reported side-effects of ENDS exposure.

Flavored ENDS e-liquids. Surveys show that sweet and fruit flavors are preferred by ENDS users over other options (Zare et al., 2018). A study by Welz et al. (2018) found that fruit flavored e-liquids showed higher toxicity than tobacco flavored e-liquids (Welz et al., 2018). Sweeteners used in ENDS thermally degrade to toxic compounds including furans, 5-hydroxymethylfurfural (HMF) and furfural (FA) (Soussy et al., 2016; Jing & Lü, 2008; Fiore et al., 2012). These flavoring agents have been shown to cause irritation and inflammation to the human respiratory tract (Arts, Muijser, Appel, Frieke, Bessems, Woutersen, 2004; Farsalinos, et al., 2013).

Flavoring agents have been found in "higher-than-safety" levels in a large portion of sweet-flavored e-liquids (Allen et al, 2015). Chronic inhalation of sweeteners may lead to respiratory complications due to irritation and inflammation, with a potentially similar effect on the larynx. Finally, levels of furans and other toxic compounds have been shown to increase relative to increased heat and sweetener concentration (Soussy et al., 2016).

ENDS battery output voltage. Increased battery output voltage affects the yield and toxicity of ENDS aerosols (Breland, Soule, Lopez, Ramôa, El-Hellani, & Eissenberg., 2017). First generation devices produce voltage at an average of 3.7 Volts (V). Second generation devices produce voltage at an average of 3-6V. Current generation devices can produce voltage of up to 8V. Studies on early or first generation devices showed that ENDS contain fewer toxic and carcinogenic compounds than conventional cigarettes (Goniewicz et al., 2014; Laugesen, 2008; Uchiyama, Inaba, & Kunugita, 2010); however, these studies were unable to take into account increases in ENDS power output ability beginning with later developed second generation devices.

Kosmider et al. (2014) found that the amount of aerosol generated by a device is directly proportional to the power of the device battery. Many later generations of ENDS products are variable voltage, which allow users to increase battery output voltage, resulting in increased aerosol density in each inhalation (Rowell & Taran, 2015). Increasing battery output voltage leads to an increased temperature of the heating coil within ENDS. The user presses and holds an on/off switch, which sends a signal to the heating coil, theoretically allowing the user to raise the temperature of the atomizer up to 662 degrees Fahrenheit (Balhas, Talih, Eissenberg, Salman, Karaoghlanian, Shihadeh, 2014).

Increased battery output voltage has been shown to rapidly increase carbonyl levels within ENDS aerosol (Kosmider et al., 2014). Additionally, the increased battery output voltage results in more e-liquid consumed per puff, so users are not only exposed to increased levels of carbonyls but generally greater amounts of toxins due to increased density of the aerosol (Havel, Benowitz, Jacob & St.Helen, 2016; Kosmider et al., 2014; Floyd, Queimado, Wang, Regens & Johnson, 2018). Kosmider et al (2014) found that increasing voltage from 3.2 to 4.8V resulted in an increase of formaldehyde, acetaldehyde and acetone levels of up to and over 200 times what it was in ENDS aerosol produced at 3.2V. Havel et al. (2016) found that levels of acetaldehyde, acrolein, and formaldehyde increased rapidly at voltages at or above 5V. Kosmider et al. (2014) found that vapors from high voltage devices, with voltage at or above 4.9V, produce levels of formaldehyde that are in the range of levels reported in tobacco smoke.

Reported health effects of ENDS. Most reported adverse effects of ENDS concern the mouth, throat, and respiratory systems (World Health Organization, 2015). In a 2012 study by Vardavas et al., researchers found that ENDS had an immediate adverse physiologic effect after short term use similar to effects caused by cigarette use and found that acute ENDS exposure may impair lung function (Vardavas, Anagnostopoulos, Kougias, Evangelolpoulou, Connolly & Behrakis, 2012). Lerner et al. (2015) found that ENDS aerosols were found to induce oxidative stress on human lung epithelial cells and trigger an inflammatory response (Lerner, Sundar, Yao, Gerloff, Ossip, McIntosh, Robinson, Rahman, 2015; Wu, Jiang, Minor, & Chu, 2014)**.** A study on the effect of PG mist revealed dry throat and cough with both acute and chronic exposure (Varughese, Teschke, Brauer, Chow, van Netten, Kennedy, 2005). Additionally, e-liquids were found to be cytotoxic to oropharyngeal tissue. The epithelium of the oropharynx is similar to the tissue within the larynx (Welz et al., 2018), which allows the possibility of a similar effect on laryngeal structures.

Anatomy and Physiology of the Larynx

The human vocal folds are located within the larynx, which is anatomically positioned at the top of the trachea at the levels of the cervical vertebrae 3 through 6 (C3 – C6) in adults (Figure 1.5). Healthy vocal folds are required for airway protection, respiration, swallowing, and phonation or voicing. To produce phonation, the airstream passes between the two vocal folds that come together and vibrate due to both myoelastic and aerodynamic forces (Figure 1.6).

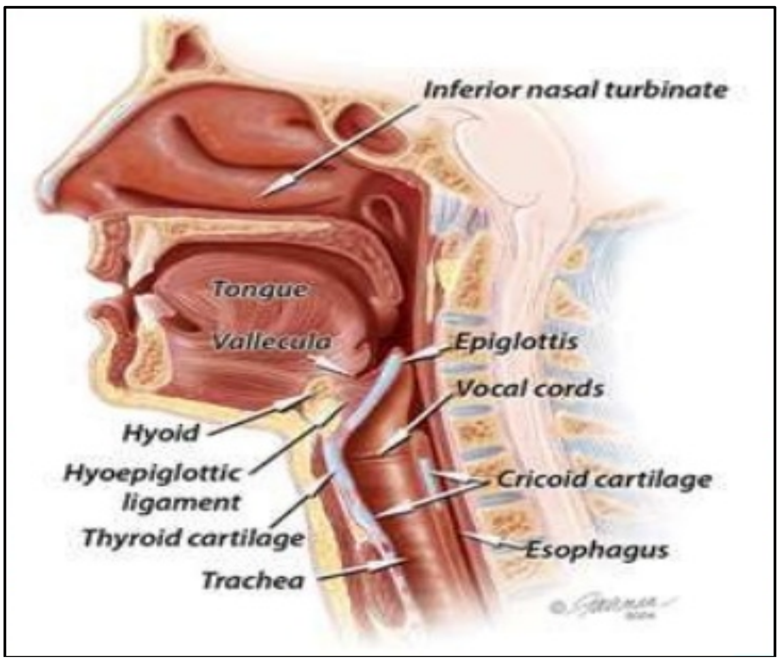


Figure 1.5 Sagittal view of vocal folds within larynx positioned at level of C3-C6
(*From: Airway Management. (n.d.). Retrieved January 13, 2019, from LSU Health shreveport website: https://LSUHealthShreveport/departments/ClinicalDepartments/OralandMaxillofacial/omfsservesprovided/airwaymanagment/OMFSAirway.aspx)*

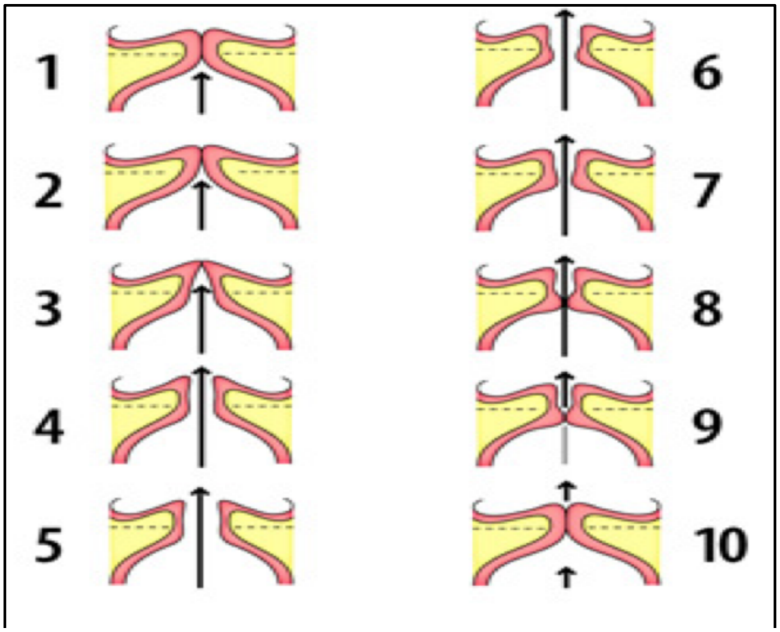


Figure 1.6 Vocal fold vibration caused by airstream from the lungs in anterior view
(From Understanding Voice Production. (2013, August 31). Retrieved January 13, 2019, from THE VOICE FOUNDATION website: https://voicefoundation.org/health-science/voice-disorders/anatomy-physiology-of-voice-production/understanding-voice-production/)

True vocal fold anatomy. The vocal folds consist of 3 layers: the epithelium of the mucosa, the lamina propria, and the vocalis muscle, with blood vessels interwoven throughout. The lamina propria is further divided into three layers: superficial, intermediate and deep. The epithelium is composed of squamous stratified epithelium. The superficial lamina propria (SLLP), or Reinke's space, is an underlying layer of loose connective tissue (Figure 1.7). The epithelium and Reinke's space make up the mucosal cover, which can be observed moving during vocal fold vibration. The movement of the mucosal cover during vocal fold vibration is known as the mucosal wave. The cover gives the vocal folds their pearly-white appearance. The mucosal cover enfolds the vocal ligament and the vocalis muscle. The intermediate and deep layers of the lamina propria make up the vocal ligament. The vocal ligament is the elastic component of the vocal folds. Reinke's space and the underlying vocal ligament together constitute the lamina propria.

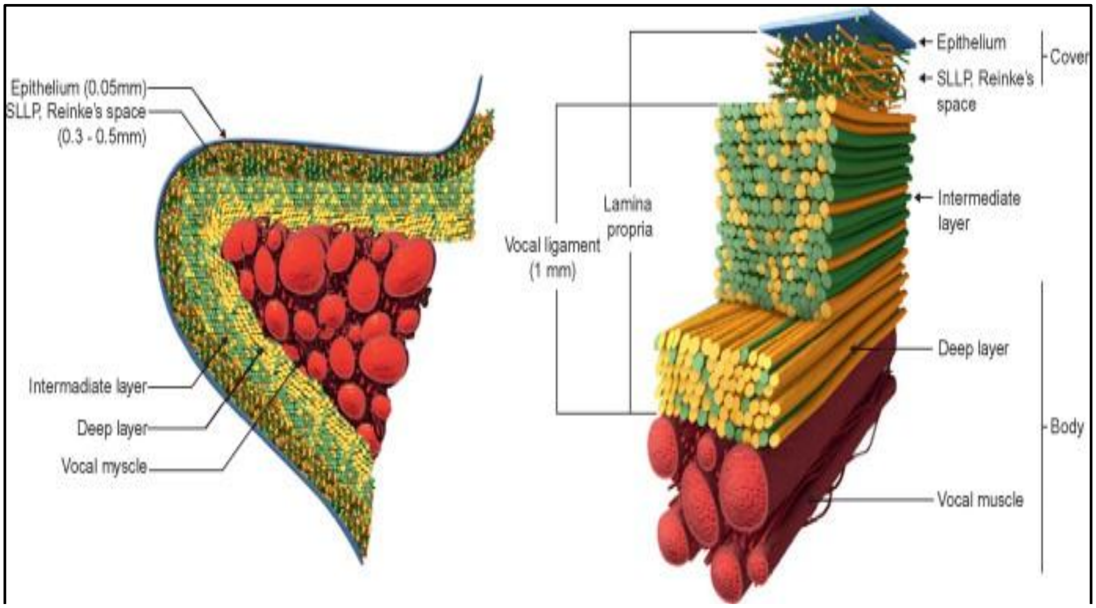


Figure 1.7 Coronal cross-section of a vocal fold (left) and three-dimensional model of the layers (right). From *Elsevier. Finck C, Lejeune L. Structure and oscillatory functions of the vocal fold. In: Stefan M. Brudzynski, ed. The Handbook of Mammalian Vocalization: An Integrative Neuroscience Approach. Chapter 10.2. Figure 2. Copyright Elsevier (2009)*

Changes in vocal fold tissue influence the function of the vocal folds for voice production. The epithelial surface of the vocal folds is covered by a thin layer of fluid/mucus, which influences the biomechanics of vocal fold vibration (Leydon, Sivansanker, Lodewyck, Atkins, & Fisher, 2009). This thin layer of fluid/mucus is continuous with the tongue, pharynx, and trachea and is covered with sensory receptors and mucus-secreting glands. Irritation or drying of this mucosa can lead to a hoarse vocal quality, or when severe, the epithelial cells within this lining can become malignant. Reinke's space also plays a major role in the vibration of the vocal folds (Hirano, 1980, 1981). Movement of the epithelium and Reinke's space form the mucosal wave. During vocal fold vibration, the mucosal wave periodically interrupts expiratory airflow, resulting in phonation. If the mucosal cover is stiff/resists movement or is overly pliable, this will alter the resulting tone produced by the wave. The majority of vocal fold pathologies develop in Reinke's space, and include polyps, nodules, and edema. The accumulation of fluid/swelling in Reinke's space due to irritation, such as smoking, is termed "Reinke's Edema".

Laryngeal cartilages. The larynx, or voice box, consists of nine cartilages, which are connected via ligaments and muscle (Figure 1.8). These include three unpaired and three paired cartilages. The large unpaired cartilages include the epiglottis, cricoid, and thyroid, which form the body of the larynx (Figure 1.9). The three paired cartilages include the corniculates, arytenoids, and cuneiforms. The cricoid, thyroid and the arytenoids have an important role in the phonatory function of the larynx. The small paired cartilages, corniculates and cuneiforms, have a minimal role in voicing, and the primary role of the epiglottis is airway protection.

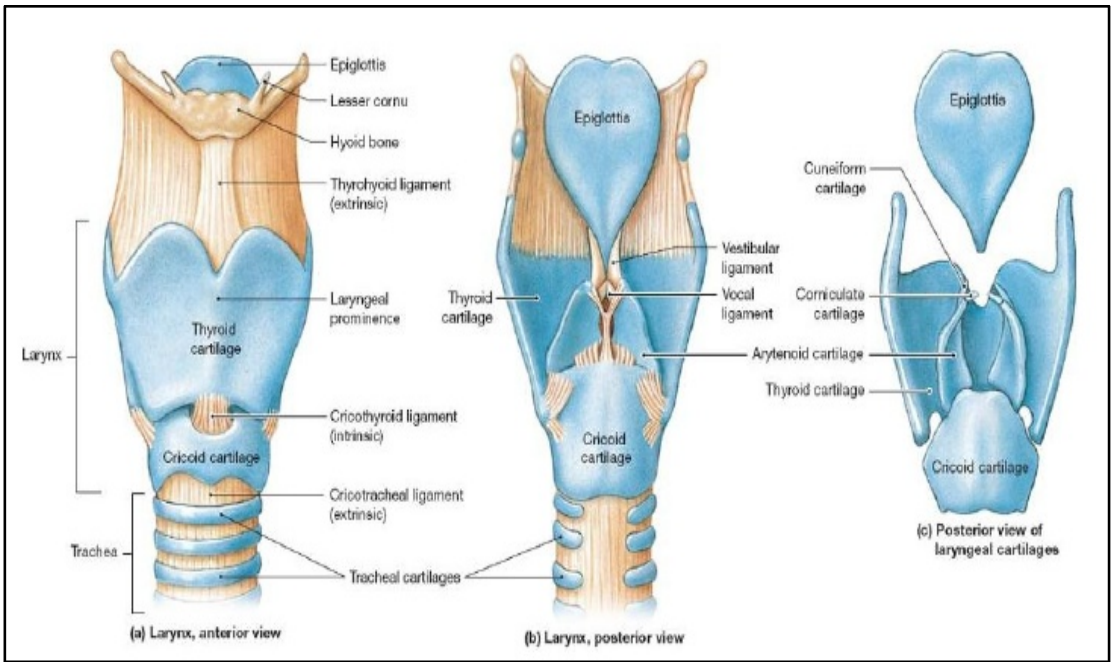


Figure 1.8 Laryngeal cartilages
(From Team, H. J. (2017, October 5). Pharynx - Anatomy & Function in Respiratory System. Retrieved January 26, 2019, from Health Jade website: https://healthjade.com/what-is-the-pharynx/)

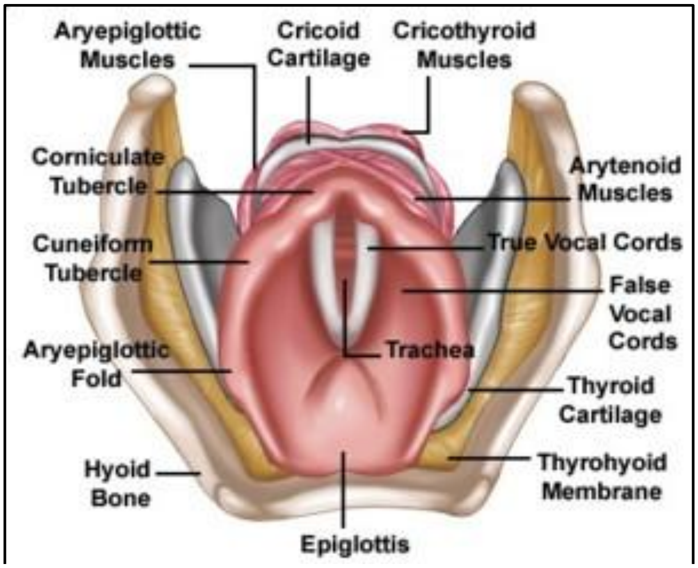


Figure 1.9 Aerial anterior view of epiglottis, cricoid and thyroid cartilages forming body of the larynx. *(From: ELLO. (n.d.). Retrieved January 27, 2019, from https://zentrum.virtuos.uni-osnabrueck.de/wikifarm/fields/english-language/field.php/PhoneticsandPhonology/JourneyAlongTheVocalTractPage2)*

Laryngeal structures. Structures that are visible during endoscopic evaluation include structures within and above the laryngeal vestibule. Structures within the laryngeal vestibule include the aryepiglottic folds, ventricular (false) vocal folds, true vocal folds, the anterior and posterior commissures of the glottis, laryngeal ventricle, interarytenoid space, and trachea. The structures located above the laryngeal vestibule include the epiglottis and other areas within the pharynx (throat).

The aryepiglottic folds extend between the arytenoids and epiglottis. The ventricular vocal folds lie superior to the true vocal folds and inferior to the aryepiglottic folds. The true vocal folds are located inferior to the ventricular folds and above the trachea at the glottis. The glottis includes the true vocal folds and the anterior and posterior commissures. The anterior commissure is the midline area of the glottis where the vocal folds meet anteriorly and where they are attached to the thyroid cartilage. The posterior commissure of the glottis lies anterior to the cricoid cartilage in between the arytenoid cartilages. The laryngeal ventricle is the area between the ventricular and true vocal folds on either side and extends nearly their entire length. The interarytenoid space is found between the arytenoid cartilages and posterior to the glottis. The trachea is located just below the vocal folds and is visualized during vocal fold abduction (opening).

The epiglottis is located adjacent to the tongue base and anterior and superior to the laryngeal vestibule. Some areas within the pharynx that may also be visualized during endoscopic evaluation include the tongue base and valleculae, which lie superior to the laryngeal vestibule. (Figure 1.10).

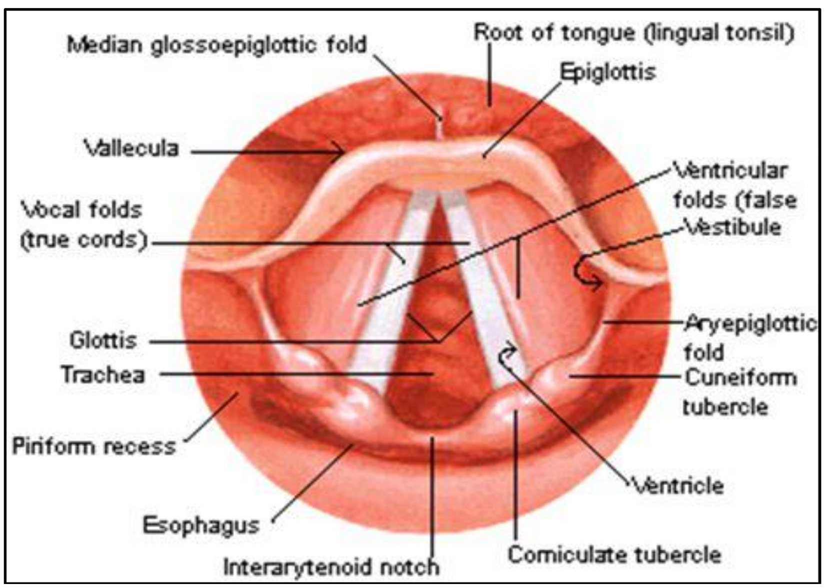
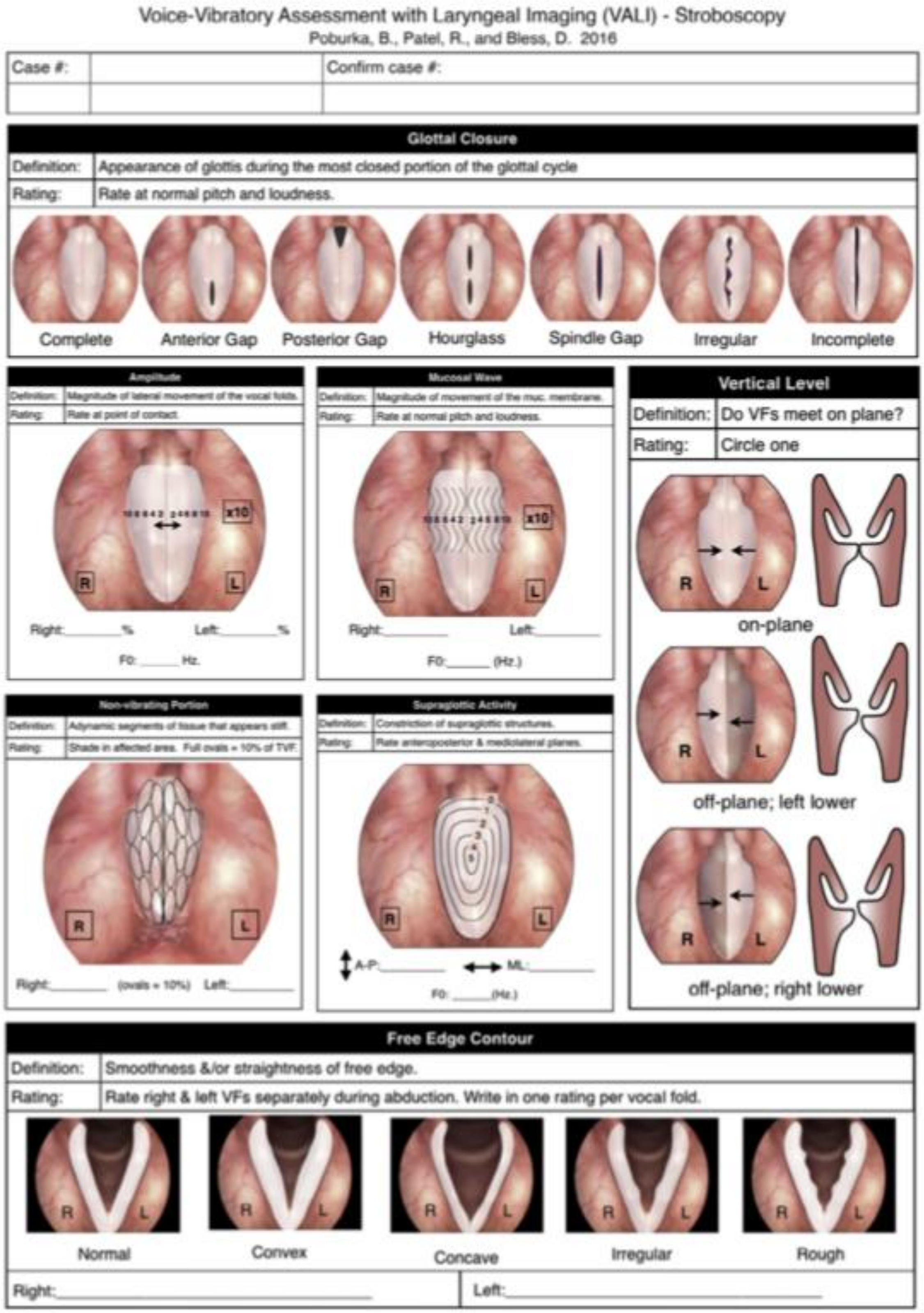


Figure 1.10 Aerial posterior view of vocal folds
(From: Anatomy of the glottis. (n.d.). Retrieved January 27, 2019, from http://www.aboutcancer.com/anatomy_glottis.gif)

Stroboscopic Assessment

Stroboscopic imaging of the vibratory function and appearance of the vocal folds plays a central role in the assessment and treatment of vocal pathologies. The effectiveness of laryngeal stroboscopy for investigation of the vibratory and nonvibratory function of the larynx is well proven (Bless, Hirano & Feder, 1987; Patel, Dailey & Bless, 2008). Videostroboscopy allows the clinician to record vocal fold patterns for visual inspection of laryngeal structure and function. Stroboscopy is a term used to indicate a specific type of light used during visualization of the laryngeal area. Because it is impossible for the naked eye to see the rapidly vibrating vocal folds, in stroboscopy, brief flashes of light at a frequency just slightly less than the rate of vocal fold vibration slow the perceived rate of movement patterns of the vocal folds to allow for assessment (Faure & Muller, 1992).

Vocal fold parameters viewed with stroboscopy. Efforts to standardize perceptual assessment of the vocal folds using videostroboscopy have resulted in rating systems of vocal fold vibratory and nonvibratory parameters. The Voice-Vibratory Assessment with Laryngeal Imaging (VALI) is one such rating form developed by Poburka, Patel, and Bless (2016) (Figure 1.11). This assessment is used to create a more reliable visuoperceptual evaluation of the videostroboscopic examination. VALI is an updated version of the Stroboscopy Evaluation Rating Form (SERF) (Poburka, 1999; Poburka & Bless, 1998). The parameters assessed using VALI include the following: glottal closure, amplitude, mucosal wave, vertical level, nonvibrating portion, supraglottic activity, free edge contour, regularity, phase symmetry, and phase contour (Figures 1.12-1.21). Additionally, nonvibratory observations, which include erythema (redness), edema (swelling), varices (vascularity), and mucus are rated according to a rating system adapted from Hirano and Bless (1993) (Figure 1.22). During examination, the clinician uses the following definitions and images of each parameter assessed according to descriptions and images provided in the VALI rating form (Poburka et al., 2016).


Voice-Vibratory Assessment with Laryngeal Imaging (VALI) - Stroboscopy

Poburka, B., Patel, R., and Bless, D. 2016

Case #:		Confirm case #:

Glottal Closure

Definition:	Appearance of glottis during the most closed portion of the glottal cycle
Rating:	Rate at normal pitch and loudness.

Complete · Anterior Gap · Posterior Gap · Hourglass · Spindle Gap · Irregular · Incomplete

Amplitude

Definition:	Magnitude of lateral movement of the vocal folds.
Rating:	Rate at point of contact.

x10 · R · L

Right:______% Left:______%

F0: _____ Hz.

Mucosal Wave

Definition:	Magnitude of movement of the muc. membrane.
Rating:	Rate at normal pitch and loudness.

x10 · R · L

Right:________ Left:________

F0:______ (Hz.)

Vertical Level

Definition:	Do VFs meet on plane?
Rating:	Circle one

R · L · on-plane

R · L · off-plane; left lower

R · L · off-plane; right lower

Non-vibrating Portion

Definition:	Adynamic segments of tissue that appears stiff.
Rating:	Shade in affected area. Full ovals = 10% of TVF.

R · L

Right:________ (ovals = 10%) Left:________

Supraglottic Activity

Definition:	Constriction of supraglottic structures.
Rating:	Rate anteroposterior & mediolateral planes.

R · L

A-P:________ ML:________

F0:_____ (Hz.)

Free Edge Contour

Definition:	Smoothness &/or straightness of free edge.
Rating:	Rate right & left VFs separately during abduction. Write in one rating per vocal fold.

Normal · Convex · Concave · Irregular · Rough

Right:______________________ Left:______________________

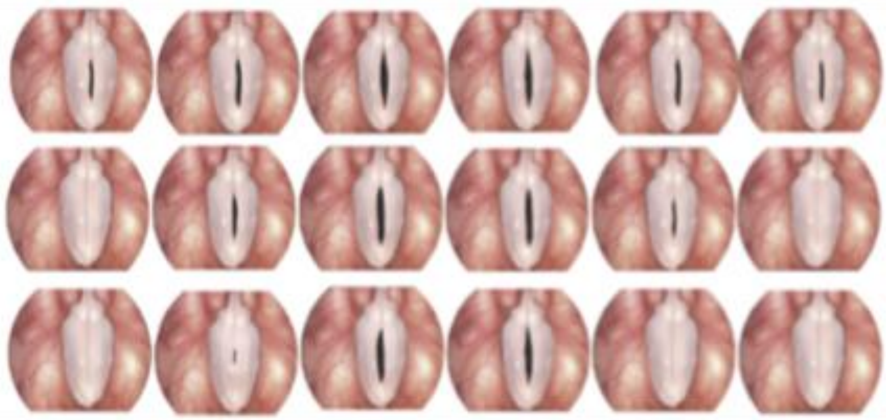
Figure 1.11 VALI rating form for stroboscopy. Voice-Vibratory Assessment with Laryngeal Imaging (VALI) -- Stroboscopy (Poburka, B., Patel, R., and Bless, D. 2016).

Phase Closure	
Definition:	The relative durations of appearance of consecutive glottal cycles.
Rating:	Circle a hash mark on the continuum below. Rate at point of contact.

Open phase predominates

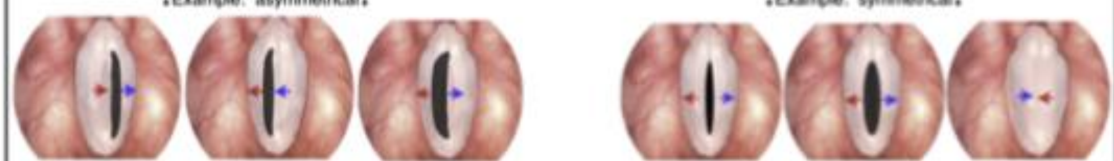
Nearly equal

Closed phase predominates



Phase Symmetry	
Definition:	The degree to which the VFs move as mirror-opposite images of each other (180° phase difference).
Rating:	Select the % of exam time that vibration is symmetrical.

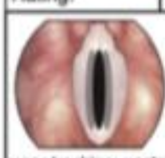
↓Example: asymmetrical↓ ↓Example: symmetrical↓



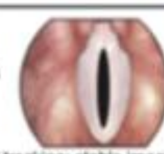
0%----------10---------20---------30---------40---------50---------60---------70---------80---------90---------100%

Key: Direction of travel: ► right VF; ► left VF

Regularity	
Definition:	Consistency of cycles.
Rating:	Circle % of time that vibration is regular



never reg. 0% -10 - 20 - 30 - 40 - 50 - 60 -70 - 80 - 90 -100% always reg.



poor tracking; unstable/blurred imaging

good tracking; stable imaging

Nonvibratory Observations	
Definition:	Report observations that were not noted elsewhere (e.g.,structural differences, erythema, varices, mucous).

Figure 1.11 (continued)

Glottal closure. Glottal closure is the appearance of the glottis during the most closed portion of the glottal cycle at normal pitch and loudness (Poburka et al., 2016). The glottis is a term that defines the vocal folds and the space between them within the larynx. Analysis of various closure patterns is helpful in diagnosing laryngeal pathology. Glottal closure patterns assessed using VALI include: complete, anterior gap, posterior gap, hourglass, spindle gap, irregular, and incomplete (Figure 1.12). Normal closure patterns include complete closure and posterior gap. Abnormal glottal closure patterns include anterior gap, hourglass closure, spindle gap, irregular closure, and incomplete closure.

In the complete closure pattern, the vocal folds fully adduct without any glottic spaces or gaps. In the posterior gap pattern, there is a small glottic gap, which is triangular in appearance and located in the posterior aspects of the vocal folds. This closure appears to be common in Caucasian and African American females more so than males, but a study by Meeks (2017) suggested that posterior gap in African American and Asian males may not be pathologic as well.

A small glottic gap exists anteriorly during vocal fold adduction in the anterior gap closure pattern. This pattern can be indicative of vocal fold pathology, including vocal nodules. In the hourglass pattern, the vocal fold closure occurs only at one point, and is typically observed in the medial to anterior one-third of the vocal folds. There is no adduction anterior or posterior to this point. The hourglass closure pattern is associated with the presence of bilateral vocal nodules located at the point of vocal fold closure.

In the spindle gap closure pattern, the vocal folds adduct in both the anterior and posterior aspects but bow outward from midline in the medial aspects leaving a glottic space. The spindle pattern is most commonly seen with hypofunctional dysphonia and presbyphonia. In the irregular closure pattern, the vocal folds do not adduct along their length and cannot complete closure during adduction due to irregular and rough margins. The irregular pattern can be indicative of vocal fold pathology, including laryngeal cancer. Incomplete closure is observed when the vocal fold margins are smooth in appearance and the vocal folds do not fully adduct along the entire length. Incomplete closure can indicate abnormality and hyperfunctional dysphonia.

In instances where the vocal folds show more than one pattern, the closure pattern is termed "variable closure" (Poburka et al., 2016). Images for comparison are provided on the rating form (Figure 1.12). Closure patterns are documented at low frequencies and high frequencies for each participant.

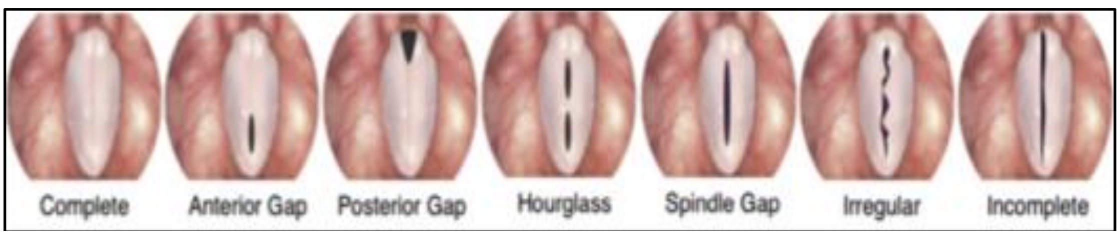


Figure 1.12 Glottal Closure patterns
From VALI rating system (Poburka et al, 2016)

Amplitude. Poburka et al. (2016) define amplitude as the magnitude of lateral movement of the vocal folds. In normal conditions, the point of contact of the lateral movement of the vocal folds is one half the width of the visible portion of the vocal folds. Increased vocal intensity or loudness will increase amplitude. Increased stiffness, mass, and pitch will reduce amplitude. VALI uses a numerical rating system that rates movement from 2-10 and corresponding images aid in documentation of the point of contact of lateral movement (Figure 1.13). A rating of 2 is given with minimal lateral separation of the vocal folds when voicing at normal pitch and loudness, indicating significantly reduced amplitude or intensity. A rating of 10 is maximal lateral separation of the vocal folds, indicating maximum amplitude or intensity. Amplitude is reported separately for the left and right vocal folds.

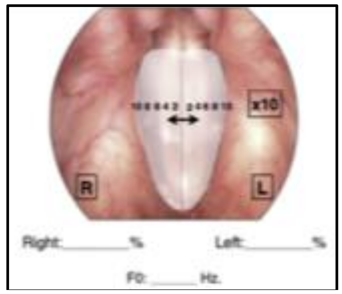


Figure 1.13 Amplitude rating system
From VALI rating system (Poburka et al, 2016)

Mucosal wave. Mucosal wave is the magnitude of movement of the mucosal membrane (Poberka et al., 2016). During vocal fold vibration, the mucosal wave originates on the medial surface of the vocal folds and moves laterally across the superior surface of the mucosal cover, spreading bilaterally (Hirano & Bless, 1993). Any change in the mucosal cover will alter the mucosal wave (Hirano, 1981). The movement of the mucosal wave also provides information about the status of the tissue that is between the cover and the vocalis muscle of the vocal folds.

If the vocal fold is stiff and resists movement or is edematous, this may reduce the mucosal wave or cause it to be absent. As said, most lesions affect the cover, causing restricted movement of the mucosal wave. Edema of the epithelium increases mass and stiffness of the cover, which interferes with the vibratory function of the vocal folds, thus also affecting the mucosal wave. Reinke's Edema can make the vocal fold cover more massive, less stiff, and more compliant, resulting in an altered mucosal wave.

Using videostroboscopy, the location of altered mucosal movement can often be specifically noted. VALI uses a numerical rating system from 2-10 for movement of the mucous membrane at normal pitch and loudness (Figure 1.14). A rating of 2 coincides with minimal motion of the mucosal wave, and would indicate increased swelling, stiffness, or otherwise restricted, nearly absent movement. A rating of 10 indicates movement of the mucosal wave across the total visible width of the vocal folds. A rating greater than 8 bilaterally indicates normal motion of the mucosal wave. Mucosal wave will be rated separately for the left and right vocal folds.

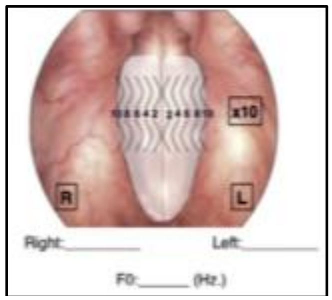


Figure 1.14 Mucosal wave rating system
From VALI rating system (Poburka et al, 2016)

Vertical level. Poberka et al. (2016) define vertical level as whether the vocal folds meet on plane. If the vocal folds do not meet on plane, the form provides options for description of off plane meeting, i.e., whether the left or right vocal fold is lower (Figure 1.15). Structural abnormalities in one or more vocal folds can lead to approximation of vocal folds off the vertical plane (Van den Berg, 1968).

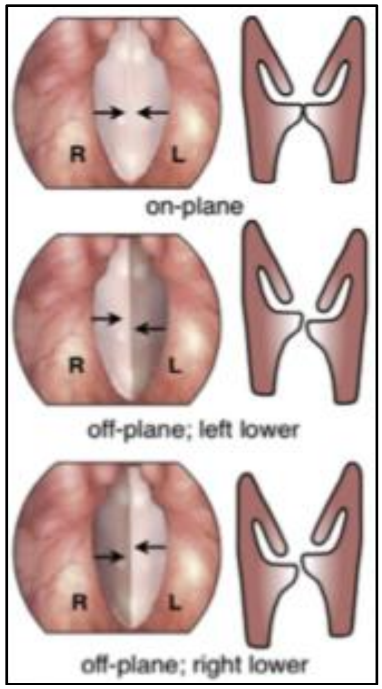


Figure 1.15 Vertical Level rating system
From VALI rating system (Poburka et al, 2016)

Non-vibrating portion. Non-vibrating portion is defined by Poberka et al. (2016) as adynamic segments of tissue that appears stiff. Stiff tissue is indicative of pathology, including nodules, polyps, edema, or early signs of laryngeal carcinoma, which originates in the mucosal cover. The gradual change in stiffness throughout the vocal fold structure is an important histological component of healthy vocal folds (Hirano & Bless, 1993). Stiffness gradually decreases from the stiff vocalis muscle to the pliable mucosal cover. Any change in the stiffness of the tissue alters how the structures function.

The superficial layer of the vocal folds is where edema often develops (Hirano, 1981). Edema and lesions increase mass and stiffness, which interferes with vocal fold vibration (Hirano, 1981). The VALI rating form shows vocal folds with 10 ovals on the surface of each fold. Each oval accounts for 10% of the true vocal fold. The evaluator shades in the ovals on the rating form that correspond to the area of stiffness or reduced or absent movement observed during videostroboscopy (Figure 1.16).

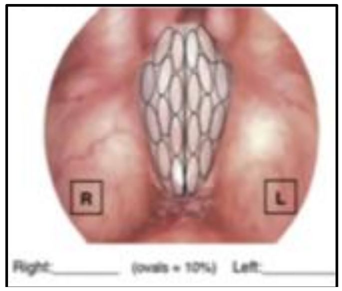


Figure 1.16 Non-vibrating portion rating system
From VALI rating system (Poburka et al, 2016)

Supraglottic activity. Poburka et al. (2016) define supraglottic activity as constriction of supraglottic structures during sustained phonation. Supraglottic structures are those that lie above the true vocal folds and below the tongue base. These include the ventricle, the ventricular folds, the epiglottis, the arytenoids, the laryngeal aspects of the aryepiglottic folds, and the vestibule (Stager, 2011). Supraglottic activity may occur due to vocal abuse or misuse, compensation of the larynx due to pathology, illness or injury, and/or the type of endoscope used in assessment (Stager, Bielamowicz, Regnell, Gupta, Barkmeier, 2000; Nospes, Kuhr, Napiontek & Keilmann, 2011). Two types of supraglottic constriction or compression may be noted: static or dynamic. Static activity initiates when phonation begins and remains until vocal fold vibration ceases. Dynamic activity changes throughout speaking tasks. A subset of dynamic activity is the occurrence of compression upon initiation of a speaking task (Stager, 2011).

Normal arytenoid movement is symmetrical during adduction and abduction. Asymmetrical movement of the arytenoids as well as hyperfunction are noted by the clinician. During phonation, the ventricular folds should vibrate minimally and be relatively stationary and remain apart. Vocal hyperfunction refers to conditions of abuse and/or misuse of the vocal mechanism due to excessive and/or imbalanced muscular forces (Hillman, Holmberg, Perkell, Walsh & Vaughan, 1989). Anterior-posterior and mediolateral ventricular fold compression are rated from 0-5 (Figure 1.17). A rating of 0 indicating normal supraglottic activity and 5 indicating maximal abnormal supraglottic activity.

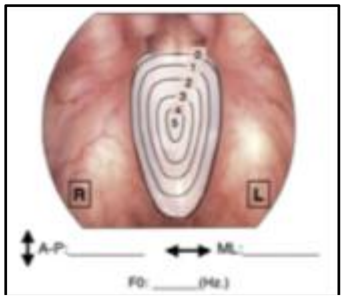


Figure 1.17 Supraglottic activity rating system
From VALI rating system (Poburka et al, 2016)

Free edge contour. Poberka et al. (2016) define free edge contour as the smoothness and/or straightness of free edge of the vocal folds. Evaluators are instructed to rate right and left vocal folds individually during abduction. Descriptions of free edge, which are accompanied by a corresponding image on the VALI rating form, include normal, convex, concave, irregular, and rough (Figure 1.18). Abnormal vocal fold edge may be due to histological alteration following chronic irritation or edema. Edematous vocal folds may have a convex appearance, whereas thinning vocal folds may have a concave appearance. Irritated vocal folds or those with lesions or other histological alterations may present with an irregular or rough appearance.

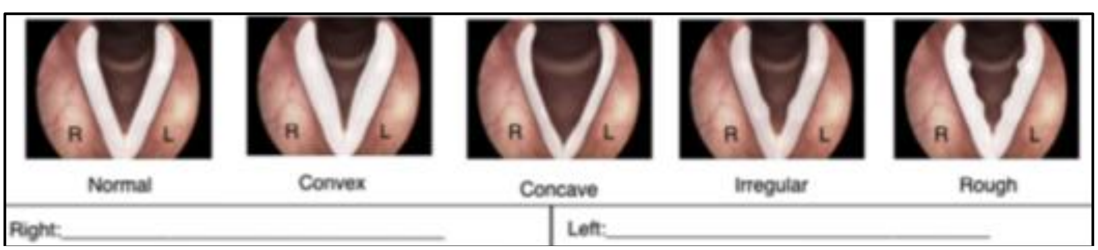


Figure 1.18 Free Edge Contour rating system
From VALI rating system (Poburka et al, 2016)

Phase closure. Phase closure is defined by Poberka et al. (2016) as the relative durations of appearance of consecutive glottal cycles. A glottal cycle consists of one open phase and one closed phase. In normal symmetrical phase patterns, the timing of the open phase and closed phase is almost equal. Symmetrical phase closure indicates that the folds have similar mechanical properties. When vocal quality is breathy or weak, as with hypoadduction or when lesions or bowing interfere with vocal fold closure, the open phase predominates. When vocal quality is harsh or strangled as with hyperadduction, the closed phase predominates.

VALI provides a continuum for rating phase closure. The clinician marks the point on the continuum where phase closure resides. The continuum includes three descriptions: phase closure when open phase predominates, phase closure when the phases are nearly equal, and phase closure when closed phase predominates. For phase closure that falls between two descriptions, the clinician identifies the appropriate point on the continuum, e.g., between nearly equal phase closure and when closed phase predominates. Phase closure is rated at the point of contact of the vocal folds (Figure 1.19).

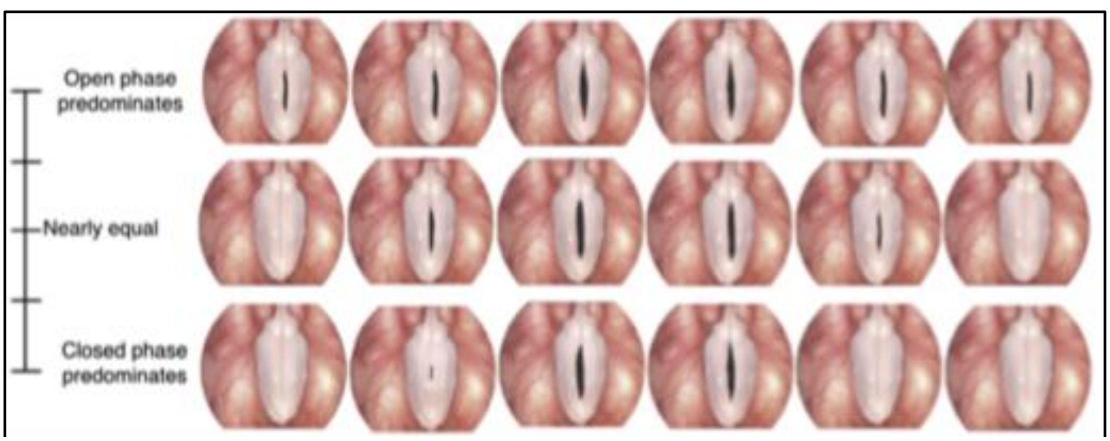


Figure 1.19 Phase Closure rating system
From VALI rating system (Poburka et al, 2016)

Phase Symmetry. Phase symmetry is defined as the degree to which the vocal folds move as mirror-opposite images of each other, or 180° phase difference (Poburka et al., 2016). In normal symmetrical phase patterns, the bilateral vocal fold movement is almost equal. Symmetry in phase indicates that the vocal folds have similar mechanical properties. The evaluator selects the percentage of exam time where the vibration is symmetrical. The form provides a visual description of asymmetry and symmetry (Figure 1.20). Phase asymmetry is indicative of unilateral changes in vocal fold tissue, e.g., lesions and edema.

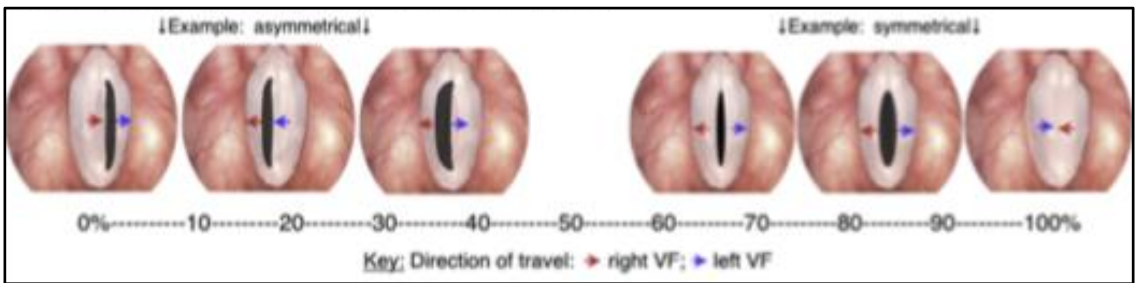


Figure 1.20 Phase Symmetry rating system
From VALI rating system (Poburka et al, 2016)

Regularity. Regularity is defined as the consistency of cycles or the type of phase closure observed (Poburka et al., 2016). The evaluator indicates the percentage of the exam that vibration is regular, from always regular to never regular. Lack of regularity in phase closure is indicative of vocal pathology, such as the presence of lesions or swollen tissue. This portion of the rating form includes images depicting regularity and irregularity (Figure 1.21).

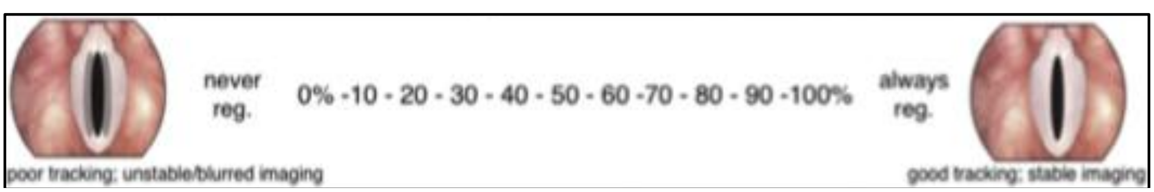


Figure 1.21 Regularity rating system
From VALI rating system (Poburka et al, 2016)

Nonvibratory observations. Poburka et al. defined nonvibratory observations as the structural observations not mentioned elsewhere. These include structural differences, such as asymmetry, erythema, vascularity, and mucus. Erythema refers to redness of the vocal folds and laryngeal structures. The presence of erythema is indicative of irritation, dehydration, and/or trauma of the tissue. Observations may involve the interarytenoid space, arytenoids, aryepiglottic folds, ventricular folds, ventricle, and epiglottis. Subjective evaluation of nonvibratory observations is rated from "within normal limits" or "none" to "severe" (Hirano & Bless, 1993) (Figure 1.23).

Parameters	VF	Rating			
Vocal fold (VF) varices	right	0 = wnl	1 = mild	2 = moderate	3 = severe
	left	0 = wnl	1 = mild	2 = moderate	3 = severe
VF edema	right	0 = no edema	1 = mild	2 = moderate	3 = severe
	left	0 = no edema	1 = mild	2 = moderate	3 = severe
VF discoloration	right	0 = pearl white appearance	1 = mild	2 = moderate	3 = profound
	left	0 = pearl white appearance	1 = mild	2 = moderate	3 = profound
VF mucus	right	0 = wnl	1 = mildly thick/thin and clear	2 = moderately thick/thin and mildly opaque	3 = severely thick/thin and moderately opaque
	left	0 = wnl	1 = mild	2 = moderate	3 = severe
Ventricular fold erythema	right	0 = wnl	1 = mild	2 = moderate	3 = severe
	left	0 = wnl	1 = mild	2 = moderate	3 = severe
Arytenoid Erythema	right	0 = wnl	1 = mild	2 = moderate	3 = severe
	left	0 = wnl	1 = mild	2 = moderate	3 = severe
Arytenoid edema	right	0 = wnl	1 = mild	2 = moderate	3 = severe
	left	0 = wnl	1 = mild	2 = moderate	3 = severe

*wnl = within normal limits

Figure 1.22 Nonvibratory observations rating form (*adapted from* Hirano & Bless, 1993).

Acoustic Analysis

Acoustic parameters provide objective and noninvasive measures of the function of laryngeal anatomy. These measures are successful at monitoring changes in voice quality over time. Among the vocal parameters assessed are Fundamental Frequency (F_0) and perturbation measures, including jitter and shimmer. Acoustic analysis software allows the clinician to record vocal productions for perceptual analysis and provides graphic imaging and statistical analysis of acoustic measurements.

F_0 provides information on the mass and stiffness of the vocal folds. F_0 is the acoustic correlate of pitch, or the perceived rate of vocal fold vibration. As F_0 changes, the listener perceives a change in pitch. F_0 refers to the frequency that occurs most often during phonation. It is the number of vocal fold phases (openings and closings) that occur in one second, as measured in Hertz (Hz) or cycles per second. A typical adult male will have a F_0 between 85 and 155 Hertz, while an adult female will have a F_0 between 165 to 255 Hertz. The norm for females ages 18-39 is 214.39 (SD 26.76), and the norm for males ages 18-39 is 106.41 (SD 13.29) (Shaefer et al., 2008). F_0 is determined by length, tension, and mass of the vocal folds (Boone et al., 2011).

Vocal perturbation measures include jitter and shimmer. Vocal perturbation refers to small changes in the vocal system. Perturbation measures provide strong measures of the basic perceptual elements of voice quality (Wolfe, Finch & Martin, 1997). Jitter refers to the abnormalities or variation in cycle-to-cycle signal frequency, which relates to pitch. The norm for males ages 18-39 for jitter is 1.41% (SD 0.32%) (Shaefer et al., 2008). The norm for females ages 18-39 for jitter is 0.82% (SD 0.28%) (Shaefer et al., 2008). Shimmer refers to the abnormalities in the amplitude of vocal fold vibration, which relates to intensity or loudness. The norm for males for shimmer is 2.14% (SD 0.32%) and the norm for females for shimmer is 1.59% (SD 0.68%) (Shaefer et al., 2008). Raised jitter and/or shimmer values may be indicative of changes that have occurred in the structure of the laryngeal epithelium and is a probable indicator for a physiological disorder (Wolfe, Cornell & Palmer, 1991; Hirano & Bless, 1993; Lourenco, Costa, & Filho, 2014).

Literature Review

A limited number of studies citing the long-term health effects of ENDS are available. This is likely due to the recent availability of ENDS products on the global market as well as a lack of regulations and standards governing ENDS production. Reports of adverse effects have included complaints of throat symptoms, including irritation of the throat during ENDS inhalation (Polosa et al., 2014). Additionally, similar types and levels of toxins to those in cigarette smoke have been found in ENDS aerosols. Despite evidence that ENDS may have an effect on the laryngeal structures, to the best of this clinician's knowledge, to date, no human studies and only one animal study on the effect of ENDS on the larynx has been published.

A study conducted by Salturk et al. (2015) assessed the effects of ENDS on the laryngeal mucosa of rats, with an additional focus on comparing effects of convection heat of ENDS aerosol to combustion heat produced by cigarette smoke on the larynx. This study assessed the histopathologic effects of ENDS on the vocal fold mucosa of 16 rats after exposure for 60 minutes per day for four weeks to ENDS with 0.9% (wt/vol) or 9 mg/ml nicotine. Rats were exposed to ENDS aerosol within a smoke drum, which contained two holes: one for inspiration and one for expiration. With the ENDS device placed in the inspiration hole, 200 mL/minute of aerosol entered the drum and was fully distributed within 20 minutes. At the end of four weeks, the rats were sacrificed, and each vocal fold was removed and examined histologically. Salturk et al (2015) found that ENDS may have effects on the vocal fold epithelium. The study found that epithelial responses, which included hyperplasia (increase in cell number) and metaplasia (change in cell type), were evident though not found to be significant.

Some gaps in this study by Salturk et al (2015) include the fact that exposure was a controlled application, which does not mirror use in humans. Humans typically use ENDS in approximately 5-minute intervals or sessions, consistent with cigarette use, and/or take single puffs frequently, potentially 100s of times, throughout the day. ENDS users puff deeply and directly from a device, hold the material briefly in the lungs and then exhale, rather than inhaling aerosol that is distributed through the air during patterns of normal respiration. The nicotine solution used in this study was 0.9% wt/vol. or (9 mg/ml), which is not representative of the wide range of nicotine levels that are currently consumed by human beings, e.g., Juul, which has 59 mg/ml. Additionally, the study did not list the battery voltage or e-liquid contents used to create the aerosol, which have been shown to alter the toxicity of ENDS aerosol (Kosmider et al., 2014; Rowell & Taran, 2015). Finally, this study was limited to four weeks duration and was also limited in the number of participants included.

While studies on the effects of ENDS on the larynx are limited to one, the effect of cigarette smoke is well studied. Reported histologic changes in the larynx in relation to smoking include atypical microscopic changes in the true vocal fold, false vocal folds, and the area above and below the level of the vocal folds, including epithelial hyperplasia and metaplasia (Auerbach et al., 1970; Levendoski, Leydon, & Thibeault, 2014; Gaafar & Al-Monsour, 1981). Smoking has been found to cause Reinke's edema, chronic laryngitis, and diffuse vocal fold polyps as well as triggering reflux that affects the larynx, termed laryngopharyngeal reflux (Öğüt & Kiliç, 2013; Stanciu & Bennett, 1972). Smoking leads to chronic irritation and can lead to an increase in vocal fold mass and thickening of vocal fold epithelium, as well as carcinomas in the epithelium (Kelleher, et al., 2014). Auerbach et al. (1970) found that effects of cigarette smoke on laryngeal epithelium were similar to previous findings of histologic change on bronchial epithelium. Similarly, studies have shown negative effects of ENDS on bronchial epithelium (Lerner et al., 2015), which further studies may find to be similar in laryngeal epithelium of ENDS users. Additionally, studies on the toxicity of ENDS reveal similar toxins to those found in cigarette smoke and, with sufficient battery output, ENDS aerosol has been shown to contain toxins at similar levels as well.

A study by Awan and Morrow (2007) compared the laryngeal characteristics of young adult female smokers versus nonsmokers using videostroboscopy. The goal of this study was to observe any early effects of smoking on the laryngeal structures and phonatory function. Participants included 34 nonsmoking females and 30 smoking females between the ages of 18 and 30 years old. Nonsmokers were operationally defined as participants who did not smoke and had not smoked for at least 5 years before the time of the study. Smokers were operationally defined as individuals who had smoked at least two cigarettes per day for at least one year. This study found a significant relationship between smoking and edema, erythema, and discoloration of laryngeal structures. It also found a significant relationship between abnormal phase symmetry and increased glottal gap size during the closed phase of phonation (Awan & Morrow, 2007).

A study conducted by Banjara, Mungutwar, Singh, and Gupta (2011) compared the larynges of smokers with that of non-smokers using videostroboscopy and acoustic voice analysis to determine the effects of smoking on the laryngeal anatomy and vocal physiology of smokers. The study's findings supported prior research that shows cigarette smoke has a significant impact on the larynx (Myerson, 1964; Auerbach, Hammond, & Garfinkel, 1970; Awan & Morrow; 2007; Banjara et al., 2011; Kelleher et al., 2014; Öğüt & Kiliç, 2013; Stanciu & Bennett, 1972).

A sampling of 50 male smokers and 50 male nonsmokers between the ages of 18 and 60 years participated in the study. Smokers were defined as any participant who, at the time of the study, had smoked at least two cigarettes per day for at least one year. Nonsmokers were defined as those who, at the time of the study, did not smoke and who had not smoked for five years before the study.

The researchers rated various aspects of the vocal folds as visualized on videostroboscopy using a rating system described by Hirano and Bless (1993). A criterion level of at least two judge agreements was required for a particular rating to be documented. Additionally, acoustic voice analysis and EGG measures were completed using Electroglottography. Acoustic analysis included the following parameters: fundamental frequency, frequency perturbation (shimmer), amplitude perturbation (jitter), F_0 tremor, normalized noise energy (NNE), harmonic-noise ratio (HNR), signal-noise ratio (SNR), amplitude tremor, maximum phonation time (MPT), and S/Z ratio. The following EGG parameters were evaluated: fundamental frequency, EGG-Jitter, EGG-

Shimmer, EGG F_0 tremor, contact quotient (CQ), contact index (CI), CQ perturbation, CI perturbation, opening rate and closing rate.

Banjara et al (2011) found a significant relationship between smoking and abnormal vocal fold edge, abnormal texture, edema, erythema, abnormal mucosal cover, abnormal phase symmetry and abnormal pliability/stiffness. Abnormal vocal fold edge in smokers included primarily swollen and rough vocal edge. These researchers found that the most common texture differences between smokers and non-smokers were mild to moderate roughness in smokers. This study also observed a highly significant relationship between the presence of edema and smoking as well as a significant relationship between increased erythema and smoking.

Banjara et al. (2011) observed a significant difference in abnormal mucosal cover in smokers as compared to nonsmokers. The most common mucosal cover abnormality observed in smokers was thick mucosal cover, which the study suggested was likely a result of the vocal folds chronic inflammatory response to cigarette smoke.

Banjara et al. (2011) observed a significant difference in abnormal phase symmetry in smokers as compared with nonsmokers. The most common abnormal phase symmetry observed in conventional smokers was asymmetrical phase symmetry (Banjara et al., 2011). Banjara et al. (2011) observed a significant relationship between changes in stiffness/pliability and smoking. Specifically, the study found mild to moderate stiffness in smokers. Researchers suggested this increase in stiffness was likely the result of long-standing edema caused by smoking. The study suggested that studies with younger participants may not see a significant relationship between smoking and stiffness due to long-standing edema occurring only after long term use.

The most common glottal closure patterns observed in the study were spindle gap and irregular glottal closure patterns; however, no significant difference was observed between abnormal glottal closure pattern findings.

Results of acoustic analysis in this study showed decreased values of F_0, significantly higher values of perturbation measures (jitter and shimmer), and significantly increased F_0 tremor (Banjara et al., 2011). These findings are consistent with other studies on the acoustic measures of cigarette smokers (Damborenea et al., 1999). The study also found raised values of NNE in smokers as compared to non-smokers, as well as lower values of SNR, and lower values of MPT. Finally, raised values of s/z ratio were discovered in smokers as compared to non-smokers in this study.

An important gap in this study by Banjara et al. (2011) as well as the study by Awan and Morrow (2007) is that while these studies provide a detailed analysis of cigarette smokers as compared to non-smokers, they do not compare smokers and non-smokers with ENDS users. ENDS use is rapidly increasing. ENDS are used by former smokers for tobacco cessation and by nonsmokers as the initial form of nicotine use (Kaisar et al., 2016). As ENDS are commonly used by both smokers and nonsmokers, comparing the laryngeal anatomy and physiology of ENDS users to smokers and nonsmokers is necessary and has the potential to provide valuable insight into the effects of ENDS on the larynx.

The purpose of the present study is to investigate the visual appearance and acoustic measures of the vocal folds and laryngeal structures of ENDS users as compared to smokers and nonsmokers. Because ENDS aerosol is inhaled in a similar manner and may contain similar types and levels of toxins as are present in cigarette smoke, it is likely that ENDS may have a similar effect on laryngeal anatomy and physiology. First, this study aims to discover whether acoustic analysis will reveal similarities in acoustic measures of ENDS users and smokers. It is hypothesized that ENDS users and smokers will experience a similar reduction in F_0 and a similar increase in perturbation measures. Second, this investigation aims to determine whether there are similarities in the mucosal wave and vibratory characteristics of ENDS users as compared to smokers. It is hypothesized that ENDS users and smokers will present with similarities in vibratory characteristics, including mucosal wave movement. Third, this study aims to investigate whether there are similarities in the visual appearance of the vocal folds of ENDS users as compared to smokers. It is hypothesized that the vocal folds of ENDS users will have a similar visual appearance to that of smokers. Finally, this investigation aims to discover whether similarities exist in the visual appearance of laryngeal structures of ENDS users and smokers. It is hypothesized that the laryngeal structures of ENDS users will have a similar visual appearance to that of smokers.

CHAPTER II

METHODS

The investigation, materials, and procedures for this study were approved by the Institutional Review Board (IRB) of Cleveland State University. This investigator recruited, screened, and collected data for all participants. All data were collected in the voice lab of the Cleveland State University Speech and Hearing Clinic. Informed consent was obtained from each participant after receiving a detailed explanation of the nature of the study (Appendix A).

A total of 20 healthy individuals participated in this study. Participants included 7 ENDS users and 4 cigarette smokers. A control group of 6 non-smokers was also included in this study. Participants' ages ranged from 18 to 35 years old, with an average of 28.14 (SD 7.10) years for ENDS users, an average of 30 (SD 2.45) years for smokers, and an average of 27.88 (SD 5.12) years for non-smokers. As far as possible, participants in each group were matched closely for age and gender. Table I lists the participants' characteristics in terms of age, gender, and grouping. Participants were recruited via word of mouth and flyers through the student investigator. The student investigator provided monetary compensation to those willing to participate and to those who helped to locate participants. Participation in this study included one sixty-minute session at Cleveland State University's voice lab located at the Center for Innovation in Medical Professions (CIMP). The entire period of data collection combined for all participants was approximately three months.

To meet the inclusionary criteria, participants had to be between 18 and 35 years old. Race was not a factor. Gender was only a factor in the investigator's attempts to match participants of a particular gender between groups. Based on their own personal account, participants were screened for conditions and daily habits known to cause visible changes in laryngeal anatomy and physiology, as well as changes in pitch and perturbation measures. All participants completed a questionnaire prior to data collection for the purpose of screening these areas (Appendix B). ENDS users and cigarette smokers were asked to use their devices or smoke within 15 minutes prior to data collection to minimize the variable of time since last use. All tasks were completed within the same visit using identical instructions (Appendix C and D), physical positioning, endoscope, and equipment.

For the purposes of this study, ENDS users were defined as individuals with daily ENDS use for at least one year and no cigarette use for at least 5 years prior to data collection. Smokers were defined as individuals with daily cigarette use of at least two cigarettes per day for at least one year with no prior ENDS use by the time of data collection. Nonsmokers were defined as individuals with no cigarette use for at least 5 years prior to data collection, and no history of ENDS use. Exclusionary criteria included: any diagnosis of a vocal fold pathology or GERD at the time of data collection, respiratory/sinus infections affecting laryngeal health within two weeks of data collection, a history of chronic overuse/abuse of the voice, and/or an overactive gag reflex.

Group	Gender		Mean Age	
	Female	Male	Age (years)	SD
ENDS	2	5	28.14	7.10
Smokers	2	2	30.00	2.45
Nonsmokers	4	2	27.88	5.12

Table I: Gender and mean age by group

Acoustic Analysis

Acoustic analysis provides information on the function of the laryngeal anatomy and an objective measure of the vibratory characteristics and patterns of the vocal folds and laryngeal structures (Hirano & Bless, 1993). Abnormal acoustic measurements are indicative of vocal fold pathologies. The voice parameters assessed in acoustic analysis included F_0, and vocal perturbation measures, including jitter and shimmer.

Quantitative measures were collected on each participant using the *Kaypentax Visi-pitch IV*. This is a clinical instrumentation tool used by speech-language pathologists for assessment, treatment, and research purposes. It provides auditory and visual feedback as well as objective data for measuring fundamental frequency, perturbation measures and other acoustic information. Abnormalities within these measures reflect dysphonia or dysfunction, and may be indicative of vocal pathologies (Lourenco, Costa, & Filho, 2014). The microphone was placed two inches from the participants' mouth while the participant sustained the vowel /a/ in a natural voice for at least five seconds according to given instructions (Appendix C).

Videostroboscopy

Videostroboscopy is used frequently by speech-language pathologists and otolaryngologists in assessment and treatment of vocal fold and laryngeal pathologies. *KayPentax 70-degree Rigid Strobolaryngoscope*™ model 9106 was used to complete a videostroboscopic assessment of the vocal folds of each participant. Topical anesthesia was not used for any of the participants. In this study, videostroboscopy was used to visualize and describe the laryngeal area of participating individuals.

The endoscope used in this study is a standard strobolaryngoscope, with mounted camera and microphone attachment. The tip of the scope contains a fiberoptic strobe light for observation of the mucosal wave. A rigid endoscope was placed just beyond the tongue base while the participant vocalized according to given instruction (Appendix D). Vocal patterns were recorded and saved on an audiovisual recording on the KayPentax equipment for further review of laryngeal structures and function. This investigator directly observed and reviewed participant's laryngeal structures and function according to the VALI rating form (Poberka et al., 2016). A criterion level of two judge agreements was required for a particular rating to be documented. The principal-investigator and student-investigator served as judges for this study. The principal-investigator is a licensed speech-language pathologist and the student-investigator is a second-year graduate student in speech-language pathology.

Questionnaire

Each participant completed a questionnaire prior to administration of instrumental evaluations (Appendix B). The questionnaire included screening questions used to assess whether participants met inclusionary criteria to be part of this study. The questionnaire also included questions regarding vocal habits and any known vocal pathologies. In addition, participants answered questions detailing ENDS or cigarette use. ENDS users provided information regarding device used, length of use, flavors of e-liquid, frequency of use, and factors that contributed to initial/current ENDS use. Each questionnaire is identical for all participants, regardless of use or lack of use of ENDS or cigarettes.

CHAPTER III

RESULTS

This study investigated the appearance and function of the vocal folds and laryngeal structures of ENDS users as compared to smokers and nonsmokers using videostroboscopic and acoustic analysis. Acoustic analysis included 3 sets of variables: fundamental frequency, jitter percent, and shimmer percent. The results of acoustic data were assessed using *SPSS Statistics 24* (IBM, Armonk, NY). Data collected were rated in comparison between groups, as well as to established norms for age and gender (Schaefer et al., 2008).

The results of videostroboscopic data were analyzed in a descriptive manner according to the VALI rating form (Poberka et al., 2016). Information gathered was then coded numerically in order to obtain data for comparison as described by Poburka et al. (2016). For example, for free edge contour, a rating of "normal" was coded as 1, "convex" was coded as 2, and so on, without these numbers otherwise containing any descriptive value. Additional observations noted on the VALI form, including discoloration, edema, erythema, and vascularity were rated from "within normal limits" to severe using a code described by Hirano and Bless (1993). For example, no abnormal presence of vocal fold edema was rated 0, mild edema was rated 1, moderate edema was rated 2, and severe edema was rated 3. Information gathered from acoustic analysis and videostroboscopy was assessed using *SPSS Statistics 24* (IBM, Armonk, NY). Videostroboscopic findings were then compared to acoustic data. The results of the self-assessment of ENDS users were used to assess whether any correlations could be made between self-assessment responses and findings from endoscopic and/or acoustic evaluation.

Acoustic Results

A one-way between subjects analysis of variance (ANOVA) was conducted to compare the effect of the nicotine device used, including ENDS, cigarettes, or non-use, on three acoustic measures: fundamental frequency, shimmer percent, and jitter percent. Fundamental frequency was assessed separately for male and female participants across the three groups (Tables II and III). Shimmer percent obtained from participants of both genders was compared across groups (Table IV). Jitter percent obtained from participants of both genders was also compared across groups (Table V). Finally, the number and percentage of ENDS users and smokers with normal and abnormal acoustic measures was presented in a table for comparison across groups (Table VI). All acoustic measures were collected at normal loudness and comfort level via Visi-pitch IV. The results are as follows:

Table II: Fundamental frequency measures using ANOVA among female ENDS users, smokers and non-smokers.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2140.187	2	1070.094	2.026	.277
Within Groups	1584.288	3	528.096		
Total	3724.475	5			

Table III: Fundamental frequency measures using ANOVA among male ENDS users, smokers and non-smokers.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	259.442	2	129.721	.493	.634
Within Groups	1578.982	6	263.164		
Total	1838.424	8			

Table IV: Shimmer Percent measures using ANOVA among all ENDS users, smokers and non-smokers.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.350	2	6.175	.916	.423
Within Groups	94.423	14	6.744		
Total	106.773	16			

Table V: Jitter Percent measures using ANOVA among all ENDS users, smokers and non-smokers.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.133	2	.566	2.058	.165
Within Groups	3.854	14	.275		
Total	4.987	16			

Table VI: Number and percentage of acoustic parameters rated as "normal" or "abnormal" compared to norms for age and gender for ENDS and smoker groups.

Rated Characteristic	ENDS		Smokers	
	Normal	Abnormal	Normal	Abnormal
Fundamental Frequency	7 (100%)	0 (0%)	3 (75%)	1 (25%)
Jitter Percent	5 (71%)	2 (29%)	2 (50%)	2 (50%)
Shimmer Percent	2 (29%)	5 (71%)	0 (0%)	4 (100%)

Comparison with norms for males and female nonsmokers, ages 18-39 years (Schaefer et al., 2008).
***Normal = within one SD of the mean; Abnormal = outside of one SD of the mean.**

No significant difference was observed between groups for any of the aforementioned acoustic parameters (significant at $P < 0.05$; marginally significant at $P < 0.09$) using ANOVA. Marginal significance is commonly used to describe data obtained from a small sample size. This indicates a pattern to be explored in future research. When compared to norms for nonsmokers of similar age and same gender, fundamental frequency and jitter percent values for ENDS users and smokers in the present study were comparable to norms (Schaefer et al., 2008); however, comparison revealed abnormal shimmer percent in a majority of ENDS users as well as smokers when compared to norms for nonsmokers (Appendix E).

Videostroboscopy Results

A one-way between subjects ANOVA was performed on data obtained using the VALI rating form and numerical coding of responses. The following areas were rated using VALI: glottal closure, amplitude, mucosal wave, vertical level, non-vibrating portion, supraglottic activity, free edge contour, phase closure, phase symmetry, and regularity. Non-vibrating portion was difficult to assess due to increased supraglottic activity in many participants. It was therefore removed from the list of parameters assessed in this study. Nonvibratory characteristics, which are a parameter assessed on VALI and distinct from non-vibrating portion, were noted on the VALI form during and after completion of videostroboscopy upon review of recorded video. Information gathered for each participant was then coded numerically for assessment using a one-way between subjects ANOVA.

ANOVA determined significant interactions between means of data collected using the VALI rating form (Table VII) and between means of data collected from descriptions of nonvibratory characteristics (Table IX). A Tukey Post Hoc test was then used to determine which relationships between the three groups were significant in the aforementioned areas (Tables VIII and X).

		Sum of Squares	df	Mean Square	F	Sig.
Glottal_closure	Between Groups	13.045	2	6.522	1.214	.326
	Within Groups	75.190	14	5.371		
	Total	88.235	16			
Amplitude	Between Groups	1.070	2	.535	.574	.576
	Within Groups	13.048	14	.932		
	Total	14.118	16			
Mucosal_Wave	Between Groups	17.025	2	8.513	4.794	.026
	Within Groups	24.857	14	1.776		
	Total	41.882	16			
Supraglottic_Activity	Between Groups	1.429	2	.715	1.815	.199
	Within Groups	5.512	14	.394		
	Total	6.941	16			
Vertical_Level	Between Groups	.000	2	.000	.	.
	Within Groups	.000	14	.000		
	Total	.000	16			
Free_edge	Between Groups	7.738	2	3.869	5.278	.020
	Within Groups	10.262	14	.733		
	Total	18.000	16			
Phase_Closure	Between Groups	81.530	2	40.765	6.011	.013
	Within Groups	94.940	14	6.781		
	Total	176.471	16			
Phase_Symmetry	Between Groups	1453.431	2	726.716	4.314	.035
	Within Groups	2358.333	14	168.452		
	Total	3811.765	16			
Regularity	Between Groups	951.261	2	475.630	3.107	.076
	Within Groups	2142.857	14	153.061		
	Total	3094.118	16			

Table VII: ANOVA comparison of videostroboscopic parameters among ENDS users, smokers, and non-smokers assessed using VALI rating form (Poberka et al., 2016).

Dependent Variable	(I) Nicotine_device_used	(J) Nicotine_device_used	Mean Difference (I-J)	Std. Error	Sig.
Mucosal_Wave	ENDS	Cigarettes	-.14286	.83518	.984
		None	-2.14286*	.74132	.030
	Cigarettes	ENDS	.14286	.83518	.984
		None	-2.00000	.86011	.085
	None	ENDS	2.14286*	.74132	.030
		Cigarettes	2.00000	.86011	.085
Free_edge	ENDS	Cigarettes	.71429	.53662	.402
		None	1.54762*	.47632	.015
	Cigarettes	ENDS	-.71429	.53662	.402
		None	.83333	.55264	.317
	None	ENDS	-1.54762*	.47632	.015
		Cigarettes	-.83333	.55264	.317
Phase_Closure	ENDS	Cigarettes	1.60714	1.63222	.598
		None	-3.80952*	1.44880	.049
	Cigarettes	ENDS	-1.60714	1.63222	.598
		None	-5.41667*	1.68095	.016
	None	ENDS	3.80952*	1.44880	.049
		Cigarettes	5.41667*	1.68095	.016
Phase_Symmetry	ENDS	Cigarettes	2.50000	8.13497	.949
		None	-18.33333	7.22080	.058
	Cigarettes	ENDS	-2.50000	8.13497	.949
		None	-20.83333	8.37786	.064
	None	ENDS	18.33333	7.22080	.058
		Cigarettes	20.83333	8.37786	.064

*. The mean difference is significant at the 0.05 level.

Table VIII: Tukey multiple comparison of videostroboscopic parameters with significant relationships on ANOVA among ENDS users, smokers, and non-smokers assessed using VALI rating form (Poberka et al., 2016).

Abnormal mucosal wave, free edge, and phase closure show significant relationships in ENDS users as compared to nonsmokers (significant at $P < 0.05$). Reduction in mucosal wave was consistently noted to occur in the presence of increased edema and observed thick mucosal cover (Figure 3.1). Abnormalities in free edge were most commonly convex or irregular (Figure 3.2). Close phase predominating was the most frequently occurring form of abnormal phase closure noted in ENDS users and was also consistently noted with the observed presence of edema. Abnormal phase symmetry and regularity show a marginally significant relationship in ENDS users as compared to nonsmokers (marginally significant at $P < 0.09$) (Figure 3.3), and again consistently noted with the observed presence of edema.

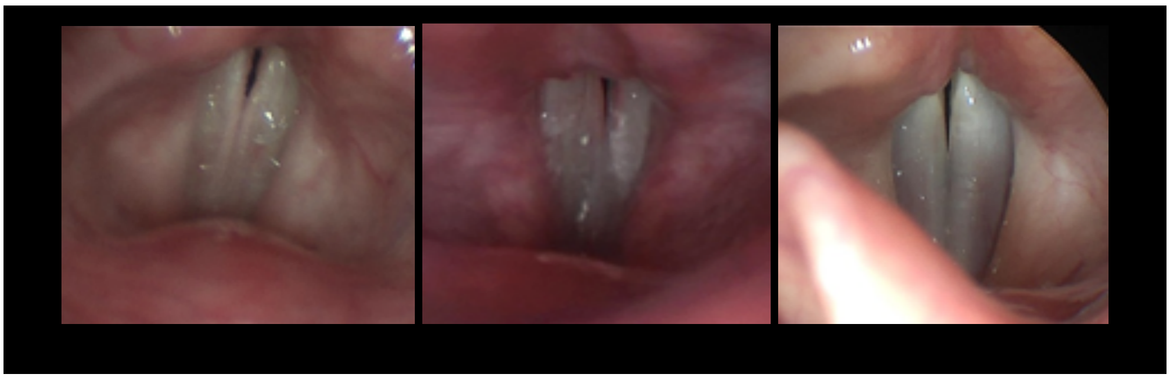


Figure 3.1: Increased edema and thick mucosal cover of ENDS users resulting in a reduction in mucosal wave (left and middle); vocal folds of nonsmoker with normal mucosal wave (right).

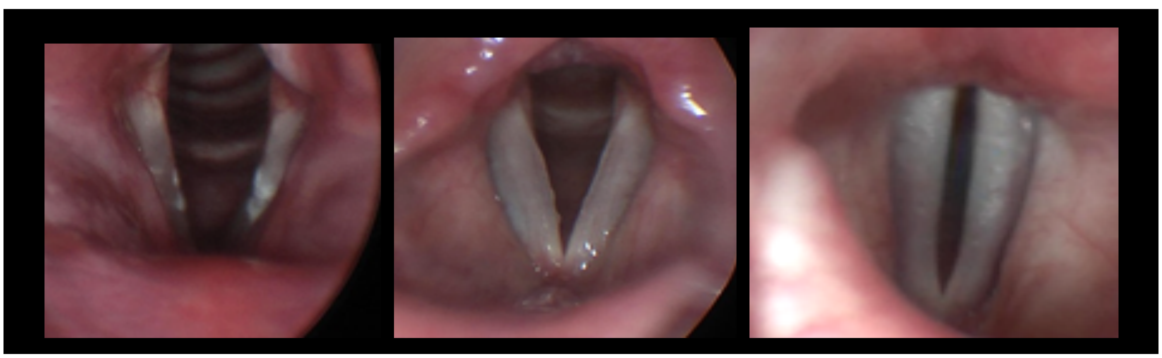


Figure 3.2: Irregular (left), convex (middle) abnormalities in free edge contour of ENDS users; normal free edge contour of nonsmoker (right)

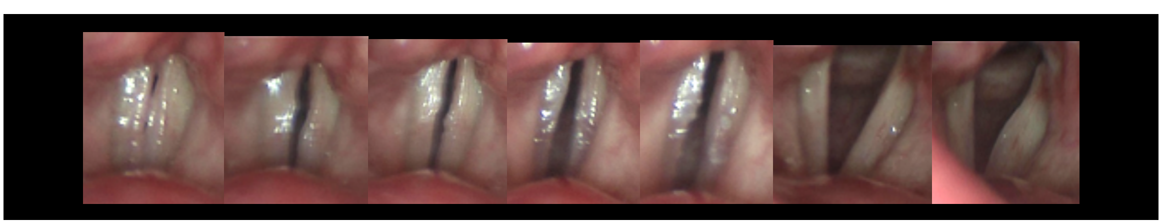


Figure 3.3: Abnormal phase symmetry in ENDS user

Abnormal phase closure also shows a significant relationship in smokers as compared to nonsmokers. Abnormal mucosal wave and phase symmetry shows a marginally significant relationship in smokers as compared to nonsmokers. Abnormal free edge was evident though not significant in smokers. No significant relationship was observed for glottal closure, amplitude, supraglottic activity, and vertical level; however, a pattern of abnormal supraglottic activity was evident in ENDS users as well as smokers (see Figure 3.4).

Figure 3.4: Abnormal supraglottic activity (top row) in ENDS users (left and middle) and smoker (right) and normal supraglottic activity (bottom row) in nonsmokers (left and middle) and ENDS user (right).

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
VF_varices	Between Groups	5.356	2	2.678	5.544	.017
	Within Groups	6.762	14	.483		
	Total	12.118	16			
VF_edema	Between Groups	12.723	2	6.362	11.856	.001
	Within Groups	7.512	14	.537		
	Total	20.235	16			
VF_Discolor	Between Groups	8.108	2	4.054	3.826	.047
	Within Groups	14.833	14	1.060		
	Total	22.941	16			
FVF_erythema	Between Groups	6.501	2	3.250	3.658	.053
	Within Groups	12.440	14	.889		
	Total	18.941	16			
Aryt_erythema	Between Groups	2.232	2	1.116	1.383	.283
	Within Groups	11.298	14	.807		
	Total	13.529	16			
Aryt_edema	Between Groups	.158	2	.079	.197	.824
	Within Groups	5.607	14	.401		
	Total	5.765	16			
VF_mucus	Between Groups	3.368	2	1.684	2.017	.170
	Within Groups	11.690	14	.835		
	Total	15.059	16			

Table IX: ANOVA comparison of nonvibratory observations among ENDS users, smokers, and non-smokers subjectively assessed according to a rating system adapted from Hirano and Bless (1993) (see Figure 1.22)

Dependent Variable	(I) Nicotine_device_used	(J) Nicotine_device_used	Mean Difference (I-J)	Std. Error	Sig.
VF_varices	ENDS	Cigarettes	-.28571	.43560	.792
		None	1.04762*	.38665	.042
	Cigarettes	ENDS	.28571	.43560	.792
		None	1.33333*	.44861	.026
	None	ENDS	-1.04762*	.38665	.042
		Cigarettes	-1.33333*	.44861	.026
VF_edema	ENDS	Cigarettes	.53571	.45912	.491
		None	1.95238*	.40753	.001
	Cigarettes	ENDS	-.53571	.45912	.491
		None	1.41667*	.47283	.024
	None	ENDS	-1.95238*	.40753	.001
		Cigarettes	-1.41667*	.47283	.024
VF_Discolor	ENDS	Cigarettes	-1.00000	.64517	.299
		None	.83333	.57267	.341
	Cigarettes	ENDS	1.00000	.64517	.299
		None	1.83333*	.66443	.038
	None	ENDS	-.83333	.57267	.341
		Cigarettes	-1.83333*	.66443	.038
FVF_erythema	ENDS	Cigarettes	-.60714	.59084	.572
		None	.97619	.52445	.186
	Cigarettes	ENDS	.60714	.59084	.572
		None	1.58333	.60848	.051
	None	ENDS	-.97619	.52445	.186
		Cigarettes	-1.58333	.60848	.051

*. The mean difference is significant at the 0.05 level.

Table X: Tukey multiple comparison of nonvibratory observations with significant relationships on ANOVA among ENDS users, smokers, and non-smokers subjectively assessed according to a rating system adapted from Hirano and Bless (1993) (see Figure 1.22).

ANOVA revealed significant relationships between groups and abnormal vocal fold varices, vocal fold edema, vocal fold discoloration, and ventricular fold erythema (Table IX). A Post-Hoc Tukey analysis revealed which relationships were significant (Table X): Abnormal varices is characterized by capillary dilation or leakage. Abnormal varices showed a significant relationship in both smokers and ENDS users when compared to the nonsmoker group (Figure 3.5). Abnormal vocal fold edema is characterized by unilateral or bilateral inflammation in the superficial layer of the vocal folds, including Reinke's space (see Figure 1.7). Abnormal vocal fold edema showed a highly significant relationship in ENDS users as compared to nonsmokers (highly significant at P < .001). Abnormal vocal fold edema showed a significant relationship in smokers as compared to nonsmokers (Figure 3.6).

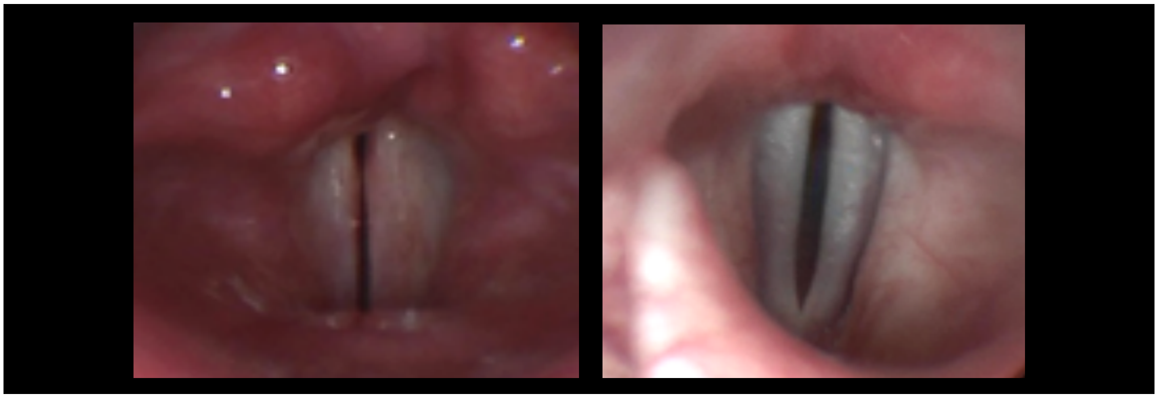


Figure 3.5: Vocal fold vascularity in smoker (left) and no vocal fold vascularity in nonsmoker (right)

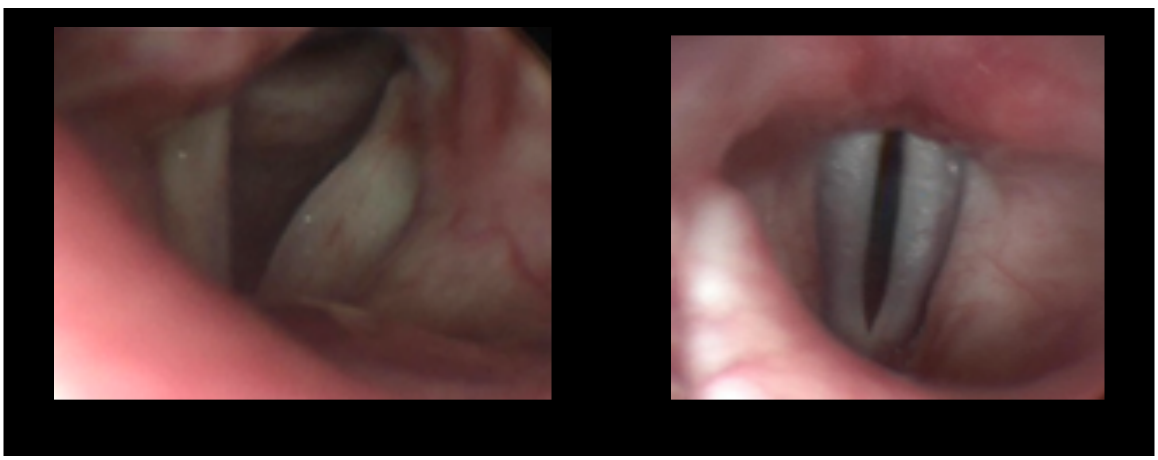


Figure 3.6: Unilateral edema of reinke's space in ENDS user vibrating upon inhalation (left); no abnormal edema in nonsmoker (right)

Vocal fold discoloration is noted when vocal folds have a yellow or pink appearance, rather than the pearl-white appearance characteristic of healthy vocal folds. A significant relationship was noted between vocal fold discoloration and cigarette use when compared to the nonsmoker group, while a relationship was evident though not significant between vocal fold discoloration and ENDS use (Figure 3.7).

Ventricular fold erythema is characterized by reddening of the tissue due to irritation. A marginally significant relationship was found between abnormal ventricular fold erythema and smokers when compared to nonsmokers (Figure 3.8); however no significant relationship was found between abnormal ventricular fold erythema and ENDS use. Additionally, no significant relationship was observed for abnormal arytenoid erythema, arytenoid edema, and vocal fold mucus between both ENDS users and smokers as compared to nonsmokers; however, a pattern of thick, white mucus was observed on the vocal folds of three of seven ENDS users, which was not observed on the vocal folds of smokers and nonsmokers (Figure 3.9).

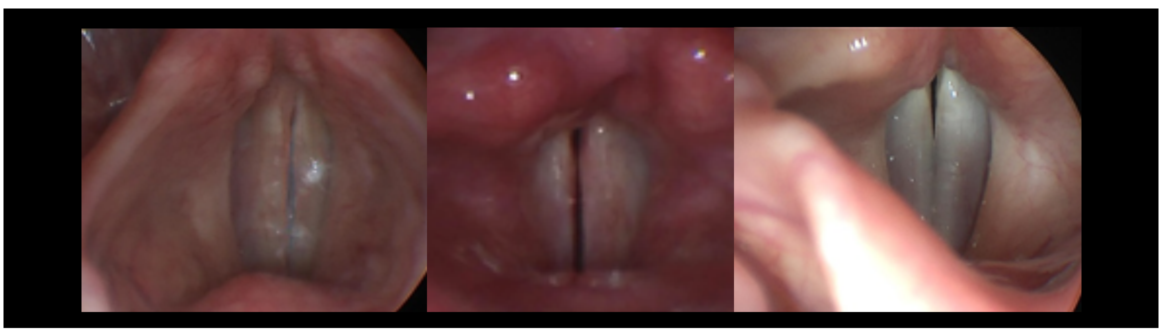


Figure 3.7: Yellowed appearance of vocal folds of ENDS user (left), reddened appearance of vocal folds of smoker (middle), and pearl white appearance of vocal folds of nonsmoker (right).

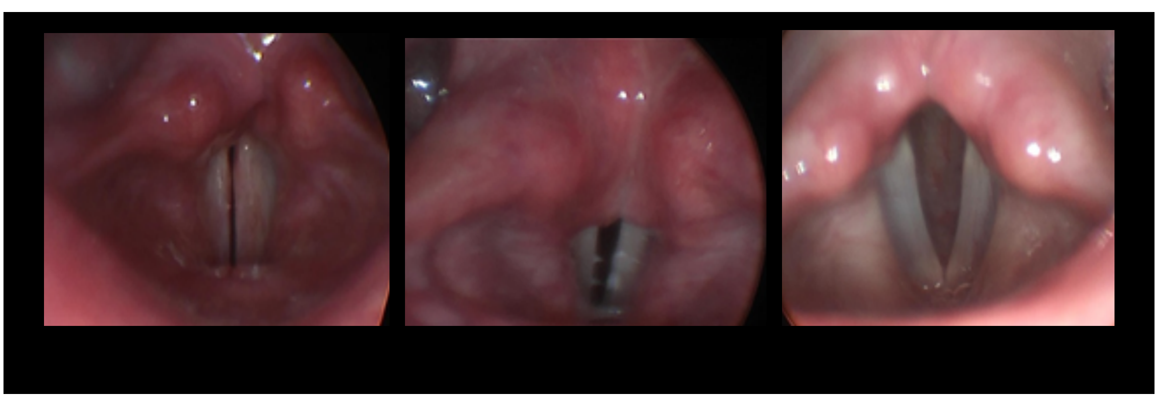


Figure 3.8: Severe ventricular fold erythema in smoker (left); moderate to severe ventricular fold erythema in ENDS user (middle); and no abnormal erythema in nonsmoker (right)

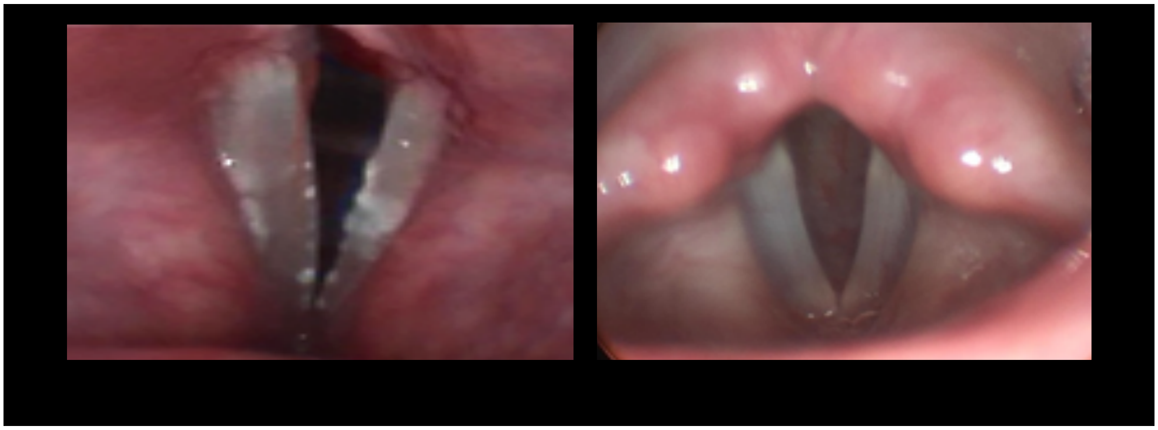


Figure 3.9: thick, white mucus of ENDS user (left); thin, clear mucus of nonsmoker (right)

Questionnaire Results

All responses provided by ENDS participants on a general questionnaire were recorded and entered into a table for comparison (Table XI). Participants reported their age, device used, duration of use in years, flavors used, frequency of use (in times per day), and contributing factors in their decision to begin and continue using ENDS.

According to ENDS user responses provided on the general questionnaire, participants used ENDS for an average duration of 3.64 (SD 2.06) years. Those who reported using ENDS because they are trendy had used ENDS for three years or less. Five of seven participants report using ENDS because they believe it is a safer nicotine alternative to smoking cigarettes. Of seven total ENDS users, three reported using sweet flavored e-liquids, three reported using menthol or mint flavored e-liquids, and one reported using tobacco flavored e-liquids. Five of seven ENDS users report using 3rd or current generation devices. Finally, five of seven ENDS users report using ENDS greater than 20 times (sessions) throughout the day.

Table XI: ENDS users' individual questionnaire responses

Age (years)	Device*	length of use (years)	Flavor	Frequency**	Factors
32	1,2	5	sweet	>20x/day	safer
31	3	6	tobacco	>20x/day	safer; indoor use
35	3	3	sweet	>20x/day	safer; flavors
19	4	1	menthol	3-10x/day	safer; trendy
35	3,4	6	sweet	10-20x/day	safer; flavors
25	1	1.5	menthol	>20x/day	trendy
18	4	3	menthol	>20x/day	trendy

*Devices: 1 = first generation, 2 = second generation, 3 = third generation, 4 = current generation
**Frequency: Sessions (x)= five minutes or less of ENDS use.

CHAPTER IV

DISCUSSION

Research Question 1: Are there similarities between acoustic measures of ENDS users and smokers?

Results indicated that no significant relation was observed between acoustic measures obtained from smokers and ENDS users; however, due to small sample size, findings were also compared to norms for age and gender. When acoustic measures of smokers and ENDS users were compared to norms, results revealed similarities in one acoustic measure of ENDS users and smokers. Both showed raised shimmer percent in a majority of participants. Increased shimmer percent is indicative of vocal pathologies, such as altered tissue due to chronic irritation. This finding is consistent with prior research on acoustic measures in smokers (Banjara et al., 2011; Damborenea et al., 1999). Irritation and epithelial changes resulting from exposure to irritants and cytotoxic compounds may be responsible for the observed increase in shimmer percent in ENDS users (Buron et al., 2009; U.S. EPA, 2003; Arts et al., 2004; Farsalinos et al., 2013; Rowell & Taran, 2015).

When compared to norms, no notable difference was observed in fundamental frequency and jitter values. This finding is not consistent with prior research on acoustic measures in smokers (Banjara et al., 2011; Damborenea et al., 1999). Fundamental frequency changes due to smoking are attributed to increased mass due to swelling and are most frequently observed in females rather than males (Banjara et al., 2011; Damborenea et al., 1999; Gilbert & Weismer, 1974). This is likely due to females having a smaller range of what is perceptually "normal" pitch as compared to males. It is likely that the female sample size in both the ENDS and smoker groups in the present study was too limited to accurately investigate whether a comparison exists with regard to a reduction in F_0.

Research Question 2: Are there similarities in the vibratory characteristics, including mucosal wave, of ENDS users as compared to smokers?

Results indicate that there are similarities in the mucosal wave and vocal fold vibratory characteristics of ENDS users and cigarette smokers. In the present study, a significant relationship was found between abnormal mucosal wave and ENDS use. A marginally significant relationship was also found between abnormal wave and cigarette smoking. As the mucosal wave is caused by movement of the mucosal cover and may be reduced when the mucosal cover is altered, this finding appears to be consistent with prior studies that have found thickened mucosal cover in smokers (Banjara et al., 2011). It is speculated that abnormal mucosal wave in ENDS users in the present study may be due to similar changes in mucosal cover caused by chronic irritation due to inhalation of ENDS aerosol.

Abnormal phase closure showed a significant relationship in both ENDS users and smokers as compared to nonsmokers in the present study. Among abnormal phase closure, the most common form noted in both ENDS users and smokers was phase closure with closed phase predominating. Phase closure with closed phase predominating may be caused by hyperfunction of laryngeal structures in response to irritation or swelling, as well as tissue changes, such as edema or the presence of lesions causing abnormalities in phase closure. In the present study, abnormal phase closure was frequently associated with increased bilateral or unilateral edema, especially in ENDS users. This may be the result of tissue changes caused by chronic irritation from ENDS aerosol similar to the tissue changes caused by chronic irritation from cigarette smoke (Salturk et al., 2015; Sataloff, 2005).

In the current study, a marginally significant relationship was found between abnormal phase symmetry and both ENDS use and smoking. This relationship between abnormal phase symmetry and smoking is consistent with prior research, which associated abnormal phase symmetry with the presence of vocal fold edema (Banjara et al., 2011; Awan & Morrow, 2008). Abnormal phase symmetry in ENDS users may be attributed to edematous vocal folds as well, or to other tissue changes that have caused the vocal folds to have different mechanical properties.

A marginally significant relationship was found in the present study between abnormal regularity and both ENDS use and smoking. This finding supports prior research on the effect of smoking on vocal fold vibration (Banjara et al., 2011; Awan & Morrow, 2008). The most frequent abnormal regularity in both groups was frequent inconsistency of cycles observed followed by inconsistency in the type of phase closure observed. As with asymmetry of phase, abnormality in regularity is indicative of differences between the mechanical properties of the two vocal folds. Vocal folds that have similar mechanical properties vibrate as mirror images of one another with observable cycle and phase closure consistency. This abnormal finding reflects probable changes in vocal fold tissue of ENDS users, which may be a result of chronic irritation from toxins present in ENDS aerosol (Buron et al., 2009; U.S. EPA, 2003; Arts et al., 2004; Farsalinos et al., 2013; Rowell & Taran, 2015).

In the current study, no significant relationship was observed between abnormal glottal closure and smoking or ENDS use. Earlier studies have mixed results regarding the effect of smoking on glottal closure. Banjara et al. (2011) found no significant difference between abnormal glottal gap findings between smoking and nonsmoking groups; however, Awan and Morrow (2007) found a significant difference, attributing observed differences in glottal closure to irritation of the arytenoid cartilages caused by smoking. The most frequently occuring glottal closure patterns for both ENDS and smoker groups was posterior gap followed by variable closure patterns. Posterior gap is a normal closure pattern. Variable closure patterns in ENDS users may be due to chronic irritation from ENDS aerosol altering the mechanical properties of the vocal folds.

No significant relationship was found in the present study between abnormal amplitude and ENDS or cigarette use. Additionally, this study found no significant relationship between abnormal vertical level and chronic ENDS aerosol or cigarette smoke exposure. Finally, no significant relationship was found between abnormal supraglottic activity and the smoker group. Prior research on supraglottic activity in smokers is consistent with the present finding in smokers. Similarly, no significant relationship was found between ENDS use and abnormal supraglottic activity; however, a pattern of both static and dynamic supraglottic activity apparently unrelated to gag response was observed in ENDS users. This supraglottic activity was consistent with observations of supraglottic edema. Abnormal supraglottic activity may be due to compensation of laryngeal structures in response to edematous and irritated vocal folds.

Research Question 3: Are there similarities in the visual appearance of the vocal folds of ENDS users as compared to smokers?

Results indicated that there are similarities in the visual appearance of the vocal folds of ENDS users and cigarette smokers, including abnormal vocal fold edge contour, varices, edema, and discoloration. In the current study, a significant relationship was found between abnormal vocal fold edge contour and ENDS use when compared to nonsmokers. Abnormal free edge may be the result of microscopic tissue changes following chronic irritation or edema, or due to the presence of lesions. Abnormal free edge contour was evident though not significant in smokers as compared to nonsmokers. This finding is consistent with prior research on the effect of smoking on the larynx (Banjara et al., 2011; Awan & Morrow, 2008). Banjara et al. (2011) suggested that abnormal free edge findings in smokers is caused by chronic irritation of the vocal folds resulting in histological changes in vocal fold epithelium as described by Sataloff (2005). Salturk et al. (2015) discovered epithelial responses, including hyperplasia and metaplasia, evident in the vocal folds of ENDS users. The observed changes in vocal fold edge contour in ENDS users may be the result of similar alterations in vocal fold epithelium caused by chronic irritation from ENDS aerosol.

In the present study, a significant relationship was observed between vocal fold varices and both ENDS use and smoking. The appearance of vascularity is characterized by capillary dilation or leakage, or thin red lines/veins on the vocal folds. Varices is known to accompany edema and is caused by irritation, including smoking and chemical exposure (Banjara et al., 2014). The current study's finding of a significant relationship between varices and smoking is consistent with prior studies (Banjara et al., 2014). It may be that the presence of vascularity in the vocal folds of ENDS users is also caused by chronic irritation due to exposure to mucous membrane and epithelial irritants within e-liquids and aerosol (Callahan-Lyon, 2014; Buron et al., 2009; U.S. EPA, 2003; Arts et al., 2004; Farsalinos et al., 2013; Rowell & Taran, 2015).

Abnormal vocal fold edema showed a highly significant relationship in ENDS users as compared to nonsmokers. A significant relationship was also found between the presence of abnormal edema and smoking as compared to nonsmokers. The most frequently observed vocal fold edema was the appearance of unilateral and bilateral edema in the superficial lamina propria as well as the epithelium of ENDS users and smokers. This edema was observed to cause reduced movement of the mucosal wave, as well as to cause tissue within Reinke's space to become more pliable and vibrate differently (see Figure 1.7). Reinke's edema is accumulation of fluid or swelling in Reinke's space due to chronic irritation and is highly correlated with chronic smoking (Jain et al., 2009). The presence of possible Reinke's edema in ENDS users in the present study suggests that effects of ENDS aerosol on the tissue of the superficial lamina propria are similar to that of cigarette smoke. Of note, one ENDS participant with significant vocal fold edema presented with audible inhalation consistent with stridor and was referred to an otolaryngologist. Stridor is the involuntary production of noise upon inhalation and is a symptom of airway obstruction.

The present study found a significant relationship between vocal fold discoloration and smoking. This finding is consistent with a prior study that found increased discoloration of laryngeal structures in smokers (Awan & Morrow, 2008). Vocal fold discoloration was evident though not significant in ENDS users. A healthy mucosal cover gives the vocal folds their pearl-white appearance. Vocal fold discoloration indicates changes in the tissue of the mucosal cover, consisting of the epithelium and superficial lamina propria or Reinke's space. Swelling, irritation, dehydration, and trauma to this area may cause vocal fold discoloration. It may be that the discoloration evident in the vocal folds of ENDS users is due to dehydration, swelling, or irritation, similarly to the effect of cigarette smoke on the mucosal cover. Although ENDS aerosol is not produced by combustion as is cigarette smoke, toxins within ENDS aerosol have known irritative and dehydrating qualities (Callahan-Lyon, 2014; Buron et al., 2009; U.S. EPA, 2003; Arts et al., 2004; Farsalinos et al., 2013; Rowell & Taran, 2015).

Research Question 4: Are there similarities in the visual appearance of laryngeal structures of ENDS users as compared to smokers?

The present study found a marginally significant increase in the presence of ventricular fold erythema in smokers as compared to nonsmokers. This finding is consistent with prior research that showed a significant increase in the presence of ventricular fold erythema in smokers as compared to nonsmokers (Banjara et al., 2011). No significant relationship was found between ventricular fold erythema and ENDS use; however, due to the small sample size of this study, it remains possible that a relationship may be found in studies with a larger sample.

In the current study, no significant relationship was observed between smoking or ENDS use and arytenoid erythema or arytenoid edema; however, past studies have found a significant relationship between smoking and arytenoid edema (Awan & Morrow, 2007). This discrepancy is likely due to the small sample size of this study. Additionally, no significant relationship was found between smoking or ENDS use and vocal fold mucus; however, a pattern of of thick, white mucus was observed on three of seven ENDS users, which was not observed on the vocal folds of smokers and nonsmokers. Thick, opaque mucus may be caused by vocal fold tissue dehydration, irritation, or other vocal pathology (Hsiung, 2004; Bonilha, White, Kuckhahn, Gerlach & Deliyski, 2012). The toxins within ENDS aerosol have known irritative and dehydrating qualities; therefore, the presence of thick, white mucus on the vocal folds of ENDS users could be indicative of a vocal pathology. More research is necessary to be conclusive in this area.

Conclusion

The purpose of the present study was to compare the laryngeal anatomy and physiology of ENDS users, smokers, and nonsmokers to determine whether similarities exist in the effect of ENDS aerosol as compared to cigarette smoke. In spite of the small sample size of this study, a pattern of similar changes in the appearance and function of the laryngeal anatomy was observed in ENDS users and smokers. These preliminary results suggest similarities may exist between the effect of ENDS use and smoking on the vocal folds; however, studies of much larger sample size are required to truly evaluate effects of ENDS on the vocal folds and make definitive determinations of safety. There have been anecdotal suggestions by ENDS companies that ENDS is a safer alternative to smoking. While this may be the case, based on seminal findings of this study, it may be beneficial for individuals to exercise caution in ENDS use as they would with cigarette use.

Limitations and Future Research

The present study has the following limitations. First, the sample size of each group was very small, despite a concerted effort to find greater numbers of participants. Recruiting participants for the ENDS group with no history of prior cigarette or other tobacco use within five years prior to data collection proved difficult. Many participants were excluded due to occasional cigarette or marijuana use. Second, the nonsmoking group primarily consisted of college students, which may present a confounding variable. Many college students are known to engage in behaviors and experiences that negatively affect the health and hygiene of the larynx, including academic stress, and poor nutrition, hydration, and sleep habits. Further studies should consider including specific questions regarding these behaviors on screening questionnaires.

Third, participants were not grouped by device type, frequency of use, battery voltage, or e-liquids used. Participants were frequently unable to determine battery voltage in order to provide information on the questionnaire. Due to the fact that research indicates these variables may impact the toxicity of ENDS, further research should control for these variables. Unfortunately, until ENDS are regulated and standardized, controlling for these variables would require testing of each device and e-liquids used rather than reliance on labeling by ENDS companies.

Fourth, participants were unable to provide details on the length of time or number of inhalations per vaping session with confidence, and thus, frequency was often determined by the number of sessions per day rather than number of inhalations. Future research should include an operational definition for frequency of use rather than depending on subjective determination by the individual participant.

Finally, this study obtained videostroboscopic data using a rigid strobolaryngoscope. Studies have shown that the type of laryngoscope used may impact the amount of supraglottic activity observed on videostroboscopy. As participants did not receive numbing spray to reduce gag reflex, it is difficult to determine whether supraglottic activity observed is a result of cigarette or ENDS use, or whether it is due to an activated gag reflex. Future studies should consider the use of a rhinolaryngoscope in videostroboscopy, as evidence shows this scope is less likely to activate a gag reflex.

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Appendix A
Informed consent

Informed Consent

My name is Hilary Sample. I am a graduate student at Cleveland State University in the speech and hearing program. I am requesting your participation in a research study. If you have any questions, please feel free to contact my advisor, Dr. Violet Cox. She can be contacted by phone at (216) 687-6909 or by email at v.cox@csuohio.edu.

The study aims to visually describe the structures of the larynx of individuals who use electronic nicotine delivery systems versus smokers and non-users. If you decide to participate in this study, you will be asked to do three things: complete a questionnaire; complete assessment using the Visi-Pitch IV; and complete assessment using videostroboscopy. With the Visi-Pitch IV, you will be asked to say "ah" into a microphone for about five seconds in a natural voice. Your larynx will then be assessed for two to three minutes using videostroboscopy. During videostroboscopic evaluation, you will be asked to open your mouth and stick out your tongue. Gauze will be used to gently hold your tongue. A rigid endoscope, which is similar to a tongue depressor in feel and placement, will be inserted into your mouth to the back of the tongue. You will be asked to say the vowels "ah" and "ee" at differing pitches. The examination is not painful, but there is a possibility that you may gag because of the placement of the scope. If the gag continues, the procedure will end. The total time involved for the entire process is less than one hour. To participate, you must be between 18 and 35 years of age.

Risks associated with participation are considered to be minimal. Such risks are largely limited to compromised confidentiality. To minimize such risks, all research documents will be secured in a locked file cabinet in Dr. Violet Cox's office at Cleveland State University, and in the password protected hard drives of the Visi-pitch IV and videostroboscopy in the CSU Voice and Swallowing Lab in room 211. You may withdraw from this study at any time without any consequence whatsoever. Only summary results may be published, presented or used for instruction. No personal identifiers will be included in such data. You will be given $10 for participating in the study upon arrival to the voice lab; however, there are no other direct benefits available to you as a participant in this research.

A copy of this Informed Consent will be provided to you for your records.

Please read the following: ***"I understand that if I have any questions about my rights as a research subject, I can contact the Cleveland State University Institutional Review Board at (216) 687-3630."***

Your signature below means that you understand the contents of this document. You also are at least 18 years of age. Finally, you voluntarily consent to participate in this research study.

____ _______________________

Signature Date

____

Name (Printed)

Appendix B
Questionnaire

Screening Questionnaire for Research Participants

Name: ________ Birthday: _______ Gender: _______ Date: ______________

Please check the appropriate box(es) for each question:

1. Have you had a history/diagnosis of any of the following? (check all that apply)

    ☐ Acid Reflux ☐ Polyps, nodules, contact ulcers ☐ Asthma or allergies ☐ Other pathologies of throat or vocal cords ________________________________

2. Have you been sick in the last two weeks with a head cold or upper respiratory infection? ☐ YES ☐NO
3. Do you frequently overuse your voice (yelling/screaming/talking over noise)? ☐ YES ☐NO
4. Which of the following have you ever used at least 2x daily, 7 days/week for at least one year?

    ☐ ENDS: juul, e-cig, vape, mods, pods. ☐ Cigarettes ☐ Other tobacco/marijuana: _______ ☐ None

5. Which of the following are you currently using at least 2x daily, 7 days/week for at least one year?

    ☐ ENDS: juul, e-cig, vape, mods, pods. ☐ Cigarettes ☐ Other tobacco/marijuana: _______ ☐ None

6. If you smoke: have you vaped within the past five years? ☐ YES ☐NO
7. If you smoke: for how many years have you smoked? ____________
8. If you smoke: what type/brand do you smoke? ______________________
9. If you smoke: how frequently do you smoke?

    ☐ <3x per day ☐ 3-10x per day ☐ 10-20x per day ☐ Other: _____________________

10. If you vape: for how many years have you vaped? _______________
11. If you vape: have you smoked within the past five years? ☐ YES ☐NO
12. If you vape: what devices do you use? (select all that apply)
    - ❑ **Cig-a-like** (same size as cigarette - disposable)
    - ❑ **Rechargeable cigalike** (same size as cigarette – rechargeable)
    - ❑ **Vape pens/Mods** (large/customizable batteries, advanced atomizers, refillable)
    - ❑ **Pods** (less vape, rechargeable, e.g., JUUL, Suorin Drop, myblu)
13. What is the brand of your device? ______________
14. What is the voltage of your device? _____________
15. Is your device: ☐ variable voltage or ☐ temperature controlled? (check one)
16. If you vape: how frequently do you vape?

    ☐ more than 20x/day ☐ 10-20x/day ☐ 3-10x per day ☐ Other: _________________

17. If you vape: what nicotine strength do you use? (check box and write amount)

    ☐ Low ___________ ☐ Medium _________ ☐ High ___________ ☐ No Nicotine

18. If you vape, what flavor(s) do you use? (Select all that apply)

    ☐ Fruit or candy(sweet) ☐ Tobacco ☐ Menthol ☐ Mint ☐ Other: ______________

19. If you have used ENDS ***daily***, what factors made you want to start?

    ☐ ENDS are safer than cigarettes ☐ Trendy/popular ☐ Flavors

    ☐ To quit smoking ☐ Indoor use ☐ Other: ___________

Appendix C
Acoustic Analysis Participant Instructions

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Visi-pitch IV Instuctions

"Please stand in front of the machine with the microphone about two inches from your mouth, like this (demonstrate). When I press record, say "ah" in a natural voice for 5 seconds, at which time I will stop the recording."

Appendix D
Videostroboscopy Participant Instruction

Videostroboscopy Instructions:

"Please sit in this examination chair. With your bottom far back in the chair, bring your shoulders forward and lift your chin. Now, open your mouth wide and stick out your tongue. I'm going to hold your tongue gently with gauze while you say "ah". Now say "ee" in a natural voice for five seconds. And now say "ee" in a high pitch and sustain for 5 seconds. Now say "ee" in a low pitch and sustain for five seconds. Now say "ee" in a low pitch and glide up to a high pitch. And finally, just breath normally for a few breaths."

Appendix E
Table of Acoustic Norms

Acoustic Parameters	Females (ages 18-39)		Males (ages 18-39)	
	Mean	**SD**	**Mean**	**SD**
Fundamental Frequency (Hz)	214.39	26.76	106.414	13.29
Jitter (%)	0.82%	0.28%	1.41%	0.32%
Shimmer (%)	1.59%	0.68%	2.14%	0.63%

Appendix E: Acoustic norms for 18-39 year olds by gender
(*From: Schaefer et al. 2008)*