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An Excised Canine Model of Anterior Glottic Web and Its Acoustic, Aerodynamic, and High-speed Measurements

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Summary

Objectives—This study aims to build an excised anterior glottic web (AGW) model and study the basic voice-related mechanisms of the AGW through investigating the acoustic, aerodynamic, and vibratory properties.

Study Design and Methods—Overall, four conditions were tested for each of the eight canine larynges used. At baseline, 10%, 20%, and 33% occlusion (as determined by the placement of the suture), acoustic, aerodynamic, and high-speed video data were collected while each larynx was phonated in a soundproof booth.

Results—The phonation threshold pressure (PTP) and the phonation threshold flow significantly increased as percent occlusion increased ($P < 0.001$). There were significant increases in jitter % and shimmer % from baseline group to AGW model groups at PTP, 1.25 PTP, and 1.5 PTP ($P = 0.039$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, and $P = 0.001$, respectively). The fundamental frequency significantly increased as percent occlusion increased at all given pressures ($P < 0.001$). Correlation dimension (D2) was significantly higher in the AGW model groups than in the baseline group at PTP, 1.25 PTP, and 1.5 PTP ($P = 0.002$, $P < 0.001$, $P = 0.01$, respectively). In high-speed videos, the left phase shift in the AGW model groups compared with the baseline at 1.25 PTP was significant ($P = 0.027$) and right phase shift at 1.5 PTP ($P < 0.001$).

Conclusions—We presented an anatomically similar model of a type 1 AGW and confirmed its validity through aerodynamic, acoustic, and high-speed video analysis in our study. We observed and investigated the glottic web movement, which may be a new explanation for the pathologic voice-related mechanism of AGW.

Keywords

Anterior glottic web; Excised larynx; Web movement; Acoustic; Aerodynamic

INTRODUCTION

An anterior glottic web (AGW) is a type of anterior glottic stenosis, in which fibrous tissue forms a bridge between the free edges of the vocal folds, involving the anterior commissure.^{1,2} Glottic webs vary based on the degree of luminal occlusion and are classified as either congenital or acquired.^{3,4} Whereas the congenital form of an AGW is a consequence of incomplete recanalization of the laryngotracheal lumen during the third month of gestation, acquired anterior glottic stenosis is secondary to laryngeal trauma or inflammatory disease as a result of laryngeal surgery, radiation therapy, or prolonged intubation, and accounts for the vast majority of cases of AGW.^{2,5–7} Unintended vocal fold injury from endoscopic or microsurgical manipulation can also induce this form of anterior glottic scarring. During the early postoperative stage, fibrin deposition in both folds meets at the midline, causes scarring, and becomes covered by a mucosal layer.⁸ The glottic web is typically localized anteriorly across the vocal folds at the level of the glottis, with varying degrees of occlusion and thickness. As originally described by Cohen,⁹ type 1 webs obstruct 35% or less of the glottis, type 2 webs cover 35%–50%, type 3 webs cover 50%–75%, and type 4 webs encompass 75%–100% of the glottal opening.

Depending on the severity and size of the web, AGW can cause abnormally elevated phonatory pitch, dysphonia, and airway constriction.¹⁰ Although some webs less than 2- to 3-mm thick can be asymptomatic, AGW symptoms range from dysphonia to severe airway obstruction, thus increasing the required phonatory effort with increasing degree of AGW.^{11,12} However, there is a lack of clinically available data on aerodynamic and acoustic outputs of patients with AGW, including perceived phonatory effort (PPE) and phonation threshold pressure (PTP) as well as jitter and shimmer. PPE provides an index of subjective vocal fatigue that can be standardized, whereas PTP is an objective quantifiable measure of the minimal lung pressure required to initiate and sustain vocal fold oscillation.¹³ Given the direct relationship between PPE and PTP, it can be concluded that patients with symptomatic AGW display an increase in PTP as the degree of occlusion increases.¹⁴

In regard to acoustic characteristics, jitter and shimmer represent cycle-to-cycle variations of fundamental frequency (F0) and waveform amplitude.¹⁵ The underlying relationship of jitter and shimmer to pathologic voices suggests that as glottal occlusion increases, irregular vibration patterns within the mucosal wave will result, causing increased jitter and shimmer in patients with AGW.¹⁶ A study by Su et al recorded acoustic data of 19 patients with AGW before and after treatment, and they discovered that percent jitter and percent shimmer significantly decreased after removal of the glottic web.¹⁷

Another valuable acoustic parameter that is critical to understanding the components of a signal is correlation dimension (D2), which measures the number of degrees of freedom in a signal. A higher D2 indicates that a signal is more complex and has a higher degree of chaos.¹⁸ This parameter may be valuable in characterizing the acoustic output in patients with glottic web, but has not been used previously in the literature. Furthermore, the F0 is reported to change with AGW. The formation of an AGW, which is being used as a technique to raise pitch in transgender patients, shortens the vibrating length of the vocal

folds.¹⁹ Given that F0 varies inversely with vocal fold length,²⁰ we hypothesized that as the glottal degree of occlusion increases, the F0 would also increase.

Research regarding AGW formation is limited and has focused primarily on treating the disorder. The application of a variety of stents, referred to as keel insertion, has become a common treatment method and demonstrated increasing success rates.^{16,21,22} Although the patients' voices are notably improved with these stents, the procedure is invasive, increases the risk of airway compromise, and may cause damage to surrounding tissue, such as granulation formation.²³ Thus, more conservative prevention strategies have been introduced, including the use of endoscopic CO2 lasers coupled with the application of mitomycin C1.¹ Clinical treatment plans are formulated based on the extent of the disorder and its impact on the patient's breathing and phonation. Surgical techniques involving the division and subsequent suturing of a curvilinear mucosal wave flap were developed in 2010 by Deganello et al and more recently, a novel mucosal flap treatment was introduced by Yang et al in 2014 that resulted in a significant improvement in voice accompanied by a faster recovery period and fewer complications.^{2,8} As management options continue to evolve, the underlying purpose of AGW procedures is to restore the airway patency and ultimately improve voice quality back toward normal levels.⁸

Although research investigating treatments of AGW has been advancing, there is a lack of understanding of its underlying mechanisms. This focus on preventing restenosis is the result of the inherent limitations in studying AGW *in vivo* in human subjects. Because of the rarity of the disorder and the invasiveness associated with its *in vivo* investigation, further advancement has begun to plateau. To facilitate research regarding the development of enhanced management strategies for AGW, suitable *ex vivo* animal models that provide physiologic information about the disorder need to be established.

This study aimed at generating an anatomically similar, *ex vivo* animal model of a type 1 AGW in an excised canine larynx. We hypothesized that the acoustic, aerodynamic, and high-speed video data results would be consistent with reported symptoms and values of patients with AGW, further validating this model as an effective representation of AGW. Additionally, we hypothesized that the symptoms of AGW (increased PTP, increased perturbation measures, and increased frequency) would increase as the percent of occlusion increased.

METHODS

Larynx preparation

Eight larynges were harvested from canines sacrificed for non-research purposes according to the method previously described by Jiang and Titze.²⁴ Following excision, the larynges were frozen in 0.9% saline solution.

In preparation for the experiment, each larynx was thawed in a 37°C bath to reintroduce pliability. The epiglottis, cuneiform cartilages, corniculate cartilages, and ventricular folds as well as the superior cornu and posterosuperior portion of the thyroid cartilage were dissected

away to expose the true vocal folds and permit the insertion of a pronged micrometer into the arytenoids.

Excised booth

The excised larynx was mounted on a flow source and secured with a hose clamp within a custom triple-walled, soundproof excised booth. This method has been previously published by Jiang and Titze² and can be seen in Figure 1. A three-pronged micrometer was inserted into the arytenoid cartilage from either side to position the vocal processes in close contact to each other and posteriorly stabilize the vocal folds for phonation. A suture was attached to midline of the anterior portion of the thyroid cartilage and maintained constant elongation of 5%, with the original resting length of the glottis as a control. A 0.9% saline solution was applied to the larynx, specifically the vocal folds, between trials to decrease the potential for dehydration. The trachea, stabilized *via* a metal pull clamp, was connected to a pseudo-lung that provided a constant airflow source. To humidify and warm the air supplied to the larynx, pressurized air was passed through a series of ConchaTherm III humidifiers. The airflow was controlled manually and measured using an Omega airflow meter and pressure transducer (airflow model FMA-1601A, pressure model MMG005V10W2MA0T1A4, Omega Engineering Inc., Stanford, CT, USA).

A Sony microphone (model ECM-88, Sony Electronics Inc., New York, NY, USA) was positioned at a 35° angle to the vocal folds approximately 10 cm from the glottis to collect acoustic data during phonation. The acoustic signals were transferred to a Symetrix preamplifier (model 302, Symetrix Inc., Mountlake Terrace, WA, USA) and then to a National Instruments data acquisition board (model AT-MIO, National Instruments Corp., Austin, TX, USA) with a customized *LabVIEW 8.5* software program designed to record airflow, pressure, and acoustic signal (wav file) for processing and analysis. The sampling rate was 40,000 Hz for the acoustic data, whereas the aerodynamic data were recorded at a sampling rate of 200 Hz. Using a high-speed digital camera with a rate of 4000 Hz and a resolution of 512 × 256 pixels, the mucosal wave of the vocal fold for each trial was recorded for approximately 200 ms during stable phonation.

AGW model

Three different procedures were performed on each larynx to create occlusions of 10%, 20%, and 33% of the length spanning the anterior commissure to the vocal process. These lengths were chosen to model type 1 AGW in varying degrees within the canine larynx. Within the tissue of an *in vivo* vocal fold, the collagen fibers are oriented in a way that permits elongation and vibration during phonation while also providing the strength to endure vibration.^{24,25} Because of the similarity in tensile strength and elasticity between this connective tissue and that of a silk suture, sutures provided a viable material that can be used to functionally model the tissue within the glottic web.^{26,27} A suture was secured at the level of the glottis superior to and on the medial surface of each of the true vocal folds, preserving the underlying vocal ligament, to create a connective bridge between the two folds. The baseline setup and AGW models used in this study are represented in Figure 2A–D.

Experimental methods

Each larynx was tested under four different conditions. The first condition, or control, was the larynx before any addition of the modeled AGW. The next three conditions modeled the AGW at 10%, 20%, and 33% occlusion, which were achieved by attaching sutures that spanned the glottal opening. To ensure consistency across the conditions, the length at which the sutures were placed on the larynx, as well as the elongation length from the anterior tip of the vocal processes to the thyroid notch, was measured using a digital caliper (model CD-4" CSX, Mitutoyo Corp., Kawasaki, Japan).

Once the vocal folds were properly aligned for phonation, the PTP was measured by manually increasing the subglottal pressure until sustained phonation was achieved, as indicated by a clear and pronounced tone. Three trials were performed at each designated pressure: PTP, 1.25 times PTP, and 1.5 PTP. For each larynx, this amounted to 45 trials, with each trial lasting approximately 5 seconds.

Data analysis

To conduct acoustic analysis, audio files obtained from the *LabVIEW* output were trimmed into three 1-second clips with a customized *Python* script (CWI, Amsterdam, Netherlands). Using the *TF32* software (University of Wisconsin, Madison, WI, USA), the F0, signal-to-noise ratio, percent jitter, and percent shimmer were obtained. D2 was calculated for each clip using the method described by Packard et al.²⁴ Values were averaged between the three trials at each designated pressure.

A customized *MATLAB* program (The MathWorks, Natick, MA, USA) generated a digital kymograph. Then, the high-speed video recordings of the mucosal wave were analyzed to obtain vibratory properties of each of the four vocal fold lips. The edges of these lips were analyzed using a nonlinear least squares curve-fitting to generate the most closely fitting sinusoidal Fourier fitted-curves, all with an R-squared value of greater than 0.9, derived from manually selected points. To maintain consistency, the same researcher was responsible for selecting the curve-fitting points for all trials. This curve was then used to calculate the phase differences of each vocal fold lip. Three phase differences were calculated, including right phase shift (right upper lip vs. right lower lip), left phase shift (left upper lip vs. left lower lip), and interfold phase shift (right upper lip vs. left upper lip).

Measuring web movement during phonation

In the AGW model groups, glottic web mass effectively served as the “fixed end” of phonation. The “fixed end” in an individual without a web, the thyroid cartilage, behaved quite differently from the web mass. Using high-speed video, we observed that the glottic web mass oscillated during phonation, whereas the thyroid cartilage had negligible movement during phonation. Thus, we quantified this amount by measuring the amplitude of the wave pattern created by the vibrational periodic movement of the web mass. In doing so, we rotated the video 90° to align the suture horizontally and subsequently selected a column at the midline. From this, we were able to quantify the deviation in position of the glottic web mass from its respective “fixed point,” defined as the point at which the glottic web

mass rests while not in motion, that is, while the larynx is not phonating, thus allowing us to obtain the amplitude of the vibration from the digital kymograph.

Statistical analysis

A repeated measures analysis of variance test was used to examine the relationships between each aerodynamic, acoustic, and high-speed video outcome measured and the treatment condition. When the statistical variance between groups was too high, an analysis of variance on ranks was conducted. An alpha value of $\alpha = 0.05$ was used to determine significance. To compare individual groups, Tukey test was used. This analysis was conducted for baseline, 10%, 20%, and 33% occlusion.

RESULTS

Aerodynamic analysis

There was a significant increase in PTP as the percent occlusion created by the modeled web increased ($P < 0.001$). A graph of this relationship can be seen in Figure 3A, and the statistical results can be seen in Table 1. Additionally, there were significant increases in PTF as percent occlusion increased ($P < 0.001$), which can be seen in Figure 3B. Statistical results for flow can be seen in Table 1.

Acoustic analysis

At PTP, 1.25 PTP, and 1.5 PTP, there were significant increases in jitter % from the baseline group to the AGW model groups ($P = 0.039$, $P < 0.001$, and $P < 0.001$, respectively). The results for shimmer % at PTP, 1.25 PTP, and 1.5 PTP demonstrated significant increases from the baseline group to the AGW model groups ($P < 0.001$, $P < 0.001$, and $P = 0.001$, respectively). Whereas there were no significant differences between the AGW model groups for either perturbation measure at PTP, there was a significant difference for both jitter % and shimmer % ($P = 0.034$ and $P = 0.014$, respectively) at 1.5 PTP. At 1.25 PTP, there were significant differences in shimmer % between AGW groups ($P < 0.001$), but not for jitter. These results are displayed in Figures 4 and 5, and in Tables 2, 3, and 4.

Additionally, the F0 was significantly higher in the AGW model groups than in the baseline condition within each pressure condition ($P < 0.001$). There was also a direct relationship between percent occlusion and F0. These results are displayed in Tables 2, 3, and 4, and in Figure 6A–C.

D2 was significantly higher in the AGW model groups than in the baseline group at PTP, 1.25 PTP, and 1.5 PTP ($P = 0.002$, $P < 0.001$, $P = 0.01$, respectively). Additionally, at 1.25 PTP and 1.5 PTP, there were significant increases in D2 as the percent occlusion increased ($P = 0.007$ and $P < 0.001$, respectively). These results are displayed in Tables 2, 3, and 4, and in Figure 7A–C.

High-speed video analysis

The results for interphase shift did not show any significant differences between the conditions at any pressure. Left phase shift was significantly higher in the AGW model

groups than in baseline at 1.25 PTP ($P = 0.027$), and right phase shift was significantly different at 1.5 PTP ($P < 0.001$). These results can be seen in Table 5 and in Figure 8A and B.

Glottic web movement

We observed a noticeable movement within the modeled web compared with the negligible amount of movement of the fixed point of the anterior commissure. These results can be found in Table 6 and Figure 9.

DISCUSSION

The results of the experiment confirmed our hypothesis that the anatomically accurate AGW model we presented would produce results consistent with what is found clinically. The direct relationship between occlusion percentage and PTP mirrors the increased PPE and PTP that is found clinically in patients with AGW.^{10–13}

Additionally, there were significant increases in percent jitter, percent shimmer, and D2 from the baseline group to the AGW model groups at varying subglottic pressure. This demonstrates that the vocal fold vibration and resulting acoustic signal become more irregular with increasing anterior glottic occlusion. This result is consistent with clinically available data.^{10–12,14,16,22}

Furthermore, the results of the experiment confirmed the presence of a direct relationship between the degree of occlusion and the F0, which indicates that decreases in the length of the glottal opening result in an increase in pitch. This is corroborated by the proportional differences in glottal size and pitch between genders^{28,29} as well as the results of Anderson's study,¹⁹ which confirmed that the formation of an AGW was accompanied by a significantly augmented F0 due to shortening of the fold and a decrease in its vibrating mass.

Although the experiment did produce significant differences between perturbation measures of AGW groups at some pressures, it did not show a direct relationship with occlusion percentage consistently at all pressures. These results can likely be attributed to the evidence that perturbation methods are not capable of accurately analyzing severely disordered signals that follow an irregular periodicity,^{30–32} even though they are capable of distinguishing between normal and pathologic voice signals. Given this, we can conclude that as the percent occlusion increased and the voice signals became increasingly nonlinear, the reliability of these traditional perturbation measures declined. Meanwhile, nonlinear dynamic methods of acoustic analysis have been able to provide stable measures of aperiodic and chaotic signals,^{31,32} which is evident in the significant relationships of D2 in all AGW groups at 1.25 PTP and 1.5 PTP.

To further investigate the pathophysiology behind AGW, we investigated glottic web displacement during phonation. In normal phonation, the anterior commissure of the thyroid cartilage serves as a fixed point. However, in AGW, the anterior “fixed point” is the glottic web, which has less relative stiffness and higher elasticity. Thus, as the vocal fold vibrates, the web oscillates in an anterior-to-posterior direction. This changes the normal,

synchronized vibration into a two-way combined movement: the anterior-to-posterior movement of the web and the vibration of the rest of the vocal fold. Vocal fold vibration is no longer a simple oscillation system, but instead a component movement system, which leads to a complex vibration of the vocal folds and chaotic acoustic signals. This observation could help explain why the acoustic signal became chaotic as the occlusion percentage increased in our model. This may also explain why GRBAS and other voice parameters improved after patients underwent glottic web removal surgery.¹⁵

In addition, two mechanisms are hypothesized to explain the increase in PTP due to the presence of the glottic web: additional mass and narrowing of the glottis. Because of the occlusion caused by the glottic web, much like the obstruction caused by mass lesions in nodules and polyps, airflow and subglottal pressure increase to overcome the increasing resistance to oscillation.³³ The increased mass of the vocal folds due to the presence of the web causes an increase in resistance that requires a higher pressure for phonation to occur.³⁴ Additionally, the obstruction and narrowing within the glottis caused by AGW causes the pressure generated in the vocal tract to rise.³⁵ Therefore, the significance of the relationship between PTP and degree of occlusion can be substantiated by the direct effect on subglottal pressure, and thus PTP, which are caused by each of these factors.

CONCLUSIONS

In this study, a novel model of an anatomically accurate type 1 AGW is presented in an *ex vivo* model. To confirm the model's validity, aerodynamic, acoustic, and high-speed video data were collected and analyzed. Consistent with the properties present in clinically symptomatic AGW, increases in the degree of occlusion were accompanied by significant increases in PTP ($P < 0.001$) and F0 ($P < 0.001$). Additionally, a significant difference was observed between baseline groups and AGW groups for jitter, shimmer, and D2 ($P < 0.001$, $P < 0.001$, and $P = 0.002$, respectively). Finally, the glottic web movement data were collected to further investigate the pathophysiology of the web. This revealed that in cases of AGW, both the web movement and the vocal fold vibration become part of a component movement system. The augmented chaos accompanied by increased occlusion is likely a result of this complex vibration pattern.

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FIGURE 1.
Visual representation of the excised booth.

FIGURE 2.

Photograph of the (A) baseline setup of excised larynx, (B) AGW model of 10% occlusion, (C) AGW model of 20% occlusion, and (D) AGW model of 33% occlusion.

FIGURE 3.

A graphical representation of the aerodynamic data: **(A)** average PTP for each condition; **(B)** average PTF for each condition.

FIGURE 4.

A graphical representation of average jitter: (A) at PTP, (B) at 1.25× PTP, and (C) at 1.5× PTP.

FIGURE 5.

A graphical representation of average shimmer: **(A)** at PTP, **(B)** at 1.25× PTP, and **(C)** at 1.5× PTP.

FIGURE 6.

A graphical representation of average fundamental frequency: (A) at PTP, (B) at 1.25× PTP, and (C) at 1.5× PTP.

FIGURE 7.

A graphical representation of average D2: (A) at PTP, (B) at 1.25× PTP, and (C) at 1.5× PTP.

FIGURE 8.

A graphical representation of average phase shift: **(A)** left phase shift for each condition at $1.25 \times$ PTP, and **(B)** right phase shift for each condition at $1.5 \times$ PTP.

Coefficients:
f = 212 Hz
a0 = 0.8714 mm
a1 = 0.06793 mm p1 = 0.2495
R-square: 0.9453

FIGURE 9.
Digital kymograph and measurements of vocal web movement.

TABLE 1

Description of Phonation Threshold Pressure (PTP) and Phonation Threshold Flow (PTF) and Statistical Tests Used in Analysis

Variable	Comparison	Test Used	<i>P</i> Value
PTP	All groups	RM ANOVA on ranks	$P < 0.001^*$
	Baseline vs. 10% occlusion	Tukey test	$P < 0.001^*$
	Baseline vs. 20% occlusion	Tukey test	$P < 0.001^*$
	Baseline vs. 33% occlusion	Tukey test	$P < 0.001^*$
	10% vs. 20% occlusion	Tukey test	$P < 0.001^*$
	10% vs. 33% occlusion	Tukey test	$P < 0.001^*$
	20% vs. 33% occlusion	Tukey test	$P < 0.001^*$
PTF	All groups	RM ANOVA on ranks	$P < 0.001^*$
	Baseline vs. 10% occlusion	Tukey test	$P < 0.001^*$
	Baseline vs. 20% occlusion	Tukey test	$P < 0.001^*$
	Baseline vs. 33% occlusion	Tukey test	$P < 0.001^*$
	10% vs. 20% occlusion	Tukey test	$P < 0.001^*$
	10% vs. 33% occlusion	Tukey test	$P < 0.001^*$
	20% vs. 33% occlusion	Tukey test	$P = 0.0089^*$

* Represents statistical significance at $\alpha = 0.05$.

Abbreviation: RM ANOVA, repeated measures analysis of variance.

TABLE 2

Description of Acoustic Analyses (Jitter %, Shimmer %, D2, and Frequency) at PTP and Statistical Tests Used in Analysis

Variable	Comparison	Test Used	P Value
Jitter %	All groups	RM ANOVA on ranks	$P = 0.039^*$
	Baseline vs. 10% occlusion	Tukey test	$P = 0.014^*$
	Baseline vs. 20% occlusion	Tukey test	$P = 0.027^*$
	Baseline vs. 33% occlusion	Tukey test	$P = 0.004^*$
	Between all AGW models	RM ANOVA on ranks	$P = 0.629$
Shimmer %	All groups	RM ANOVA on ranks	$P < 0.001^*$
	Baseline vs. 10% occlusion	Tukey test	$P = 0.002^*$
	Baseline vs. 20% occlusion	Tukey test	$P < 0.001^*$
	Baseline vs. 33% occlusion	Tukey test	$P < 0.001^*$
	Between all AGW models	RM ANOVA on ranks	$P = 0.092$
D2	All groups	RM ANOVA on ranks	$P = 0.002^*$
	Baseline vs. 10% occlusion	Tukey test	$P = 0.015^*$
	Baseline vs. 20% occlusion	Tukey test	$P = 0.008^*$
	Baseline vs. 33% occlusion	Tukey test	$P < 0.001^*$
	Between all AGW models	ANOVA on ranks	$P = 0.410$
Frequency	All groups	RM ANOVA on ranks	$P < 0.001^*$
	Baseline vs. 10% occlusion	Tukey test	$P = 0.049^*$
	Baseline vs. 20% occlusion	Tukey test	$P < 0.001^*$
	Baseline vs. 33% occlusion	Tukey test	$P < 0.001^*$
	Between all AGW models	RM ANOVA on ranks	$P < 0.001^*$
	10% vs. 20% occlusion	Tukey test	$P = 0.012^*$
	10% vs. 33% occlusion	Tukey test	$P < 0.001^*$
	20% vs. 33% occlusion	Tukey test	$P < 0.001^*$

* Represents statistical significance at $\alpha = 0.05$.

Abbreviation: RM ANOVA, repeated measures analysis of variance.

TABLE 3

Description of Acoustic Analyses (Jitter %, Shimmer %, D2, and Frequency) at 1.25 PTP and Statistical Tests Used in Analysis

Variable	Comparison	Test Used	P Value
Jitter %	All groups	RM ANOVA on ranks	$P < 0.001^*$
	Baseline vs. 10% occlusion	Tukey test	$P = 0.067^*$
	Baseline vs. 20% occlusion	Tukey test	$P < 0.001^*$
	Baseline vs. 33% occlusion	Tukey test	$P < 0.001^*$
	All AGW models	RM ANOVA on ranks	$P = 0.088$
Shimmer %	All groups	RM ANOVA on ranks	$P < 0.001^*$
	Baseline vs. 10% occlusion	Tukey test	$P = 0.067^*$
	Baseline vs. 20% occlusion	Tukey test	$P < 0.001^*$
	Baseline vs. 33% occlusion	Tukey test	$P < 0.001^*$
	All AGW models	RM ANOVA on ranks	$P < 0.001^*$
	10% vs. 20% occlusion	Tukey test	$P = 0.072$
	10% vs. 33% occlusion	Tukey test	$P = 0.046^*$
	20% vs. 33% occlusion	Tukey test	$P = 0.501$
D2	All groups	RM ANOVA on ranks	$P < 0.001^*$
	Baseline vs. 10% occlusion	Tukey test	$P < 0.001^*$
	Baseline vs. 20% occlusion	Tukey test	$P < 0.001^*$
	Baseline vs. 33% occlusion	Tukey test	$P < 0.001^*$
	All AGW models	RM ANOVA on ranks	$P = 0.007^*$
	10% vs. 20% occlusion	Tukey test	$P = 0.399$
	10% vs. 33% occlusion	Tukey test	$P < 0.001^*$
	20% vs. 33% occlusion	Tukey test	$P = 0.017^*$
Frequency	All groups	RM ANOVA on ranks	$P < 0.001^*$
	Baseline vs. 10% occlusion	Tukey test	$P = 0.005^*$
	Baseline vs. 20% occlusion	Tukey test	$P < 0.001^*$
	Baseline vs. 33% occlusion	Tukey test	$P < 0.001^*$
	All AGW models	RM ANOVA on ranks	$P < 0.001^*$
	10% vs. 20% occlusion	Tukey test	$P = 0.007^*$
	10% vs. 33% occlusion	Tukey test	$P < 0.001^*$
	20% vs. 33% occlusion	Tukey test	$P < 0.001^*$

* Represents statistical significance at $\alpha = 0.05$.

Abbreviation: RM ANOVA, repeated measures analysis of variance.

TABLE 4

Description of Acoustic Analyses (Jitter %, Shimmer %, D2, and Frequency) at 1.5 PTP and Statistical Tests Used in Analysis

Variable	Comparison	Test Used	P Value
Jitter %	All groups	RM ANOVA on ranks	$P < 0.001^*$
	Baseline vs. 10% occlusion	Tukey test	$P = 0.045^*$
	Baseline vs. 20% occlusion	Tukey test	$P < 0.001^*$
	Baseline vs. 33% occlusion	Tukey test	$P < 0.001^*$
	All AGW models	RM ANOVA on ranks	$P = 0.034^*$
	10% vs. 20% occlusion	Tukey test	$P = 0.090$
	10% vs. 33% occlusion	Tukey test	$P = 0.034^*$
	20% vs. 33% occlusion	Tukey test	$P = 0.335$
Shimmer %	All groups	RM ANOVA on ranks	$P = 0.001^*$
	Baseline vs. 10% occlusion	Tukey test	$P = 0.538$
	Baseline vs. 20% occlusion	Tukey test	$P < 0.001^*$
	Baseline vs. 33% occlusion	Tukey test	$P = 0.001^*$
	All AGW models	RM ANOVA on ranks	$P = 0.014^*$
	10% vs. 20% occlusion	Tukey test	$P = 0.175$
	10% vs. 33% occlusion	Tukey test	$P = 0.113$
	20% vs. 33% occlusion	Tukey test	$P = 0.191$
D2	All groups	RM ANOVA on ranks	$P = 0.01^*$
	Baseline vs. 10% occlusion	Tukey test	$P = 0.697$
	Baseline vs. 20% occlusion	Tukey test	$P = 0.867$
	Baseline vs. 33% occlusion	Tukey test	$P = 0.001^*$
	All AGW models	RM ANOVA on ranks	$P < 0.001^*$
	10% vs. 20% occlusion	Tukey test	$P < 0.001$
	10% vs. 33% occlusion	Tukey test	$P < 0.001^*$
	20% vs. 33% occlusion	Tukey test	$P = 0.122$
Frequency	All groups	RM ANOVA on ranks	$P < 0.001^*$
	Baseline vs. 10% occlusion	Tukey test	$P = 0.002^*$
	Baseline vs. 20% occlusion	Tukey test	$P < 0.001^*$
	Baseline vs. 33% occlusion	Tukey test	$P < 0.001^*$
	All AGW models	RM ANOVA on ranks	$P < 0.001^*$
	10% vs. 20% occlusion	Tukey test	$P = 0.003^*$
	10% vs. 33% occlusion	Tukey test	$P < 0.001^*$
	20% vs. 33% occlusion	Tukey test	$P < 0.001^*$

* Represents statistical significance at $\alpha = 0.05$.

Abbreviation: RM ANOVA, repeated measures analysis of variance.

TABLE 5

High-speed Video Analyses (Left, Right, and Interphase Shifts) and Statistical Tests Used in Analysis

Variable	Pressure	Comparison	Test Used	P Value
Left phase shift	PTP	All groups	RM ANOVA on ranks	$P = 0.531$
	1.25 PTP	All groups	RM ANOVA on ranks	$P = 0.027^*$
	1.25 PTP	Baseline vs. 10% occlusion	Tukey test	$P = 0.261$
	1.25 PTP	Baseline vs. 20% occlusion	Tukey test	$P = 0.047^*$
	1.25 PTP	Baseline vs. 33% occlusion	Tukey test	$P = 0.452$
	1.25 PTP	Between AGW groups	RM ANOVA on ranks	$P = 0.079$
	1.5 PTP	All groups	RM ANOVA on ranks	$P = 0.183$
Right phase shift	PTP	All groups	RM ANOVA on ranks	$P = 0.125$
	1.25 PTP	All groups	RM ANOVA on ranks	$P = 0.276$
	1.5 PTP	All groups	RM ANOVA on ranks	$P < 0.001^*$
	1.5 PTP	Baseline vs. 10% occlusion	Tukey test	$P = 0.062$
	1.5 PTP	Baseline vs. 20% occlusion	Tukey test	$P = 0.007^*$
	1.5 PTP	Baseline vs. 33% occlusion	Tukey test	$P = 0.004^*$
	1.5 PTP	Between AGW groups	RM ANOVA on ranks	$P = 0.311$
Interphase shift	PTP	All groups	RM ANOVA on ranks	$P = 0.432$
	1.25 PTP	All groups	RM ANOVA on ranks	$P = 0.327$
	1.5 PTP	All groups	RM ANOVA on ranks	$P = 0.522$

* Represents statistical significance at $\alpha = 0.05$.

Abbreviation: RM ANOVA, repeated measures analysis of variance.

TABLE 6

Glottic Web Movement Results

Condition	Average Movement (mm)
Baseline	NA
10% Occlusion	0.053698995
20% Occlusion	0.073907149
33% Occlusion	0.058010659