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What is This?
Evaluation of True Vocal Fold Growth as a Function of Age

Derek J. Rogers, MD1, Jennifer Setlur, MD2, Nikhila Raol, MD1, Rie Maurer, MA3, and Christopher J. Hartnick, MD1

Abstract

Objective. To evaluate change in true vocal fold length as a function of age.

Study Design. Prospective study.

Setting. Tertiary aerodigestive center.

Subjects and Methods. In total, 205 patients (aged 1 month to 20 years), of whom 87 (42.4%) were female and 118 (57.6%) male, were included. Lengths of the total vocal fold (TVFL), membranous vocal fold (MVFL), and cartilaginous vocal fold (CVFL) were measured during direct laryngoscopy. Membranous-to-cartilaginous (M/C) ratios were calculated.

Results. For patients younger than 1 year, mean (SD) MVFL was 4.4 (1.3) mm for females and 4.9 (1.8) mm for males. At age 17 years, mean (SD) MVFL was 12.3 (2.1) mm for females and 14.0 (1.4) mm for males. Mean TVFL, MVFL, and CVFL increased an average of 0.7 mm, 0.5 mm, and 0.2 mm per year in linear fashion, respectively (linear regression, \( P < .0001 \)). The M/C ratio did not significantly change with age (\( P = .33 \)). Mean TVFL, MVFL, and CVFL showed no statistical difference between males and females (\( P = .27, .11, \) and .75, respectively).

Conclusion. This is the largest longitudinal pediatric study specifically examining vocal fold length as a function of age. Each length of the true vocal fold appeared to linearly increase for both females and males. The M/C ratio remained relatively constant, unlike previously reported data, possibly due to in vivo vs cadaveric measurements. These findings suggest that critical periods of development in females and males are not explainable by changes in vocal fold length alone, and other factors such as vocal fold layers need further exploration.

Keywords

vocal fold length, pediatric voice, pediatric laryngology

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Understanding the anatomic development of the pediatric vocal fold and how changes in anatomy affect acoustic and aerodynamic properties remains paramount to the evolving field of pediatric laryngology. As vocal tasks become more sophisticated throughout development, the length of the true vocal fold increases, and the composition of the lamina propria changes. It remains unclear whether the increase in vocal fold length or the number of layers in the lamina propria is responsible for changes in fundamental frequency.

The first step to increase our understanding of the pediatric voice was to establish normative pediatric voice data. Campisi et al developed the first normative pediatric voice database, which suggested that prepubescent females and males share a similar vocal profile until the fundamental frequency of males dramatically decreases at age 12 years. However, this study derived the normative data from only 100 patients. Maturo et al established a more comprehensive database by recording 335 children sustaining the phrase “ah” to develop an age- and sex-based growth chart to track the pediatric voice as it changes with maturation. Unlike the study by Campisi et al, this study found that discrete fundamental frequency changes occurred at ages 11 and 14 years in girls and ages 12 and 16 years in boys. Hill et al then evaluated the consistency of sustained utterances in measuring pediatric voice frequency and perturbation with the Voice Evaluation Suite (VES) and Multi-Dimensional Voice Program (MDVP). They found that fundamental...
frequency had excellent reliability in both VES and MDVP, but jitter, shimmer, and noise-to-harmonic ratio were poorly reliable in the MDVP and more reliable in the VES. Next, Dierckx et al\(^6\) found that fundamental frequency and frequency-based analyses demonstrated excellent reliability for continuous speech across 2 time points, suggesting that frequency-based analysis of continuous speech may be more representative of a child’s actual voice. We are currently repeating the study by Maturo et al by using continued speech sampling to analyze whether similar discrete fundamental frequency changes occur. Further work by Maturo et al\(^7\) resulted in a normative database of pediatric laryngeal diadochokinetic rates, which suggested that neurolaryngeal development approaches adult maturation during early adolescence.

Now that normative pediatric voice data have been established that suggest critical periods of development, a more thorough knowledge of the anatomic maturation of the pediatric larynx and how these changes in anatomy affect the acoustic and aerodynamic qualities remains imperative. Most theories of vocal mechanics have been transferred from adult studies with minimal data arising from the first 20 years of life. Although it has been recognized that the vocal folds lengthen with age, little is known regarding the details of these changes. Moreover, the impact of the change in the microstructure of the vocal fold lamina propria on acoustic and aerodynamic measurements remains to be elucidated.

Our current understanding of the changes in both vocal fold length and layers in the lamina propria hinges on the seminal work of Hirano. In 1983, Hirano et al\(^1\) reported changes in the length and the inner structure of the true vocal fold as a function of age in 88 normal Japanese larynges (Table 1). However, the data came from cadaveric larynges, most of which were fixed in 10% formalin between the 7th and 10th days postmortem. Furthermore, only 39 (44%) of the larynges were from subjects younger than 20 years. Eckel et al\(^8\) studied the development of 43 larynges from children aged 1 to 60 months, but these were cadaveric specimens treated via plastination before measurements were taken. The plastination process involved freezing the specimens, treating them with multiple chemicals, and then slicing the specimens with a diamond band-saw, which presumably caused alterations in the delicate vocal fold tissue.

The objective of this study was to further evaluate the change in true vocal length as a function of age. By specifically focusing on ages younger than 20 years and obtaining data in vivo, we hope to more accurately characterize the changes in true vocal fold length as we age. Our hypothesis is that this study will help explain the critical periods of development in females and males and lead to a better anatomic laryngeal model in which to correlate the changes seen in acoustic and aerodynamic vocal properties.

### Methods

#### Patients

This study was approved by the institutional review board of the Massachusetts Eye and Ear Infirmary. Written, informed consent was obtained for each patient before enrollment in this study. Patients were gathered consecutively and were included if they were aged 20 years and younger and required a direct laryngoscopy as part of their operative procedure. Exclusion criteria consisted of age older than 20 years, vocal fold pathology such as a mass or paralysis, prior laryngeal or tracheal surgery, and presence of a known syndrome.

#### Measurement Technique

After informed consent, the patients were brought to the operating room and placed supine on the operating table. Anesthesia was induced with inhalational sevoflurane and transitioned to intravenous propofol and remifentanil. Direct laryngoscopy was performed with a Miller blade as long as a view of the entire glottis was possible. Otherwise, a Lindholm laryngoscope was inserted and placed on suspension. Approximately 5 patients required suspension laryngoscopy. A metal vocal fold measuring stick was then used to measure the membranous vocal fold length (MVFL) and cartilaginous vocal fold length (CVFL) of one of the true vocal folds (Figure 1). The measuring sticks were sized 5.0 mm, 7.5 mm, 10 mm, and 15 mm (Figure 2). The appropriately sized measuring stick was selected based on the size of the patient’s glottis. The MVFL was measured from the vocal process of the arytenoid to the anterior commissure and the CVFL from the vocal process of the arytenoid to the presumed posterior insertion point. The actual vocal fold lengths were estimated, beginning with the size of the measuring stick.

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**Table 1. Summary of Vocal Fold Measurements from Hirano et al.**

<table>
<thead>
<tr>
<th>Age</th>
<th>Total Vocal Fold Length, mm</th>
<th>Membranous Vocal Fold Length, mm</th>
<th>Cartilaginous Vocal Fold Length, mm</th>
<th>Membranous-to-Cartilaginous Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn</td>
<td>2.5-3.0</td>
<td>1.3-2.0</td>
<td>1.0-1.4</td>
<td>1.1-1.8</td>
</tr>
<tr>
<td>Adult female</td>
<td>11-15</td>
<td>8.5-12</td>
<td>2.0-3.0</td>
<td>3.3-4.5</td>
</tr>
<tr>
<td>Adult male</td>
<td>17-21</td>
<td>14.5-18</td>
<td>2.5-3.5</td>
<td>4.7-6.2</td>
</tr>
</tbody>
</table>

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[5] MDVP: Modified Speech Analysis Program
Statistical Analysis

Total vocal fold length (TVFL), MVFL, and CVFL were recorded for all patients. The TVFL was calculated by adding the MVFL and CVFL. The membranous-to-cartilaginous (M/C) ratio was determined for each patient by dividing the MVFL by the CVFL. Mean TVFL, MVFL, CVFL, and M/C ratio were calculated for each age group. These data were plotted with error bars for initial visual inspection. Simple linear regression appeared to be an accurate fit for each vocal fold length. A nonparametric smoothing, or LOESS fit, was performed on the data for MVFL, which confirmed that the linear regression model was a good fit over the entire age range. Multiple linear regressions were performed for each vocal fold length (TVFL, MVFL, and CVFL) and the M/C ratio, including age, sex, and interaction between age and sex. The Bonferroni correction was applied, and a reduced P value of .0125 was considered statistically significant. All statistical analyses were conducted using SAS version 9.2 statistical software (SAS Institute, Cary, North Carolina).

Results

A total of 205 patients were included in this study. Eighty-seven (42.4%) were female, and 118 (57.6%) were male. Ages ranged from 1 month to 20 years. Mean TVFL, MVFL, CVFL, and M/C ratio for each sex and age group are presented in Supplemental Tables S1 and S2 (available at otojournal.org).

Linear regressions were performed on the data for TVFL, MVFL, CVFL, and M/C ratio (Figure 3 and Table 2). Mean TVFL increased by an average of 0.7 mm each year ($P < .0001$) and showed no statistical difference between females and males ($P = .27$). Mean MVFL increased by an average of 0.5 mm each year ($P < .0001$) and demonstrated no statistical difference between females and males ($P = .11$). Mean CVFL increased by an average of 0.2 mm each year ($P < .0001$). Once again, no statistical difference was detected between males and females ($P = .75$). The mean M/C ratio did not significantly change with age ($P = .33$). Furthermore, no significant difference was found in the M/C ratio between males and females ($P = .27$).

Discussion

Although our understanding of pediatric dysphonia continues to evolve, pediatric laryngology remains in its nascency. Developing a normative pediatric voice database marked a considerable advancement in this field. However, the next step is to determine what is responsible anatomically for these different critical periods of vocal development in both females and males.

To address this fundamental question, one must be familiar with the physics of vocal fold vibration. Traditionally, it was thought that vocal fold length, thickness, and mass were the key variables involved, and the equations were inferred from the formula for a mass coupled to a spring or the formula for a vibrating string. However, the most recent theory deduced by Titze provides the following equation for fundamental frequency, or $F_0$:

\[
F_0 = \frac{1}{2L_m} \sqrt{\frac{\sigma_p}{\rho}} (1 + \frac{d/a_{TA}}{\sigma_m a_{TA}})^{1/2}.
\]

$L_m$ represents membranous vocal fold length; $\sigma_p$, passive (noncontractile) tissue stress; $\rho$, tissue density; $d$, medial-lateral depth of vibration; $a_{TA}$, depth of vibration of the thyroarytenoid muscle; $\sigma_m$, maximum active stress; and $a_{TA}$, the activation level in the thyroarytenoid muscle. In the above equation, Titze stated that soft tissue density, $\rho$, remains constant at 1.04 g/cm$^2$.

This study specifically assessed changes in true vocal fold length as we age. Titze’s equation assumed that the primary oscillator contributing to fundamental frequency is the membranous vocal fold and that the contribution from the cartilaginous vocal fold is negligible. We evaluated TVFL, MVFL, CVFL, and the M/C ratio as a function of...
age in case the growth pattern of any portion of the true vocal fold suggested it may correlate with changes in fundamental frequency.

Various methods have been used in the past to measure true vocal fold length. Several studies used cadaveric larynges to measure vocal fold dimensions, but each of these often used a fixation or plastination process for their specimens.\(^1,12-14\) A few studies have attempted to measure true vocal fold length in living individuals. The methods used include photography,\(^15\) plain films,\(^16\) ultrasound,\(^17\) and laser.\(^18\) We chose to acquire our measurements in vivo using vocal fold measuring sticks. This ensured that all individuals were in a similar physiologic state (under the same type of anesthetic and spontaneously ventilating) and allowed direct visualization of the vocal folds during the measuring process.

Our data revealed some interesting results compared with previously published data. Mean MVFL for females in our study was 4.4 mm (2.5-7.0 mm) for those younger than 1 year and 12.3 mm (10.0-14.0 mm) at age 17 years; for males, it was 4.9 mm (2.0-7.5 mm) for those younger than 1 year and 14.0 mm (13.0-15.0 mm) at age 17 years. This compares well to the data by Hirano et al\(^1\) presented in Table 1. For subjects younger than 1 year, Eckel et al\(^8\) found a mean MVFL of 2.9 mm (2.6-4.7 mm) in 24 male and female cadaveric specimens, and for their 4 oldest subjects aged 49 to 60 months, they reported a mean MVFL of 5.9 mm (5.3-6.7 mm). These measurements were shorter than ours at both of these age groups, possibly due to loss of elasticity during their measurement process.

Mean CVFL for females in our study was 2.8 mm (1.3-5.0 mm) for those younger than 1 year and 7.5 mm (7.5-7.5 mm) at age 17 years; for males, it was 3.0 mm (1.0-5.0 mm) for those younger than 1 year and 8.8 mm (7.5-10.0 mm) at age 17 years. These values are approximately twice as long as those reported by Hirano et al.\(^1\) Eckel et al\(^8\) reported a mean CVFL of 4.1 mm (2.9-5.1 mm) in children younger than 1 year and 4.8 mm (4.2-5.2 mm) in children aged 49 to 60 months. Our mean CVFL was about 1 mm shorter in children younger than 1 year compared with these data, but it was quite similar for patients aged 4 to 5 years.

The mean M/C ratio for females in our study was 1.7 (1.0-2.8) for those younger than 1 year and 1.6 (1.3-1.9) at

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**Figure 3.** Linear regressions for each portion of the true vocal fold and M/C ratio. \(P\) value was <.0001 for the total vocal fold length (TVFL), membranous vocal fold length (MVFL), and cartilaginous vocal fold length (CVFL) and <.033 for the membranous-to-cartilaginous (M/C) ratio. Correlation coefficient (\(R\)) was 0.79, 0.82, 0.62, and 0.10 for the TVFL, MVFL, CVFL, and M/C ratio, respectively.
Membranous-to-cartilaginous ratio

<table>
<thead>
<tr>
<th>Variable</th>
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<th>SE b</th>
<th>P Value</th>
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<td>R²</td>
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<td>P value</td>
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<tr>
<td>Membranous vocal fold length</td>
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<tr>
<td>Intercept</td>
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<td>P value</td>
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Table 2. Summary of Multiple Regression Analyses.

Age 17 years; for males, it was 1.8 (1.0-2.0) for those younger than 1 year and 1.7 (1.3-2.0) at age 17 years. Compared with the data from Hirano et al, these values are similar for children younger than 1 year, but they are approximately half as large when comparing 17-year-olds in our study with adults in Hirano et al. The mean M/C ratio in our study did not increase significantly with age as opposed to the data reported by Hirano et al. We found that the cartilaginous vocal fold was not only longer but also continued to grow enough along with the membranous vocal fold to keep the M/C ratio relatively constant.

A few factors may be responsible for the difference in our data compared with previously published data. First, we obtained our measurements in vivo under the same type of anesthetic for each patient. As noted earlier, Hirano et al and Eckel et al both used cadaveric larynges, preparing them with formalin and a plastination process, respectively. Our method likely resulted in a more physiologic state of the larynx during the measurements. Second, we had to estimate the posterior insertion point of the cartilaginous vocal fold in our patients. No clear demarcation exists along the arytenoid mucosa to delineate this exact location. Last, using a Miller or Lindholm laryngoscope might have placed some tension on the glottis during the measurement process, possibly resulting in slight lengthening of the true vocal folds.

When looking at the growth patterns for each vocal fold length in both sexes, we found that the TVFL, MVFL, and CVFL all increase in a linear manner as we age. The TVFL, MVFL, and CVFL were not statistically different between males and females. Hirano et al also found no evidence that there is a rapid increase in the length of any portion of the vocal folds corresponding to the age of vocal mutation (puberty), but they reported that the TVFL and MVFL were longer in males than in females at about ages 10 to 15 years. Likewise, Harries et al followed males progressing through puberty with serial vocal fold ultrasounds and observed no significant increase in vocal fold length to account for their patients’ sudden drop in fundamental frequency.

Our study had a few limitations. Although we measured more than 200 patients, fewer were older adolescents. Despite this fact, we had many patients at the critical ages of fundamental frequency change for both females and males. If we had had approximately 120 females rather than 87, we might have been able to assess whether the MVFL of males indeed increased more quickly compared with females. Otherwise, our sample size appeared to be adequate in showing a linear increase in the MVFL as well as the other vocal fold lengths. Next, our method of vocal fold length measurement required some estimation. We estimated our lengths based on the vocal fold measuring sticks but could not ensure that we were measuring exactly at the posterior insertion point of the cartilaginous vocal fold. Despite this estimation, our MVFLs were similar to previously published data; however, the CVFLs were roughly twice as long, which could have been the result of overestimating the CVFL. Last, our patients were under a general anesthetic, which has been shown to elongate vocal folds in adults. Evaluating younger children while awake would not be possible given our current measurement devices.

In conclusion, this is the largest longitudinal pediatric study specifically examining vocal fold length as a function of age. Each length of the true vocal fold appeared to linearly increase for both females and males. The M/C ratio remained relatively constant, unlike previously reported data, possibly due to in vivo vs cadaveric measurements. These findings suggest that the critical periods of vocal development in females and males are not explainable by changes in vocal fold length alone, and other factors such as vocal fold layers need further exploration.

Authors’ Note

Major Rogers is a military service member. This work was prepared as part of his official duties. Title 17 U.S.C. 105 provides
that “copyright protection under this title is not available for any work of the United States Government.” Title 17 U.S.C. defines a “United States Government work” as a work prepared by a military service member or employee of the US government as part of that person’s official duties.

**Author Contributions**

Derek J. Rogers, concept and design, wrote article, acquisition/analysis/interpretation of data, critical revision for intellectual content, final approval, agree to be accountable for all aspects of work; Jennifer Setlur, collected data, critical revision for intellectual content, final approval, agree to be accountable for all aspects of work; Nikhila Raol, collected data, critical revision for intellectual content, final approval, agree to be accountable for all aspects of work; Rie Maurer, acquisition/analysis/interpretation of data, formal statistical analysis, critical revision for intellectual content, final approval, agree to be accountable for all aspects of work; Christopher J. Hartnick, concept and design, acquisition/analysis/interpretation of data, critical revision for intellectual content, final approval, agree to be accountable for all aspects of work.

**Disclosures**

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**Supplemental Material**

Additional supporting information may be found at http://otojournal.org.

**References**