MORPHOMETRIC PARAMETERS OF LARYNX IN YOUNG NORMOPHONIC ADULTS USING ULTRASONOGRAPHY: PRELIMINARY STUDY

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JULY, 2024

CERTIFICATE

This is to certify that this dissertation entitled **"Morphometric Parameters of Larynx in Young Normophonic Adults Using Ultrasonography: Preliminary Study"** is a bonafide work submitted as a part fulfilment for the Degree of Master of Science (Speech-Language Pathology) of the student with the Registration Number: P01II22S123026. This has been carried out under the guidance of a faculty of this institute and has not been submitted earlier to any other University for the award of any other Diploma or Degree.

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CERTIFICATE

This is to certify that this dissertation entitled **“Morphometric Parameters of Larynx in Young Normophonic Adults Using Ultrasonography: Preliminary Study”** has been prepared under my supervision and guidance. It is also been certified that this dissertation has not been submitted earlier to any other University for the award of any other Diploma or Degree.

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DECLARATION

This is to certify that this dissertation entitled **"Morphometric Parameters of Larynx in Young Normophonic Adults Using Ultrasonography: Preliminary Study"** is the result of my own study under the guidance of Dr. R Rajasudhakar, Associate professor in Speech Sciences, Department of Speech- Language Sciences, All India Institute of Speech and Hearing, Mysuru, and has not been submitted earlier to any other University for the award of any other Diploma or Degree.

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Dedicated to my LORD JESUS CHRIST and my PARENTS

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“தேவன் அருளிய சொல்லிமுடியாத ஈவுக்காக அவருக்கு ஸ்தோத்திரம்- 2 கொரிந்தியர் 9:15”

“Thanks be unto God for his unspeakable gift”- 2 Corinthians 9:15

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Please enjoy this study, acknowledging that "Every study is a learning experience, building on trial and error to advance knowledge"

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CHAPTER I

INTRODUCTION

Ultrasonography (USG) has gained popularity as it is a non-invasive, simple diagnostic tool for laryngologists and speech-language pathologists (SLPs) to assess laryngeal functioning. Other imaging modalities, like high-speed digital imaging, videokymography, and stroboscopy, directly visualizes the vocal folds but they have limitations like high imaging data and are more expensive. Computerized Tomography (CT) and Magnetic Resonance Imaging (MRI) are relatively longer procedures and presents with exposure of radiation. USG serves as a convenient and accessible alternate because it is non-invasive, patients can tolerate it better and it can be used safely. According to recent research, USG offers augmented methodological homogeneity for assessing laryngeal functioning (Allen et al., 2021) and can be a dependable technique for evaluating vocal folds (Kumar et al., 2018).

The severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) that caused the COVID-19 global pandemic has had a significant impact on otolaryngology practice (Cho et al., 2020). Through droplets and aerosols produced during aerosol generating procedures (AGPs) like endoscopy, the virus is highly contagious. Since they are frequently exposed to viral transmission through aerosol particles during ENT clinical examinations, endoscopic procedures, and head and neck surgeries, otolaryngologists are well-known to be at-risk subjects for potential infection (Anagiotos et al., 2021). The goal of current recommendations is to reduce aerosol generation as much as possible. From this standpoint, endoscopic examinations should be avoided whenever possible and only performed strictly when necessary (risk of malignancy, obstruction of the airway) in both emergent and elective situations (Reddy et al., 2020). According to Balakrishnan et al. (2020), otolaryngologists are mandated to seek alternative approaches for diagnosis and treatment, and to prioritize non-aerosol-generating procedures whenever feasible. The transcutaneous laryngeal ultrasonography (TLUSG) has gained popularity for laryngeal assessment and airway management as it is a non-invasive and well-tolerated technique. Because TLUSG doesn't produce any aerosol, it has been suggested as a safer examination to direct flexible laryngoscopy, particularly in light of the current COVID-19 pandemic (Noel et al., 2020; Sciancalapore et al., 2021). The assessment of vocal fold mobility, the execution of cricothyroidotomy or laryngeal electromyography, the evaluation of swallowing behavior, the treatment of pediatric dysphonia and stridor, and the evaluation of laryngeal cancers are just a few of the otolaryngological diagnostic and therapeutic procedures in which TLUSG may be involved (Sciancalapore et al., 2023).

The physiological and anatomical characteristics of the laryngeal system, including the length of muscles, the adaptability of the speech valve, and the examination of mucosal waves, can be investigated using USG (Barberena et al., 2014). Due to its high visibility rate of the thyroid and cricoid structures, studies have found that USG is a viable, supplemental, and alternative technique for assessing the larynx (Arruti et al., 2010). When diagnosing vocal fold palsy, USG has proven to be a non-invasive, economical and reliable method for evaluating vocal folds with high sensitivity and specificity. Preoperatively, vocal fold movement can be assessed via refolded video examination or by measuring the interarytenoid distance. In individuals who have had thyroidectomy, USG has also been shown to have a high sensitivity for identifying recurrent laryngeal nerve palsy (Lazard et al., 2018). It has been used as a benchmark for calculating the vocal folds' areas and angles in adults with good health condition. In addition to providing information on vocal fold mobility, USG can provide highly specific characteristics such as vocal fold lengths (VFL) and vocal fold displacement velocity (VFDV). The mucosal wave velocity is a component represented by the VFDV. This measure has been suggested as a substitute for evaluating the stiffness of the vocal folds, which is necessary for the production of mucosal waves. VFDV is a measure of the stiffness of the vocal fold cover and physiological state (Rai et al., 2023). It has been demonstrated that VFL and VFDV measurements taken during active and passive maneuvers are useful in identifying certain laryngeal diseases (Wong et al., 2015). Internal structural movement may limit the precision of conventional techniques for measuring VFL, but USG can measure VFL accurately even during motion-based tasks like singing and phonation (Cho et al., 2012). The VFL which is commonly used to investigate the vocal fold behavior, is generally measured from the edge of the vocal process to the anterior commissure of the arytenoid cartilage. Metrics like VFL and VFDV have been studied with different tools like direct laryngoscopy and high-speed video endoscopy but only few studies have examined the utility of USG for assessing such parameters. Currently there are very few studies available for laryngeal examination on phononormals, despite the fact that USG has shown to be a very effective tool for laryngeal assessment in clinical practice.

A laryngologist or speech language pathologist will typically evaluate voice disorders subjectively (Toran and Lal, 2009). Of the various vocal assessments available, acoustic analysis is the one that gives an understanding of the process of sound signal generation and provides an estimate of the vocal fold vibration patterns, vocal tract conformation, and laryngeal physiology of populations with varying age groups and genders in an indirect manner (Hirano and Bless, 1997). Furthermore, this evaluation draws conclusions about laryngeal function and has been applied extensively in clinical practice and research for diagnosis and treatment monitoring (Demirhan et al., 2016; Kent et al., 2021). It also sheds light on the normal aging and growth changes that occur in the voice of healthy individuals (Sthathopoulus et al., 2011; Spazzapan et al., 2022). Age as well as gender are important factors to consider when creating databases to be used as a reference for the analysis of voice disorders because these variables have a significant impact on voice (Kent et al., 2021). Acoustic voice analysis gives measures like fundamental frequency (F0), intensity (amplitude), perturbation (jitter and shimmer), harmonic to noise ratio (HNR), and dynamic vocal range (Wang et al., 2004). The number of times a sound wave produced by the vocal folds repeats in a given amount of time is the fundamental frequency (F0), and it is measured in Hertz. It also refers to the number of cycles in which the glottis opens and closes (Teixeira et al., 2013). According to Casper and Leonard (2006), F0 reflects the patient's vocal physiological limitations. The vocal fold will exhibit a small fluctuation in both F0 and amplitude from cycle to cycle during sustained vibration; these phenomena are referred to as jitter (frequency perturbation) and shimmer (amplitude perturbation), respectively (Wang et al., 2004). These changes are a reflection of minor changes in the neural control of vocal folds as well as their mass, tension, and biochemical characteristics. The perception of voice roughness and hoarseness is correlated with perturbation (Casper and Leonard, 2006).

Cepstral measurements can recognize and quantify noise and periodicity in an acoustic wave without determining the sound wave's individual cycles (Hillenbrand et al., 1994). In Cepstral Peak Prominence (CPP), the cepstral peak stands out in relation to noise level in acoustic signal, and cepstral measurements present the harmonics emerging from Fundamental Frequency (F0). Consequently, the degree of sound energy and periodicity both have an effect on the cepstral peak. As a result, acoustic signals with higher noise levels and less periodicity have a cepstral peak with less definition and amplitude (Hillenbrand et al., 1994; Sujitha and Pebbili, 2022). Because cepstral measures are strong indicators of the presence of vocal deviation, they have been reported to be more reliable for evaluation purposes than time-based acoustic measures of disturbance and noise (such as jitter, shimmer, and noise-harmonic ratio) (Lopes et al., 2022). A modification to the CPP algorithm, the Cepstrum Peak Prominence Smoothed (CPPS) measure involves smoothing the cepstrum prior to extraction of the cepstral peak. In other words, the CPPS value is smoothed prior to extraction, making it more accurate when analyzing voices with disorders (Maryn et al., 2015). The cepstrum is calculated every 2 ms in CPPS, whereas in CPP, it is calculated every 10 ms. Yang et al. (2014) delineates that there is insufficient data on CPPS for children with velopharyngeal insufficiency following cleft palate surgery, despite the fact that CPPS is recognized for its higher accuracy in assessing individuals with voice disorders. They explain that this gap exists because authors also add that this is because the majority of studies examining the acoustic characteristics of healthy children's voices did not incorporate the CPPS into their examination.

Need for the Study

USG has been used to visualize the true vocal fold mobility since late 1980’s. The high-frequency ultrasound gives visualization of the airway structures and trachea. It is a quick, easy, and widely accessible noninvasive technique and can also be used for emergency larynx investigation at patient’s bedside. A recent study by Wong et al. (2017) using Transcutaneous Laryngeal Ultrasonography, showed 88.9% sensitivity in detecting the vocal fold palsy before thyroidectomy. Vats et al. (2004) insisted that since USG is non-invasive in nature, it aids in the diagnosis of vocal fold paralysis in children. USG has the advantage of real-time motion analysis during breathing or phonation with high temporal resolution.

Study done by Rai et al. (2023), sought to use USG to establish normative data for two anatomical characteristics of the vocal folds (vocal fold displacement velocity and vocal fold length) in young normophonic individuals aged from 18 to 30 years. More research is required to determine the accuracy of this quantification method because there is a paucity of literature in the reference standard available to assess the accuracy of sonographic measurements of laryngeal structures. The broad use of USG as a tool for assessing patients has been hampered by the absence of sufficient normative data. It is necessary to investigate the acoustic correspondence of the vocal fold parameters obtained through USG with parameters like fundamental frequency, frequency range, jitter, and cepstral peak prominence smoothed in normophonic adolescents because the USG parameters may probably have an association with the acoustic voice correlates. Thus, the present investigation was conducted to bring to light the importance of utilization of USG in the diagnosis by healthcare professionals.

Aim of the study

The present study aimed to investigate the morphometric parameters of Larynx in young normophonic adults using Ultrasonography.

Objectives of the study

1) To document or measure the morphometric parameters of Larynx in young normophonic adults using Ultrasonography in the age range of 18 to 25 years.

2) To investigate the following on the morphometric parameters of adult larynx;

- ➢ To document the differences between the groups (Males and Females)

- To document the differences between the tasks/ maneuvers (Silent Breathing and /a/ phonation)
- To document the differences in the Left and Right sides (or Laterality of VFs) with respect to VFDV, VFL, VFW and A-P TC length.

3) To determine the correspondence or correlation between the laryngeal parameters acquired using USG (VFL and VFDV of /a/ phonation) and the acoustic voice parameters like F0, jitter and CPPS of /a/ phonation.

4) To postulate the gender differences in the acoustic parameters of voice (F0, Jitter and CPPS).

CHAPTER II

REVIEW OF LITERATURE

2.1 USG in Voice profiling

Ultrasound has been used for diagnosing laryngeal pathologies since 1960 (Hamlet and Reid, 1972). The high-frequency ultrasound gives visualization of the airway structures and trachea. It is a quick, easy, and widely accessible non-invasive technique and can also be used for emergency larynx investigation at a patient's bedside. Echoes from the free margins of the vocal folds were discovered by Tamura et al. in 1973. Larynx was effectively visualized by Noyekin (1977) using ultrasonography. Since the late 1980s ultrasound has been used for visualization of true vocal fold mobility. In 2007, Huang et al. reported the use of high frequency ultrasound probes for improved image resolutions of the larynx. A recent study by Wong et al. (2016) using Transcutaneous Laryngeal Ultrasonography, showed 88.9% sensitivity in detecting the vocal fold palsy before thyroidectomy. Vats et al. (2004) insisted that since USG is non-invasive in nature, it aids in the diagnosis of vocal fold paralysis (VFP) in children. USG has the advantage of real-time motion analysis during breathing or phonation with high temporal resolution.

2.2 USG Vs Laryngoscopy and Imaging procedures

Laryngoscopy is considered gold standard in evaluation of vocal and valvular functions of the glottis but is invasive and an aerosol (minute particles that are suspended in the air which can aid in the transmission of pathogens) generating procedure (Wang et al. 2011). Though Computerized Tomography (CT) and Magnetic Resonance Imaging (MRI) play important roles in the diagnosis of head and neck diseases, they cannot reveal the mobility of the vocal folds in real time. Respiratory and motion artifacts often occur in CT and MRI examination and impact the image quality, especially in children with vocal fold immobility, so sedation is usually required. The laryngeal cartilages remain uncalcified in children and appear the same as soft tissue in a CT image, making them difficult to distinguish (Hudgins et al., 1987). Further disadvantages are the need for contrast agents and the radiation load of CT. It has the potential to deliver significantly greater radiation doses to children than to adults and in view of their greater susceptibility to radiation effects, care should be taken to avoid unnecessary CT examinations (Khursheed et al., 2002). In an ultrasound image, the rim was so clear that it was easy to distinguish the vocal fold and arytenoid cartilage. Hence, it enables the sonographer to precisely interpret the laryngeal landmarks in which the lesion is present. Since it is a dynamic examination, laryngeal structures can be easily visualized during respiration and phonation thereby allowing us to view vocal folds in abduction as well as in adduction (Wang et al., 2011). Few studies have described the ability of ultrasound to detect laryngeal diseases examined Laryngeal Ultrasonography (LUS) results alongside indirect laryngoscopy (Schade et al., 2003, Rubin et al.,2004, Sirikci et al., 2007). Also, a couple of Indian studies have been done where they determine the accuracy of USG in assessing the VF mobility and comparing it with the Laryngoscopy (Subramaniasamy et al., 2023, Murthy and Bhatia, 2019).

The first study to describe the application of Laryngeal Ultrasonography (LUS) for analyzing benign lesions in children was done by Bisetti et al. (2009). They attempted to compare the findings of fiberoptic-flexible laryngoscopy (FFL) with those of laryngeal ultrasonography (LUS) in order to assess the potential of LUS to identify benign vocal fold lesions in children. 16 children in the age range of 3 to 13 years were included in the study. A Pentax FNL-7RP3 fiberscope (Pentax S.r.l., Milano, Italy) coupled to an ATMOS Medizin Technik Gmb H& CoKG, Leuzkirch, Germany, was used to carry out an FFL procedure. To prepare for the nasal endoscopy, three drops of 4% lidocaine hydrochloride and 1/4% Neo-Synephrine were sprayed in each nostril. The larynx was examined during respiration and phonation tasks. FFL took about ten to fifteen minutes on average, including topical anaesthesia and the explanation of the process. Following the FFL, LUS was carried out using an ATL 5000 (ATL, Bothell, Washington) ultrasound with a linear 7.5–12.0 MHz broadband probe. The laryngeal structures were viewed by making the subject lie in supine position with a fully extended neck. The LUS was carried out using axial and sagittal scanning with anterior-posterior and cranio-caudal ultrasonic bean incidence during normal respiration, followed by the phonation of vowels /a/, /e/, and /i/. The procedure took less than ten minutes on average to complete, including the explanation of the process to the subject. Fourteen of the sixteen patients had vocal fold lesions on FFL. Nine patients had bilateral vocal fold nodules, two more had vocal fold cysts, and two other children's vocal folds appeared normal. There was only one patient with laryngeal papillomatosis, vocal fold polyp, and irregular vocal folds. Without any complaints, all 16 of the study's participants underwent LUS. For each of the 14 patients with vocal fold lesions, LUS made the appropriate diagnosis. Three patients had hypoechoic lesions, while ten patients had bilateral hyperechoic lesions. Two children had no lesions found, and one patient had a monolateral hyperechoic lesion. The images of both the FFL and LUS images showed the nodules clearly. LUS was even able to measure the size of the nodules. The LUS and FFL findings matched in the majority of the cases. The results of this study indicate that LUS may be a promising substitute for FFL not only in regards to vocal fold paralysis but also in regards with the vocal fold lesions. The small sample size, its variability, and the potential impact of other uncontrolled factors like age, on the LUS data, are the limitations of this study.

Wong et al. (2016) aimed to evaluate the accuracy of hoarseness, voice related questionnaire [Voice handicap Index (VHI- 30)] and transcutaneous laryngeal ultrasound (TLUSG) in diagnosing Vocal fold palsy (VFP), as well as the role of TLUSG in the evaluation of high-risk patients. Patients were considered as “high-risk” if they had voice complaints, history of head & neck or mediastinal operation at risk of RLN palsy, or if they were operated for a suspicion of malignancy. 1000 patients with a median age of 53 years (males=213 and females=787) undergoing thyroidectomy or other endocrine-related neck procedures were prospectively included. A preliminary interview was conducted by a nurse with each patient, asking the standard question, "Did you have any hoarseness of voice?" Subsequently, they were given a validated Chinese version of the Voice Handicap Index - 30 (VHI-30) questionnaire to complete. The influence of voice impairment on the emotional, physical, and functional domains is measured using the validated 30-item VHI-30 questionnaire. Then the patient underwent TLUSG which was performed by a surgeon using a portable USG machine (iLookTM 25 Ultrasound System, Sonosite®, SonoSite Inc., Washington, United States) and a 5-10 MHz linear transducer (L25). Whenever feasible, three sonographic landmarks—false vocal folds, true vocal folds, and arytenoids—were identified. TLUSG involved both passive (silent breathing) and active (phonating by sustaining the vowel, "aa") maneuvers from June '12 to January '14 (phase 1). Furthermore, a Valsalva maneuver was carried out along with the other two maneuvers from February '14 to September '15 (phase 2). Right after the TLUSG procedure, laryngoscopic examination (LE) was done which was performed by a competent laryngologist using flexible trans-nasal laryngoscopy (Olympus BF-P40, Bronchoscope, Olympus®, Tokyo, Japan). Both the laryngologist and the surgeon assessing TLUSG were not informed about the mutual results, scores of VHI-30, or the quality of voice.

The accuracy of VHI-30, TLUSG, and hoarseness in the diagnosis of Vocal fold palsy (VFP) were assessed. The study found out that the sensitivity of hoarseness is low in determining the presence of VFP. Using Laryngoscopic examination (LE), 3 patients were diagnosed to have VFP out of 64 patients who had hoarseness of voice. Compared to hoarseness, VHI-30 was more sensitive and capable of distinguishing VFP from normal; however, it saved fewer patients from LE (79.1% vs. 93.9%) but it has certain limitations when it comes to VFP identification. 38 patients in this study were illiterate, which made it impossible for them to complete the questionnaire. Another reason was the failure to comply with the thirty questions in the questionnaire. TLUSG saved more than 90% of patients from LE as it had the highest accuracy in identifying VFP and typical vocal folds when compared to hoarseness and VHI-30. Additionally, it had a high sensitivity of about 88.9% in diagnosing VFP. The examination takes only about 30 seconds to 2 minutes, and it is not dependent on patient effort or literacy. Compliance by patients is not an issue as it causes very little discomfort and is non-invasive in nature.

Maneuvers offering better assessability and diagnostic accuracy were also assessed in this study. Passive maneuver typically yields more false positive results than either an active or Valsalva maneuver (5.4% vs. 2.3% vs. 2.5%, p=0.054); sensitivity and accuracy were similar. However, compared to active and Valsalva maneuvers, passive maneuver was better able to distinguish VFP from typical mobile VFs. Hence the study strongly advises carrying out all three maneuvers. A VF would be regarded as abnormal or as having VFP if movement is restricted or does not move at all during any of the maneuvers. The study finds that TLUSG is more sensitive and precise in identifying VFP pre-operatively than both hoarseness and a validated questionnaire. TLUSG was able to assess the VFs of 92.4% of the patients under examination, and had a high sensitivity of 87.5%. TLUSG could prevent an LE in more than 87% of the patients if it was carried out as the vocal fold's initial line of pre-operative examination. It is recommended that TLUSG be included in the head and neck ultrasound examination.

Few limitations of the above study are that since all TLUSG were carried out by a single examiner, it is yet to be seen if more examiners will be able to produce results that are satisfactory. Secondly, this study lacks sufficient power to evaluate the accuracy of diagnosis of pre-operative TLUSG. However, this research represents the largest series on pre-operative TLUSG with laryngoscopic validation that has been published to date. Only nine pre-operative VFP were found in this study, despite the inclusion of 1000 patients. Because pre-operative VFP is not so common, it is very challenging to carry out a study on an assessment tool with adequate power. Lastly, the VFs of about 6% of patients were unable to evaluate using TLUSG due to calcification. Especially for male subjects, a technique that maximizes assessability needs to be developed.

2.3 USG in Children

Although Flexible Laryngoscopy (FLS) is considered as the gold standard in the evaluation of laryngeal disorders especially in the pediatric population, there are some limitations in this procedure. The most common limitation is poor cooperation by the patient especially in neonates and infants. Furthermore, the risk of laryngospasm increases when FLS is performed on children with obstruction of the larynx. These limitations can be overcome by the use of USG as it is non- invasive in nature and aids in the proper visualization of vocal folds (Vats et al., 2004), unlike FLS where the ENT might get only a glimpse of the vocal folds due to poor cooperation of children (Wang et al., 2011).

Garel et al. in 1990 took the initiative to view and locate the normal sonographic anatomy of the larynx in children. Their purpose was to explore the potential of ultrasound imaging for assessing the larynx in healthy individuals, from fetuses to children to evaluate the clinical relevance of this new way of imaging the normal and pathological larynx. Normal sonographic anatomy of the larynx was established by studying 40 healthy children, aged from 2 days to 15 years. The sonograms were performed with real-time equipment (HITACHI EU B 410 and 450) using a 7.5 MHz transducer and an equivalent-fluid path interposed between the neck of the patient and transducer. Patients underwent sonography in the supine position, with the neck well extended, during quiet breathing, deep inspiration and expiration and phonation for the older children. Ultrasonographic images that were obtained from three 22 weeks-old foetuses (dead in utero during a multiple pregnancy) were correlated with the anatomic preparations to discover the normal ultrasonographic anatomy of the fetal larynx. They identified various cartilages by measuring the distance separating them from the skin and the anterior margin of the cervical vertebra. The true vocal folds appear as two triangular hypoechoic structures on the ultrasound image. During deep inspiration, the glottis is widely open and the vocal folds are abducted. The glottis is closed by the adduction of the vocal folds during deep expiration. The vocal folds tighten and adduct during phonation and this is easily observed by the vibration movement. The false vocal folds, positioned just above the true vocal folds appear as two hyperechoic triangles. The thyroid cartilage appears as a reversed V-shaped structure and is hypoechoic at the centre with a hyperechoic margin. Its shape changes as the child ages. The angle of thyroid cartilage appears smoother in neonates than in the elder children. The arytenoids appear as two hypoechoic structures surrounded by a hyperechoic margin. They are located on either side of the midline, within the posterior part of the thyroid lamina, behind the vocal folds. During quiet breathing, little change in the position of the vocal folds was observed whereas during deep breathing, the abduction-adduction motions of those cartilages were well appreciated.

The cricoid cartilage appeared as a round, hypoechoic structure. Due to the subglottic air that creates an acoustic shadow, imaging of the posterior aspect of the cricoid is hindered. But in the fetal specimen, the posterior aspect of the cricoid could be easily seen. The epiglottis is hyperechoic. The base was visible and its antero-posterior movements during swallowing was seen. The shape of the epiglottis varies according to the age of the child. In the neonates, the shape of the epiglottis was sharper than in older children. It was concluded that laryngeal sonography appears as a novel, highly reproducible and non-invasive method of examining these structures. Additionally, they mentioned that it permits good analysis of the cartilaginous structures, false and true vocal folds and can even study the dynamic aspect of the larynx.

Garel et al. in 1992 studied normal and pathological findings in infants and young children using Laryngeal USG. They briefly described the normal sonographic anatomy; the pathological findings, its advantages and its drawbacks were put forth. Normal sonographic anatomy of the larynx was established by studying 40 healthy children, aged from 2 days to 15 years and three fetuses of 22-weeks-old. Thirty- three children presenting a laryngeal pathology were studied, including 5 functional disorders and 28 organic disorders. This study put forth that this method is less invasive than endoscopy and does not require any preparation. Also, it allows sometimes to foresee the diagnosis before the endoscopic examination and especially a good technique for functional disorders and space-occupying lesions.

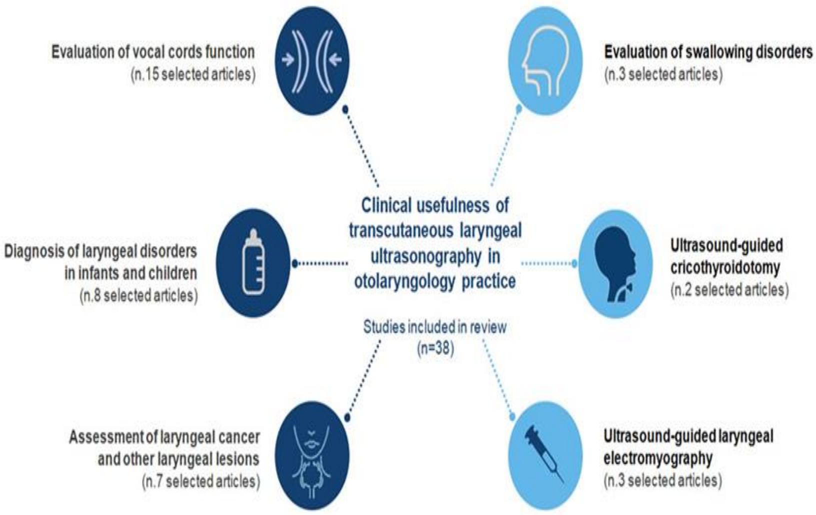
Non-invasive nature of ultrasound was stressed by Vats et al. (2004) to diagnose vocal fold paralysis in children. The study assessed the practicality and validity of laryngeal ultrasound to establish vocal fold movement in children with suspected vocal fold palsy. Fifty- five consecutive patients (age range varied from three days to 12 years) with suspected vocal fold palsy underwent both laryngoscopy and laryngeal ultrasound. Ultrasonographic findings correlated with endoscopic findings in 81.2 percent of cases. Authors concluded that Laryngeal ultrasound is well-tolerated, safe and non-invasive and the authors feel that it is a useful adjunct to endoscopy in the diagnosis of vocal fold palsy.

Sciancalepore et al. (2022) did a review of literature upto March, 2022

with the aim of investigating the clinical usefulness of TLUSG in otolaryngology practice, since the SARS- CoV-2 had a profound impact on the utility of endoscopy in laryngeal assessments. Thirty- eight studies with a total of 5056 participants were eligible to be included in the review. The selected papers were divided into 6 topics of interest, depending on the field of application of Transcutaneous Laryngeal Ultrasonography (TLUSG), namely, a) evaluation of the function of vocal folds, b) evaluation of swallowing disorders, c) Ultrasound- guided cricothyroidotomy, d) Ultrasound- guided laryngeal electromyography, e) assessment of laryngeal cancer and other laryngeal lesions and f) diagnosis of laryngeal disorders in infants and children. The same has been described in Figure 2.3.1 below.

Figure 2.3.1

Articles pertaining to the clinical uses of transcutaneous laryngeal ultrasonography (TLUSG) in otolaryngology practice



Sciancalepore et al. (2023)

Alexander et al. (2021) stated that TLUSG decreases aerosolization (process of converting a liquid or solid substance into minute particles known as aerosoles that become suspended in the air which can potentially increase the transmission risk for pathogens that can spread via these aerosols) during COVID-19 pandemic and may serve as an alternative tool of visualizing the larynx in children who cannot tolerate laryngoscopy. Many authors like Wang et al. (2011), Alexander et al. (2021), and Vats et al. (2004), have demonstrated that laryngeal ultrasound can be used to evaluate vocal fold function, allowing early detection of vocal fold paralysis in children. Zawadzka-Glos et al. (2013) reported that the evaluation of vocal folds mobility is more accurate if compared with laryngoscopy under general anesthesia and easily leads to the diagnosis of fractures of cartilages without dislocation. COVID-19 pandemic brought to the attention of otolaryngologists the need to practice diagnostic and treatment strategies alternative to aerosol generating endoscopic procedures. The results of this review demonstrated that transcutaneous laryngeal ultrasonography has proven to be a valid method for dynamic laryngeal assessment and airway management.

2.4 USG in Adults

The first attempt to obtain the sonographic anatomy of the Larynx, with particular reference to the vocal folds was done by Raghavendra et al. in 1987. There was no previous report concerning the use of B-scan ultrasound for the assessment of vocal fold morphology. So, the study aimed at describing the normal sonographic anatomy of the vocal folds using an ultrasound scanner. Sonographic images of the vocal folds were obtained in 41 healthy human subjects using a phased array real time ultrasound scanner. The symmetry of movement of the vocal folds during respiration becomes apparent on examination in real time. Hence, the authors concluded that Sonography may prove to be a potentially useful technique for the examination of the vocal folds.

Hu et al. (2010) attempted to identify the normal sonographic values of human true and false vocal folds on 229 chinese healthy individuals who were divided into 8 groups according to their age (<18 years, 18-39 years, 40-59 years, 60 years and older) and gender (males and females). Length, width, and thickness of the true and false vocal folds were measured using high-frequency ultrasound. Measurements were compared between groups, and correlations with age were analyzed. Forty of the 229 volunteers also participated in reliability and reproducibility studies. The sonographic measurements had good reproducibility, with intraclass correlation coefficient ranging between 0.736 and 0.903 for interobserver reliability; and between 0.723 to 0.943 for intraobserver reliability. Measurements for the 3 parameters of both true and false vocal folds in male adults were greater than those in female adults ($p < 0.001$). The length, width, and thickness of true and false vocal folds in participants younger than 18 years were obviously correlated with age ($r = 0.835–0.957$; $p < 0.001$), but no significant correlation was found in the adult groups. The rates of visualization in male groups were significantly lower than those in female groups ($p < 0.001$) and gradually decreased with increasing age. It was concluded that both true and false vocal folds can be visualised, assessed, and measured quantitatively by using high frequency sonography, which can with good reliability and reproducibility.

Nasser and Aleem (2019) evaluated the validity of sonography as an imaging tool in identifying normal ranges and abnormalities of laryngeal dynamics and anatomic structures and to determine the optimal scanning technique in terms of transducer selection and orientation. A cross-section study was done on 60 patients (25 males and 35 females) in the age range of 21- 62 years who were scheduled for neck surgery for different causes. Thirty patients who had no clinical signs or symptoms that are indicative of laryngeal pathology or paralysis of the vocal folds were clustered into the first group. Patients who had abductor type of unilateral vocal fold paralysis (n=30) were included in the second group. All the 60 patients underwent full clinical history, complete head and neck examination, flexible fiberoptic and/or indirect laryngoscopy and sonographic examination. The sonography was performed using Ultrasound Logiq P5 with a linear 7-12 MHz high-frequency ultrasound transducer. All the patients were instructed to lie down with their necks extended in supine position. The larynx was scanned in the Transverse plane and in the Oblique-transverse plane. Patients were asked to do full inspiration and phonation.

Authors identified immobile arytenoid cartilages on the paralyzed side during both inspiration and phonation with sensitivity (80%) and specificity (90%). Another important finding is that the percent of change in interarytenoid distances was much less in group II (patients with unilateral vocal fold paralysis) than in group I (participants with no clinical pathology) which could be attributed to limited mobility of paralyzed vocal fold. The interarytenoid distance was observed to be greater during respiration than during phonation with a mean percent of change in interarytenoid length of 57.57±14.41%. In patients with unilateral fold abductor paralysis, the interarytenoid distances during phonation was lower with mean value of 0.39±0.21cm which was significantly different than normal group with mean value of 0.43 ±0.18cm. This finding is due to the fact that in case of unilateral abductor VF paralysis, the vocal fold is fixed near mid line due to unopposed adductor effect, so the distance will be less than the control group.

Hence, it is concluded that examining the vocal fold mobility preoperatively proved to be valid in identifying the various laryngeal structures with high potency in judging vocal fold mobility either by short real time cine videos or measuring interarytenoid distances. Few limitations reported by the authors include non-fitting surface of the linear probe to neck contour, and concurrent movement of thyroid cartilage. Patients were instructed not to hold their breath and avoid swallowing movement. Usually, vocal fold movement can be plainly observed during quiet respiration. Laryngeal cartilage calcification also leads to variable imaging and poor visualization of intra laryngeal structure such as the vocal folds and arytenoid cartilage.

2.5 Indian Studies

Few Indian studies have been carried out where the indications, findings and limitations of USG in the evaluation of laryngeal and laryngopharyngeal cancers were postulated (Desai et al., 2004); tested the accuracy of USG and Computed Tomography (CT) while assessing thyroid cartilage invasion in patients with airway cancer (Dhoot et al., 2017); evaluated the feasibility of USG for assessing vocal fold mobility as an alternative to videolaryngoscopy to rule out recurrent laryngeal nerve injury following thyroidectomy (Shah et al., 2019). Kumar et al. (2018) assessed the functionality of vocal folds using ultrasound before and after thyroid surgery.

Subramaniasamy et al. (2023) examined the diagnostic accuracy by correlating the vocal fold mobility determined by ultrasonography with laryngoscopy results, incorporating patients from varying age groups. For one year, a prospective pilot study was conducted. The study comprised of 60 patients in total (33 males and 27 females), who were divided into two groups according to the results of the Hopkin's 70° rigid laryngoscopy. Patients in group I had bilateral normal vocal fold mobility who were scheduled for intrathoracic surgery, thyroidectomy, or any other procedure that could result in vocal fold paralysis. Patients in group II had vocal fold palsy, either unilaterally or bilaterally, for any reason. The two groups comprised thirty patients each. All the patients underwent Ultrasonography of the larynx. The ultrasonologists were blinded to the laryngoscopy results. An ultrasound system of model RS-80A (Samsung Medison, Republic of Korea) was used for the performing ultrasonography. Using a linear probe at a frequency of 3–16 MHz, the imaging was carried out in B mode. The patient was advised to extend their neck while lying in supine position. To see the vocal folds in the transverse plane, a linear probe was positioned over the thyroid cartilage at various angles. Vocal fold imaging in real time was evaluated. During silent breathing, the vocal folds were detected and the mobility of the VF was determined during sustained phonation of the vowel "e". The movement was verified in the Sagittal plane also.

In 56 of these patients (i.e. 93%), the vocal folds had been examined and the mobility was analyzed. The results were found to be consistent with the laryngoscopy findings in each of these patients. USG was unable to visualize the vocal folds in four patients (three in group II and one in group I) (i.e. 6.6%). This resulted from severe calcification of the thyroid cartilage, which prevented the sonography waves from passing through any acoustic window in any angulation of the probe. The vocal folds could be visualized properly for all the females. However, four of the 33 males who were older than 40 had calcified thyroid cartilage and hence were unable to visualize their vocal folds. In children and adults under 40, there was a 100% correlation with the results of the vocal fold examination and the mobility test. It was discovered that patients older than 40 years of age had a higher incidence of thyroid cartilage calcification. Out of the 17 patients who were above the age of 40, 13 patients (76.5%) were able to undergo vocal fold assessment using the previously described angulation technique, even though they had calcified thyroid cartilage. In group I, findings of 29 individuals were confirmed with ultrasonography and one was not well appreciated in the USG. Whereas in group II, 27 findings were confirmed with ultrasonography and three were not well appreciated in the USG. USG had a sensitivity of approximately 90% and a specificity of 96.67% in diagnosing vocal fold palsy. The authors conclude that USG can be optimally utilized because it is not an invasive procedure that produces aerosols like laryngoscopy and it can be used effectively during the COVID-19 pandemic or any other respiratory disease pandemic with appropriate precautions, minimizing the risk of respiratory infection transmission. Also, when a patient is unable to undergo laryngoscopy or when the necessary equipment is unavailable, this tool may be used to its full potential.

Another study done by Murthy and Bhatia (2019), reported that in suspected cases of vocal fold paralysis (VFP), USG can be used as the main instrument or as an alternative to laryngoscopy to assess the movements of vocal folds. They assessed the usefulness of USG in diagnosing the movements of vocal folds in suspected cases of VFP and then its results were compared with the findings of fiberoptic laryngoscopy. A total of 73 clinically suspected cases of VFP were studied (of which 32 were females and 41 were males). The study utilized GE Voluson Ultrasound equipment from the Radio-diagnosis department, JSS Hospital, Mysore. The vocal folds were examined using a high-resolution linear probe (9–12 MHz). The movements of the vocal folds during phonation and breathing were observed. The outcomes were compared to those from fiberoptic laryngoscopy, and statistical analysis was performed. During fiberoptic laryngoscopy, 45 (61.64%) of the 73 clinically suspected patients with vocal fold palsy were diagnosed with VFP. Compared to fiberoptic laryngoscopy, USG had the ability to identify the same condition in 42 (93.33%) cases, but it was unable to do so in 3 (6.67%) cases because the cartilage was calcified excessively. The utility of ultrasound to diagnose vocal fold palsy was found to have 93.33% sensitivity and 100% specificity. When it comes to identifying vocal fold palsy, USG has more sensitivity and specificity than fiberoptic laryngoscopy. Hence, the authors concluded that ultrasound is an inexpensive, non-invasive, and reliable method which is extensively available to evaluate movements of vocal folds with the advantage of real-time visualization of the vocal folds during silent breathing and normal phonation.

Study done by Rai et al. (2023), sought to use USG to establish normative data for vocal fold biomarkers like vocal fold morphology, vocal fold symmetry, gender and task-specific data for vocal fold length (VFL) and vocal fold displacement velocity (VFDV) in young normophonic adults in the age range of 18–30 years. Initially 54 participants (27 males and 27 females) were recruited for the study. Individuals who scored ‘normal’ grade on GRBAS scale and whose phonatory functions are normal were taken into the study. Subjects who engaged in vocal abuse, smoking, had vocal fold pathologies or irregularities in their vocal tract, who reported a history of voice disorders or infection of the upper respiratory tract, neck pathology, reflux conditions, dense thyroid cartilages, or poor visualization during USG were excluded from the study. Based on the above-mentioned criteria, four participants were excluded leaving 50 individuals were finally involved in the study. Participants were made to lie in supine position with their necks stretched and USG was performed using 3D/4D GE LogiqV5 transcutaneous B-mode ultrasound with a multi-frequency linear array transducer that operates at frequencies between 5-12 MHz, positioned horizontally on the sides of the thyroid cartilage. USG parameters like vocal fold morphology and symmetry, VFL and VFDV were acquired across three maneuvers- normal breathing, phonation of /a/ vowel and phonation of the vowel /i/. Acoustic analysis was done using Praat software (Version 6.2.03) and a headphone [Jabra Evolve (40 UC)] which has a built-in microphone was used to record the samples in a noise-free environment. The subjects were asked to maintain a relaxed, upright sitting position with the microphone being placed at a distance of 5 cm away from the mouth at an angle of 45°. They were asked to sustain the phonation of the vowels /a/and /i/ and for 3 to 5 seconds. Three trials were given for each maneuvers and the best sample was selected for further analysis of acoustic measures like fundamental frequency (F0), jitter, and cepstral peak prominence (CPP). Furthermore, the participants were asked to glide their pitch while sustaining on the vowel /a/ and the range of frequency was analyzed. Correlation analysis was done to determine if there is any relationship between the acquired parameters of Ultrasonography and acoustic measures.

Authors reported that males have longer vocal folds than females in all tasks for the left and right vocal folds. VFL was significantly longer during /a/ phonation, followed by /i/ phonation, and quiet breathing for the left and right vocal folds. The mean VFL for females during silent breathing (SB) was reported to be 2.08 cm (SD=0.24 cm) and 2.06 cm (SD=0.25 cm) for left and right VF and it was 2.56cm (SD=0.30 cm) and 2.56 cm (SD=0.28 cm) for left and right VF, respectively in males. During /a/ phonation, VFL was reported to be 2.31cm (SD=0.35 cm) and 2.31 cm (SD=0.34 cm) among females; and 3 cm (SD=0.32 cm) and 2.99 cm (SD=0.32 cm) among males for left and right VF, respectively. The length of the vocal folds during /i/ phonation was 2.22 cm (SD=0.33 cm) and 2.21cm (SD=0.33 cm) in females and 2.81cm (SD=0.28 cm) and 2.8 cm (SD=0.28 cm) in males for left and right VF, respectively. Overall greater velocities were observed in /a/ phonation, followed by /i/ phonation, with the lowest velocity observed in the quiet breathing task. The mean VFDV for females during SB was 8.52 cm/s (SD=1.15 cm/s) and 8.78 cm/s (SD=2.30 cm/s) and for males, it was 7.64 cm/s (SD=1.79 cm/s) and 7.82 cm/s (SD=2.05 cm/s) for the left and right VF. During /a/ phonation, the velocity was found to be 14.5 cm/s (SD=6.09 cm/s) and 14.14 cm/s (SD=6.19 cm/s) among women; and 13.22 cm/s (SD=4.0 cm/s) and 13.15 cm/s (SD=3.93 cm/s) among men for left and right VF, respectively. The VFDV for /i/ phonation was 12.36 cm/s (SD=2.75 cm/s) and 12.28 cm/s (SD=2.88 cm/s) among females; and 11.48 cm/s (SD=2.74 cm/s) and 11.83 cm/s (SD=2.72 cm/s) among males for left and right vocal fold, respectively. The study used Pearson’s correlation to investigate the relationship between ultrasound parameters (VFL AND VFDV) and the acoustic measures (F0, frequency range, jitter and CPP) for /a/ and /i/ phonation. For /a/ phonation, a statistically significant moderate negative correlation was reported between VFL and F0. No significant correlations were observed between VFL, jitter, and CPP. Furthermore, a statistically significant weak inverse correlation was obtained between the frequency range derived during pitch glide /a/ and the VFL, indicating that, as the VFL increased, the frequency range decreased. For /i/ phonation, a statistically significant moderate negative correlation was obtained between VFL and F0, indicating that as VFL increased, F0 decreased. No such correlations were reported between other parameters. It was also found that there was no significant correlation between USG parameter, VFDV and acoustic parameters (F0, frequency range, jitter and CPP).

The authors concluded that USG provides specific parameters like VFDV and VFL which are useful in diagnosing voice disorders. The authors delineate that the obtained norms can serve as a quantitative benchmark for evaluating vocal fold function in young adults. Few limitations of the study are that the study used a smaller sample size. Larger sample sizes are necessary to achieve better population representation. Also, vocal loudness was not monitored during USG assessment as it provides better insights.

However, while this sounds promising the reproducibility of these results need to be confirmed. Also, most of the studies available in literature focussed on assessing the accuracy and feasibility of USG in evaluating the vocal fold mobility and has been compared with the results of CT and VLS. But there is a dearth of Indian literature regarding the data for laryngeal parameters like vocal fold length, vocal fold displacement velocity, vocal fold width, horizontal length of thyroid cartilage and interarytenoid space in adults using USG. Also, since USG and acoustic parameters may be related, there is a need to study the correlation between USG parameters and acoustic parameters like fundamental frequency, jitter and cepstral peak prominence smoothed as it would augment the voice researchers and enhance the clinician's diagnostic skills. Hence, the present study is carried out to bring to light the importance of utilization of USG in the diagnosis by healthcare professionals.

CHAPTER III

METHOD

3.1 Participants

A total of 53 phononormal individuals in the age range of 18 - 25 years were enrolled in the study. The study employed convenient sampling method to choose participants. Study subjects were classified as Group A (30 males) and Group B (23 females).

3.1.1 Inclusion criteria

- Participants in the age range of 18 to 25 were taken into the study.
- Participants with normal phonatory functions ensured with perceptual voice rating scale (GRBAS) were included in the study. Those who scored 'Zero' (0) on 'G' in GRBAS rating scale were considered.

3.1.2 Exclusion criteria

- Participants reporting to have a history of any voice problems or respiratory infections, pathologies surrounding the neck, reflux disorder, vocal tract irregularities, vocal fold pathologies, smoking or vocal abuse were excluded from the study.
- At the time of USG examination, participants with high-dense laryngeal cartilages or poorly visualised laryngeal structures were excluded from further investigations of the study.

Based on the above-mentioned criteria, few individuals (8 males and 1 female) were excluded from the study during the USG refolding due to poor visualization of the laryngeal structures. Hence, a total of 44 participants (22 males and 22 females) were considered for the acoustic voice analysis.

3.2 Instrumentation

3.2.1 USG examination

USG testing was performed with 3D/4D Mindray DC-80 Transcutaneous B-mode Ultrasound using L9-3E probe (a multifrequency linear array transducer with the frequency range of 3 MHz -9 MHz) from the Department of Radiology of Mysore Medical College and Research Institute (MMC & RI), Mysore.

3.2.2 Acoustic voice analysis

Acoustic analysis was done using Praat software (Version 6.4.12) which was installed on a laptop (DELL Inspiron 15 3000, core i5 11^{th} generation) with Boya ByM1 microphone and was saved with a sampling frequency of 44.1 kHz and was saved in .wav file format.

3.3 Procedure

Each participant was informed about the aim and objectives of the research and then obtained a written informed consent which is attached in Appendix A. The entire data collection procedure was carried out adhering to AIISH ethical committee guidelines for Bio-Behavioural Sciences for Human Subjects (AEC, 2009). In order to collect details on the study's selection criteria, a thorough case history was taken. Perceptual voice screening was carried out using the GRBAS scale. Participants who met the selection criteria were taken to MMC & RI where the USG examination was done. Subjects who successfully completed the USG examination then underwent acoustic voice analysis.

3.3.1 USG examination

The Ultrasound procedure was performed by a qualified, experienced radiologist from (MMC & RI), Mysore. The participants were made to lie in supine position with their neck stretched out for better visualization of laryngeal structures. They were instructed to stay still until the procedure is done in order to get clear images. A thin layer of gel was applied to the skin in the Laminae of Thyroid cartilage (Acoustic window) and the radiologist moved the gel transversely over the laryngeal area with the transducer to get the image. The radiologist also used transverse oblique and longitudinal planes when the visualisation was poor. Doppler gate was set at 3 to 5 mm and the scale and line shift were adjusted to achieve the highest amplitude possible with a frame rate of 52 Hz. USG was performed across two maneuvers- Silent breathing (SB) and /a/ phonation. Measurement of the vocal fold length (VFL), width of the vocal folds (VFW), antero- posterior length of the thyroid cartilage (A-P TC), vocal fold displacement velocity (VFDV) and interarytenoid (IA) space were done during silent breathing. During phonation of /a/ vowel, VFL and VFDV were measured. Measurements of both right and left vocal folds were obtained individually for all the parameters except for the IA space. Vocal Fold Length was measured between the anterior commissure and the tip of the vocal process (Oyamada et al., 2005). The width of the vocal fold was measured at the midportion, perpendicular to the length of the fold (Hu et al., 2010). The A-P length of the thyroid cartilage was measured from the laryngeal prominence (Adam’s apple) to the posterior part of the thyroid cartilage. The distance between the hyperechoic arytenoid cartilages was measured as the Interarytenoid distance (Nasser et al., 2020). The procedure was carried out for 5-7 minutes approximately for each individual.

3.3.2 Acoustic voice analysis

The participants were made to sit comfortably and straight in a quiet testing room while refolding the data. A microphone, angled to 45° was positioned 8 cm away from the participant's mouth to refold the samples. For three trials, participants were asked to phonate the vowel /a/ for about five to eight seconds. The best out of the three samples was selected for analysis. In each sample, leaving the first and the last one second, the middle 3 to five seconds were considered for acoustic voice analysis and measures such as Fundamental frequency (F0), jitter and cepstral peak prominence smoothed (CPPS) were extracted.

3.4 Statistical Analysis

The statistical analysis was performed using SPSS (version 26) software. Mean and Standard deviation of the morphometric parameters of larynx were obtained using descriptive statistics. To assess the normality of the collected data, the Shapiro-Wilk’s test of normality was applied. Seven outliers were present, out of which 3 were males and 4 were females After confirming normal distribution, suitable parametric tests were chosen for conducting the subsequent statistical analysis. Three-way Mixed ANOVA was carried out to study the main effect factors and interaction effects of the laterality of vocal folds (Rt vs Lt), gender (Males vs Females) and tasks (Silent breathing vs Phonation of /a/ for VFDV and VFL. Tasks and Laterality of vocal folds were considered as factors varying within subjects, while gender was a factor varying between subjects. To study the main effect factors and interaction effects of gender

(Males vs Females) and Laterality of vocal folds (Rt vs Lt) and thyroid cartilage (Rt vs Lt) for parameters like VFW and A-P TC length, Two- way mixed ANOVA was used. Vocal folds were considered as a factor varying within subjects, and gender was a factor varying between subjects. Comparison of IA space between genders was done using Independent samples t-test. Gender differences on acoustic parameters (F0, Jitter and CPPS) was analysed using MANOVA test. Pearson's correlation was conducted to see if any correlation exists between the USG parameters like VFL and VFDV during phonation of vowel /a/ and acoustic parameters like F0, jitter and CPPS during /a/ phonation.

CHAPTER IV

RESULTS

The aim of the current study was to investigate the morphometric parameters of larynx in young normophonic adults using Ultrasonography.

The objectives of the study were

1) To document or measure the morphometric parameters of larynx in young normophonic adults using Ultrasonography in the age range of 18 to 25 years.
2) To investigate the following on the morphometric parameters of adult larynx;
   - To document the differences between the groups (Males and Females)
   - To document the differences between the tasks/ maneuvers (Silent Breathing and /a/ phonation)
   - To document the differences between the Left and Right vocal folds with respect to VFDV, VFL, VFW and A-P TC length.
3) To determine the anatomical correspondence of the laryngeal parameters acquired using USG (VFL and VFDV of /a/ phonation) with the acoustic voice parameters such as F0, jitter and CPPS of /a/ phonation.
4) To postulate the gender differences in the acoustic parameters (F0, jitter and CPPS)

The following statistical tests were performed to analyze the above-mentioned objectives using the software “Statistical Package for Social Sciences (SPSS, Version 26.0)”.

1. Shapiro-Wilk’s test of normality
2. Descriptive statistics

3. Three- way Mixed ANOVA
4. Two- way mixed ANOVA
5. Independent samples t-Test
6. MANOVA
7. Pearson's correlation

The results of this study are discussed in the following sub-headings;

4.1 Test of normality

4.2 Morphometric parameters of larynx

4.3 Comparison of VFDV and VFL using three- way Mixed ANOVA

4.4 Comparison of VFW and A-P TC using two- way Mixed ANOVA

4.5 Comparison of IA space between genders

4.6 Comparison of acoustic parameters between genders

4.7 Correlation of acoustic parameters with USG parameters

4.1 Test of normality

Shapiro-Wilk's test of normality was used to find out the nature of distribution of the data. All the parameters like VFDV, VFL, VFW, IA space, A-P TC length, F0, jitter and CPPS followed a normal distribution (p-value>0.05). Hence, Three-way Mixed ANOVA was carried out to study the main and interaction effects of maneuvers (Silent Breathing and /a/ phonation), gender (Males and Females) and vocal folds (Right and Left) on parameters like VFDV and VFL. Two- way mixed ANOVA was used to study the main and interaction effects of gender (Males and Females) and vocal folds and thyroid cartilage (Right and Left) on parameters like VFW and A-P TC length. Comparison of IA space between genders was done using Independent samples t-test. Gender differences on acoustic parameters (F0, jitter and CPPS) were acquired using MANOVA. Pearson's correlation was conducted to see if any correlation exists between the USG parameters such as VFL and VFDV during /a/ phonation and acoustic parameters like F0, jitter and CPPS during /a/ phonation.

4.2 Morphometric parameters of larynx

To obtain the morphometric parameters of larynx like VFDV, VFL, VFW, A-P TC and IA space across two tasks for males and females, descriptive statistics was performed. The mean, standard deviation, 95% confidence interval for mean, minimum and maximum values for the refolded parameters are enumerated separately for males in Table 4.2.1 and for females in Table 4.2.2. The mean values for all the parameters are illustrated individually for males in Figure 4.2.1 and for females in Figure 4.2.2.

Table 4.2.1

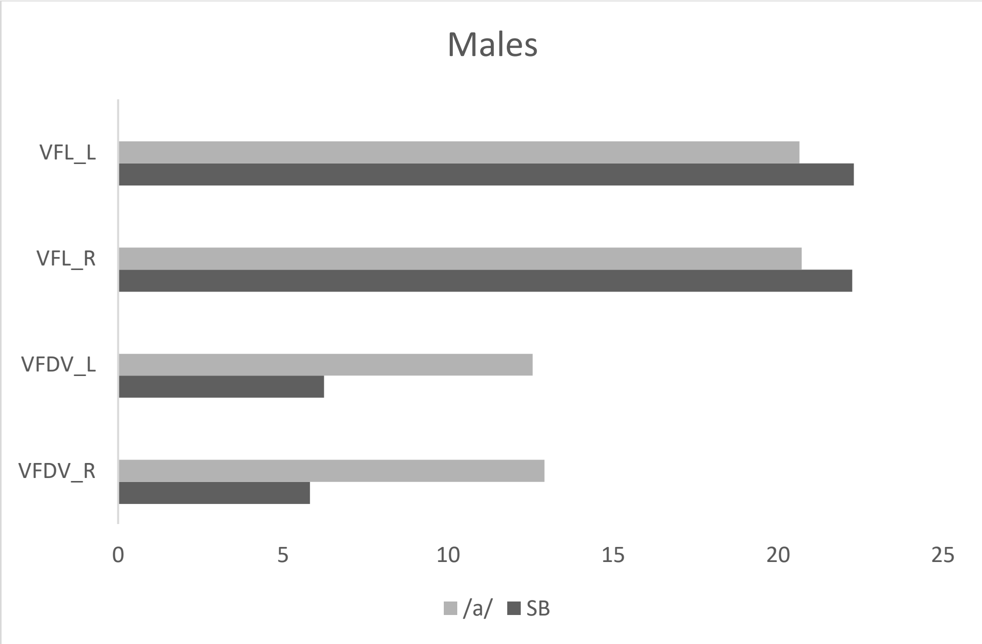
Mean and SD of USG and acoustic voice parameters of males during the two tasks

Parameters	Task	Mean	SD	95% confidence interval for mean		Minimum	Maximum
				Lower bound	Upper bound		
VFDV_R (cm/sec)	SB	05.81	0.74	05.45	06.17	04.50	06.86
VFDV_L (cm/sec)	SB	06.24	0.74	05.88	06.60	04.58	07.44
VFDV_R (cm/sec)	/a/	12.92	0.92	12.48	13.37	10.91	14.30
VFDV_L (cm/sec)	/a/	12.56	1.04	12.05	13.06	10.30	14.46
VFL_R (mm)	SB	22.25	2.06	21.26	23.25	17.40	24.90
VFL_L (mm)	SB	22.30	2.18	21.24	23.36	17.10	26.60
VFL_R (mm)	/a/	20.72	2.31	19.61	21.84	16.90	24.80
VFL_L (mm)	/a/	20.65	2.39	19.49	21.80	17.90	25.50
VFW_R (mm)	SB	05.46	0.69	05.12	05.79	04.10	06.50
VFW_L (mm)	SB	05.78	0.72	05.43	06.13	04.40	07.00
APTC_R (mm)	SB	20.28	1.65	19.48	21.08	17.20	23.30
APTC _L (mm)	SB	20.47	2.01	19.50	21.44	17.30	25.10
IA Space (mm)	SB	12.66	2.40	11.50	13.82	07.30	18.50
F0 (Hz)	/a/	139.58	16.10	131.81	147.34	106.07	170.86
Jitter (%)	/a/	0.293	0.114	0.237	0.348	0.15	0.51
CPPS (dB)	/a/	13.92	1.92	12.99	14.85	10.57	18.20

(*VFDV= Vocal Fold Displacement Velocity, VFL= Vocal Fold Length, VFW= Vocal Fold Width, APTC= Antero-posterior Thyroid cartilage length, IA Space= Interarytenoid space, R=Right, L= Left, F0= Fundamental frequency, CPPS= Cepstral peak prominence smoothed, SB= Silent breathing, /a/= /a/ phonation, SD= Standard Deviation, mm=millimeter, Hz= Hertz, dB= decibel).

Figure 4.2.1

Mean values of VFL and VFDV between the two tasks in males



(*VFL= vocal fold length, VFDV= Vocal Fold Displacement Velocity, R=Right, L= Left, SB= Silent breathing, /a/= phonation of /a/ vowel)

During Silent breathing, the VFDV for right and left VF was found to be 5.81cm/s (SD=0.74cm/s) and 6.24cm/s (SD=0.74cm/s). VFDV during /a/ phonation was found to be higher than VFDV during SB with a mean of 12.92cm/s (SD=0.92cm/s) for right and 12.56cm/s (SD=1.04cm/s) for left VF. The mean length of right and left VF was 22.25mm (SD=2.06mm) and 22.30mm (SD=2.18mm) respectively, which was higher than the mean of right (20.72mm with SD=2.31mm) and left (20.65mm with SD=2.39mm) VFL during /a/ phonation. The mean of right (mean=5.46mm, SD=0.69mm) and left (mean=5.78, SD=0.72mm) VFW did not differ much in their thickness. The same trend was seen in the A-P thyroid cartilage length, where the mean of right and left A-P TC was identified as 20.28mm (SD=1.65mm) and 20.47mm (SD= 2.0mm), respectively. The mean IA space was measured to be 12.66mm (SD= 2.40mm) during SB.

Table 4.2.2

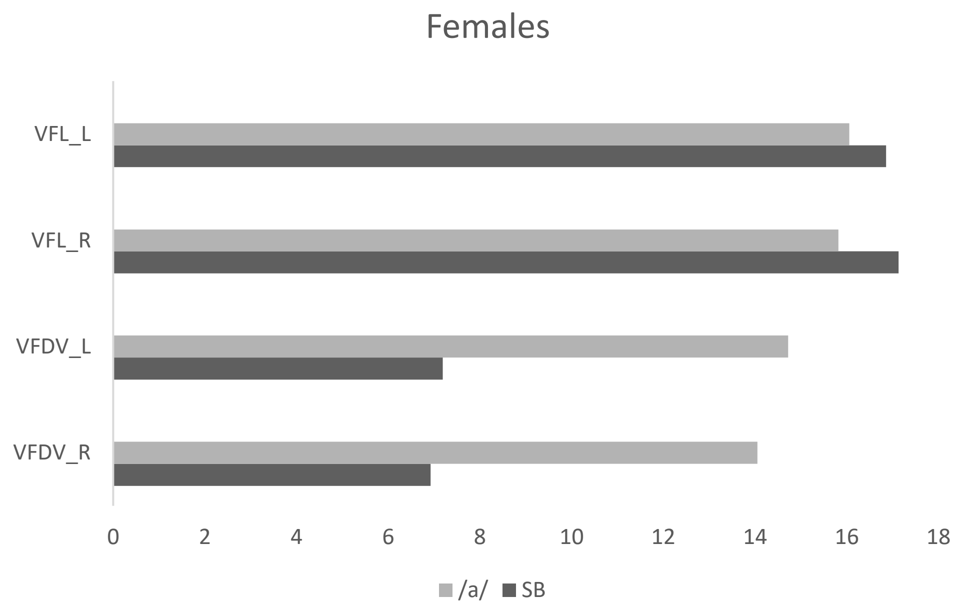
Mean and SD of USG and acoustic voice parameters of females during the two tasks

Parameter	Task	Mean	SD	95% confidence interval for mean		Minimum	Maximum
				Lower bound	Upper bound		
VFDV_R (cm/sec)	SB	06.92	1.31	06.27	07.57	05.15	09.50
VFDV_L (cm/sec)	SB	07.19	1.42	06.48	07.90	04.80	09.97
VFDV_R (cm/sec)	/a/	14.05	2.52	12.80	15.31	08.80	17.45
VFDV_L (cm/sec)	/a/	14.72	2.36	13.54	15.89	09.91	19.07
VFL_R (mm)	SB	17.13	2.01	16.13	18.13	13.80	19.80
VFL_L (mm)	SB	16.86	2.17	15.78	17.94	14.00	21.00
VFL_R (mm)	/a/	15.82	1.49	15.07	16.56	12.80	17.90
VFL_L (mm)	/a/	16.06	1.68	15.22	16.90	12.70	19.00
VFW_R (mm)	SB	05.85	1.05	05.33	06.37	03.50	07.70
VFW_L (mm)	SB	05.75	1.12	05.19	06.30	04.10	07.80
APTC_R (mm)	SB	17.27	1.47	16.53	18.00	15.20	20.20
APTC _L (mm)	SB	17.66	1.64	16.84	18.48	13.80	19.90
IA Space (mm)	SB	10.93	1.68	10.10	11.77	07.50	14.00
F0 (Hz)	/a/	220.82	10.43	215.63	226.01	197.69	238.44
Jitter (%)	/a/	0.32	0.15	0.24	0.40	0.13	0.68
CPPS (dB)	/a/	12.13	2.26	11.07	13.32	8.22	16.98

(*VFDV= Vocal Fold Displacement Velocity, VFL= Vocal Fold Length, VFW= Vocal Fold Width, APTC= Antero-posterior Thyroid cartilage length, IA Space= Interarytenoid space, R=Right, L= Left, F0= Fundamental frequency, CPPS= Cepstral peak prominence smoothed, SB= Silent breathing, /a/= /a/ phonation, SD= Standard Deviation, mm=millimeter, Hz= Hertz, dB= decibel).

Figure 4.2.2

Mean values of VFL and VFDV between the two tasks in females



(*VFL= vocal fold length, VFDV= Vocal Fold Displacement Velocity, R=Right, L= Left, SB= Silent breathing, /a/= phonation of vowel /a/)

The VFDV for right and left VF was found to be 6.92 cm/s (SD=1.31cm/s) and 7.19cm/s (SD=1.42cm/s) during Silent breathing. VFDV during /a/ phonation was found to be higher than VFDV during SB with a mean of 14.05cm/s (SD=2.52cm/s) for right and 14.72cm/s (SD=2.36cm/s) for left VF. The mean length of right and left VF was 17.13mm (SD=2.01mm) and 16.86mm (SD=2.17mm) respectively, which was higher than the mean of right (15.82mm with SD=1.49mm) and left (16.06mm with SD=1.68mm) VFL during /a/ phonation. The mean of right (mean=5.85mm, SD=1.05mm) and left (mean=5.75, SD=1.12mm) VFW did not differ much in their length. The same trend was seen in the A-P thyroid cartilage length, where the mean of right and left A-P TC was identified as 17.27mm (SD=1.47mm) and 17.66mm (SD=

1.64mm), respectively. The mean IA space was measured to be 10.93mm (SD= 1.68mm) during SB.

Ultrasonographic images for all parameters are attached in Appendix B.

4.3 Comparison of VFDV and VFL using Three- way Mixed ANOVA

The Vocal Fold Displacement Velocity was compared across tasks (silent breathing and /a/ phonation), laterality (R and L vocal folds) and gender (males and females) using Three- way Mixed ANOVA. Similarly, the Vocal Fold Length also was compared across the above mentioned three independent variables using the same statistical test. Initially, the main effects for variables such as task, laterality (R-L VFs) and gender are presented. Later on, interaction effects between any of the above-mentioned variables, if present, are discussed. Table 4.3.1 shows the results of Three-way Mixed ANOVA for VFDV parameter.

a) Comparison of VFDV across tasks, sides and gender

Table 4.3.1

Results of Three-way Mixed ANOVA on VFDV across task, laterality and gender

Variables	df	F	Sig. (p value)	Partial Eta Squared
Task	1	579.39	0.00**	0.94
Laterality	1	3.71	0.06	0.09
Gender	1	13.32	0.00**	0.27
Task* Gender	1	1.10	0.30	0.03
Laterality* Gender	1	2.78	0.10	0.07
Task*Laterality		0.82	0.37	0.02
Task*Laterality* Gender	1	6.97	0.01**	0.16

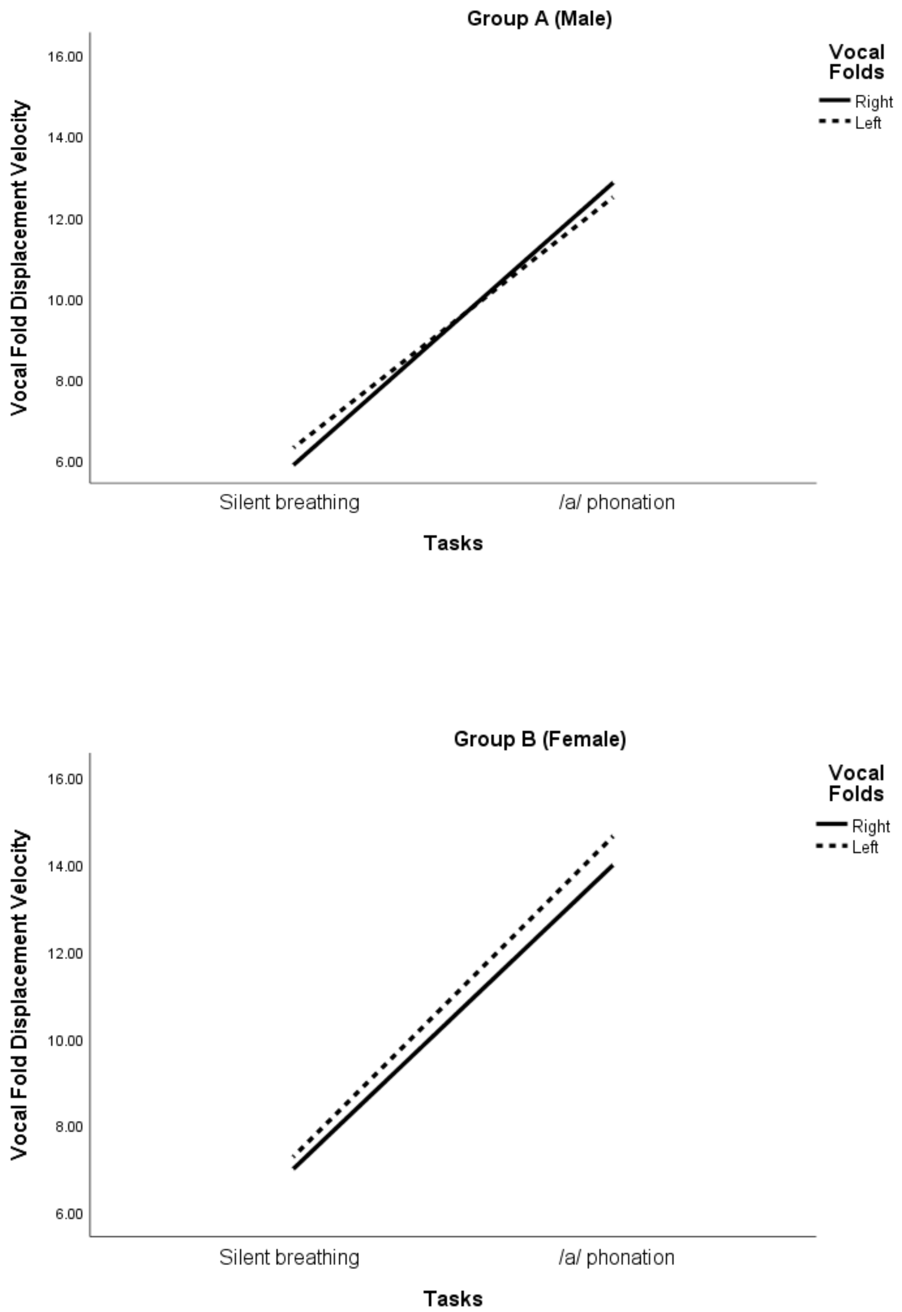
(‘**’indicate significant difference at 0.05 level)

Main Effect. Analysis revealed that the task [F(1,35)= 579.392, p<0.05] and gender [F(1,35)= 13.329, p<0.05] had statistically significant effects on the vocal fold displacement velocity [/a/ > SB, females > males], with task having a large effect size (0.943) and gender having a small effect size (0.276). This signifies that task and gender as a separate entity, influences the VFDV. Also, it was found that there is no significant effect of laterality (right and left vocal folds) on the displacement velocity of the vocal folds [F(1,35)=3.711, p>0.05].

Interaction Effect. Along with the main effects observed for tasks and gender on VFDV, an interaction effect was also observed. It was observed between the *task*laterality* gender* [F (1,35) = 6.979, p < 0.05] has interaction effect with a smaller effect size (0.166). In figure 4.3.1, profile plot option of SPSS 26 has been used to display this as a 3-way interaction plot for better understanding. The figure indicates that VFDV was significantly higher for /a/ phonation task compared to silent breathing in both males and females.

Figure 4.3.1

A three-way interactional plot of Vocal fold Displacement Velocity between two tasks, laterality and gender



The statistically significant three-way interaction effect ($p < 0.05$) indicates that task, laterality, and gender all have a significant influence on the VFDV. This implies that the effects of task and laterality on the VFDV differ depending on gender, indicating a complex interplay between these variables.

b) Comparison of VFL across tasks, laterality and gender

Table 4.3.2

Results of Three-way Mixed ANOVA on VFL across tasks, laterality and gender

Variables	df	F	Sig. (p value)	Partial Eta Squared
Task	1	22.27	0.00**	0.38
Laterality	1	0.01	0.90	0.00
Gender	1	70.59	0.00**	0.66
Task* Gender	1	0.90	0.34	0.02
Laterality* Gender	1	0.00	0.99	0.00
Task* Laterality	1	0.63	0.43	0.01
Task*Laterality* Gender	1	1.64	0.20	0.04

(‘**’indicate significant difference at 0.05 level)

Main Effect. The results showed that the task [F(1,35)= 22.271, p<0.05] and gender [F(1,35)= 70.596, p<0.05] had statistically significant effects on the vocal fold length [SB > /a/; males > females] with task having a smaller effect size (0.389), [/a/ > SB] and gender having a relatively larger effect size (0.669). This signifies that both task and gender independently impact the VFL. Also, it was found that there is no significant effect of laterality (right and left vocal folds) on the length of the vocal folds [F(1,35)=0.015, p>0.05]. That is, the vocal fold length was similar between right as well as left vocal folds.

Interaction Effect. No significant interaction effects were found between the variables for Vocal fold length. Table 4.3.2 depicts the results of 3- way ANOVA for the variables tasks, laterality of VFs and gender on VFL parameter.

4.4 Comparison of VFW and A-P TC length using Two- way Mixed ANOVA

a) Comparison of VFW across gender and laterality (R-L vocal folds)

Two- way Mixed ANOVA was used to compare the Vocal Fold Width across gender (males and females) and across laterality (the right and left vocal folds). The results of the same are tabled in Table 4.4.1.

Table 4.4.1

Results of Two- way Mixed ANOVA on VFW when comparing across gender and laterality (R-L vocal folds)

Variables	df	F	Sig. (p value)	Partial Eta Squared
Laterality	1	1.61	0.21	0.04
Gender	1	0.38	0.53	0.01
Laterality* Gender	1	6.31	0.01**	0.15

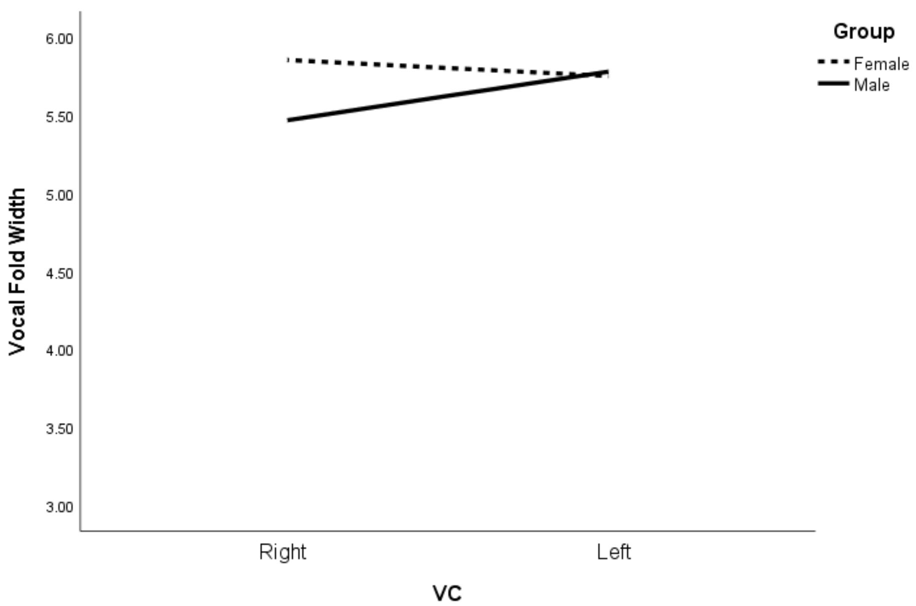
(** indicate significant difference at 0.05 level)

Main Effect. The findings suggested that laterality (right and left vocal folds) have no statistically significant effect on the width of the vocal folds [$F(1,35)=1.612$, $p>0.05$]. Also, it was found that there is no significant effect of gender on the width of the vocal folds [$F(1,35)= 0.386$, $p>0.05$]. This signifies that gender and laterality (R-L VF) does not have an impact on the width of the vocal folds.

Interaction Effect. Although main effect was not observed, interaction effect was present between the laterality (R-L VF) * gender groups [F (1,35) = 6.317, p<0.05] with a poor effect size of 0.15. Figure 4.4.1 uses the profile plot option of SPSS 26 to display this as a 2-way interaction plot for better understanding.

Figure 4.4.1

Results of two- way ANOVA (interactional plots for vocal fold width across gender and laterality [R-L VFs])



The interaction effect indicates that the relationship between gender and laterality of the right and left vocal folds has a significant influence on vocal fold width. This finding suggests that the effect of laterality on vocal fold width may vary by gender.

b) Comparison of A-P TC length across gender and R-L thyroid laminae

Two- way Mixed ANOVA was used to compare the antero-posterior length of the thyroid cartilage across gender (males and females) and laterality (right and left sides of the thyroid cartilage). The results of two- way- mixed ANOVA is depicted in Table 4.4.2.

Table 4.4.2

Results of Two- way Mixed ANOVA on A-P TC length

Variables	df	F	Sig. (p value)	Partial Eta Squared
Laterality	1	3.21	0.08	0.08
Gender	1	29.10	0.00**	0.45
Laterality* Gender	1	0.38	0.54	0.01

(** indicate significant difference at 0.05 level)

Main Effect. The data indicated that laterality (right and left thyroid cartilage) have no statistically significant effect on the antero-posterior length of the thyroid cartilage [F(1,35)= 3.217, p>0.05]. The main effect of gender on the A-P thyroid cartilage length was statistically significant [F(1,35)= 29.103, p<0.05] with a good effect size of 0.454. This signifies that gender has an impact on the antero-posterior length of the thyroid cartilage (Males> Females).

Interaction Effect. The interaction effect of Gender and laterality on the antero-posterior length of the thyroid cartilage was not found to be significant statistically- [F (1,35) = 0.382, P>0.05].

4.5 Comparison of IA space between genders

The IA space was compared between males and females using independent samples t - test. The results are tabulated in Table 4.5.1

Table 4.5.1

Results of Independent samples t-test for gender comparison on IA space.

Parameter	t	df	Sig. (2-tailed) (p value)
IA space	-2.516	35	.017*

(IA= Interarytenoid space, * indicate significant difference at 0.05 level)

Results showed significant difference between the two groups [t(35)= -2.516, p<0.05 on Interarytenoid space]. The mean IA space for group A (males) was 12.66mm (SD=11.50mm) and the mean IA space for group B (females) was10.93mm (SD= 10.10mm), indicating that the IA space was significantly larger for males than females during silent breathing.

4.6 Comparison of acoustic parameters between genders

MANOVA was used to compare acoustic parameters like F0, jitter and CPPS between genders. The findings are displayed in table 4.6.1 and 4.6.2

Table 4.6.1

Results of MANOVA for effect of group (Males and Females) on F0, Jitter and CPPS.

Acoustic Parameters	df	F	Sig. (p value)	Partial Eta Squared
F0	1	327.547	0.000*	0.903
Jitter	1	0.398	0.532	0.011
CPPS	1	6.248	0.017*	0.151

(F0= Fundamental Frequency, CPPS= Cepstral Peak Prominence smoothed, * indicate significant difference at 0.05 level)

Results showed significant effect of group on F0 (p<0.05) and CPPS (p<0.05) with F0 having larger effect size (0.903) and CPPS having smaller effect size (0.151), whereas there was no significant effect of group on jitter (p>0.05) [mean= 0.293, SD=0.11 in males; mean=0.322, SD= 2.261 in females]. It is inferred that the gender had profound influence on F0 followed by CPPS. The mean F0 in males was found to be 139.58 Hz (SD= 16.1) whereas in females it was found to be 220.82Hz (SD= 10.43). Regarding CPPS, the mean was about13.92 (SD=1.93) in males and 12.13 (SD= 2.26)] in females. Hence, it is interpreted that females had higher F0 than males and for CPPS, the results is vice versa. That is, males had higher CPPS than females.

4.7 Correlation of acoustic parameters with USG parameters

To examine the relationship between the acoustics parameters of /a/ phonation and the USG parameters of /a/ phonation, Pearson's correlation coefficient (r) was used for both males and females separately. The results for the same are presented in table 4.7.1.

Table 4.7.1

Results of Pearson's Correlation between USG and Acoustic parameters in males

USG parameters	Pearson's Coefficient	Acoustic parameters		
		F0	Jitter	CPPS
VFDV_R	r	-0.110	-0.039	-0.227
	p	0.655	0.873	0.350
VFDV_L	r	-0.417	0.346	0.010
	p	0.076	0.147	0.966
VFL_R	r	-0.346	-0.115	0.049
	p	0.147	0.639	0.843
VFL_L	r	-0.439	-0.021	-0.004
	p	0.060*	0.932	0.987

(VFDV= Vocal Fold Displacement Velocity, VFL= Vocal Fold Length, F0= Fundamental Frequency, CPPS= Cepstral Peak Prominence smoothed, R=Right, L= Left, * indicate significant correlation at 0.05 level)

Marginal moderate negative correlation was noted between left VFL and F0 (r= -0.439, p= 0.060) whereas no significant correlation was observed between the right VFL and F0. No statistically significant correlation was found between other parameters of USG and acoustic measures.

Table 4.7.2

Results of Pearson's Correlation between USG and acoustic parameters in females

USG parameters	Pearson's Coefficient	Acoustic parameters		
		F0	Jitter	CPPS
VFDV_R	r	0.073	0.315	-0.092
	p	0.773	0.203	0.718
VFDV_L	r	-0.147	0.136	0.142
	p	0.561	0.592	0.574
VFL_R	r	0.381	0.163	-0.442
	p	0.119	0.518	0.066
VFL_L	r	0.220	0.164	-0.546
	p	0.380	0.517	0.019*

(VFDV= Vocal Fold Displacement Velocity, VFL= Vocal Fold Length, F0= Fundamental Frequency, CPPS= Cepstral Peak Prominence smoothed, R=Right, L= Left, * indicate significant correlation at 0.05 level)

Statistically significant correlation was found between the left VFL and CPPS in females. A moderate negative correlation was observed between left VFL and CPPS (r= -0.546, p=0.019) whereas a marginal moderate negative correlation was found between right VFL and CPPS (r= -0.442, p=0.066). There was no statistically significant correlation between any other USG and acoustic paarmeters.

CHAPTER V

DISCUSSION

The present study investigated the morphometric parameters of Larynx in young normophonic adults using Ultrasonography. The study encompassed thirty-seven participants who were in the age range of 18 – 25 years were divided into two groups (Group A- males [19 in number] and Group B- females [18 in number]). These participants were subjected to Ultrasonography testing and acoustic analysis. USG was performed during silent breathing and /a/ phonation; acoustic analysis was performed during /a/ phonation. The study aimed at (i) measuring and investigating the morphometric parameters of Larynx using USG across tasks, sides and gender; (ii) to find out if there is any correlation between the USG parameters and acoustic parameters; and (iii) to postulate the gender differences seen in the acoustic parameters.

Descriptive statistics was used to measure Mean, Standard deviation, Minimum & Maximum and 95% confidential interval for the morphometric parameters of larynx. Since all the parameters followed normal distribution, Three – way Mixed ANOVA, Two- way Mixed ANOVA and Independent samples t- test were performed to analyse the USG parameters across the tasks, sides and gender. MANOVA was used to determine the difference in genders across the acoustic parameters. Finally, Pearson's correlation was used to find out if there is any correlation between USG and acoustic parameters. Three – way Mixed ANOVA revealed that task and gender had significant effects on the VFDV and VFL whereas no significant difference was found in the right and left vocal folds (i.e., laterality of VF as a factor). The results of Two – way Mixed ANOVA suggested that there is no significant effect of gender and laterality of VFs on VFW and that, a significant effect of gender was seen on the A-P TC length but the laterality of thyroid cartilages did not have a significant effect on the A-P length of TC. The Independent samples t- test indicated a significant difference in the IA space across gender. The MANOVA analysis yielded a significant difference between males and females across all the acoustic parameters except for Jitter. Pearson's r showed significant correlation between VFL and CPPS in females. There was no statistically significant correlation found for other parameters between USG and acoustic measures in both males and females.

The results of the current study are discussed under three headings: -

1) Comparison of USG parameters across tasks, sides and gender.
2) Correlation of USG parameters with Acoustic parameters
3) Comparison of Acoustic parameters between gender.

5.1 Comparison of USG parameters across tasks, sides and gender

Five parameters were measured using Ultrasonography, namely, Vocal Fold Displacement Velocity (VFDV), Vocal Fold Length (VFL), Vocal Fold Width (VFW), A-P Thyroid cartilage length (A-P TC) and Inter-arytenoid space (IA space). Each of the 5 parameters are discussed below in detail.

For both females and males, the VFDV was found to be higher during /a/ phonation and lower during silent breathing; and no significant difference was seen in the left and right vocal folds. But in terms of gender, females had higher velocities than males across both the tasks. Studies in the literature reported higher ranges in the displacement velocities of vocal folds (Kumar et al., 2018; Dedecjus et al., 2010). The current study found lower VFDV values, possibly due to the lateral displacement of the USG probe. Placing the USG probe medially may result in poor image quality because of reverberation artifacts (like echoes) and anisotropic ultrasound reflection (where the ultrasound waves bounce back unevenly) at the medial air-mucosa interface of vocal folds. Hence the probe was placed laterally to avoid such issues (Jing et al., 2017; Woo et al., 2017). However, the results of the current study are in acfoldance with another study wherein a similar lateral placement was used. During the production of /a/ vowel, the vocal tract opens more, following which there would be a higher vocal fold vibratory displacement. During silent breathing, vocal folds experience minimal changes and therefore, the VFDV is higher during /a/ phonation and lower during Silent breathing (SB).

The vocal fold length varied depending on the task, with longer lengths observed during SB and relatively shorter lengths during the phonation of the vowel /a/ in both males and females. No significant differences were found between the right and left vocal folds (i.e., laterality of VFs). Notably, in both tasks, males had longer vocal fold length when compared to females. The mean vocal fold length obtained in this study align with previous research (Eckel et al., 1994; Eckel et al., 1995; Su et al., 2002; Rawal et al., 2015; Mobashir et al., 2018; Rai et al., 2023). The results obtained for task factor was not in accordance with the previous study done by Rai et al. (2023) and this might be due to the difference in sample size (smaller sample size compared to the study mentioned above) or the plane in which the transducer was placed while measuring the structures.

In the present study, during silent breathing, there were no variations noted in the width of the vocal folds across gender and laterality of VFs. But the investigation done by Hu et al. (2010) reported significant difference across gender in the width of the vocal folds. The results of the present study did not support the findings of Hu t al.

(2010) study. The differences in the findings may probably be due to methodological differences between the studies, like instrument employed and sample size. The A-P length of the TC differed across gender where the males were having longer TC length than females, but there was no significant difference in the right and left side (laterality) of the thyroid cartilage. Also, The Inter-arytenoid space was found to be more in males than females. The changes across gender in all the 5 parameters could be attributed to the anatomical variations and hormonal changes (effect of testosterone) during puberty which leads to larger muscles of the vocal folds, increased mass and prominent thyroid structures in males (Hunter et al., 2011).

5.2 Correlation of USG parameters with acoustic parameters

In males, a marginal moderate negative correlation was found between the length of the left vocal fold and F0, indicating that as left VFL increases, F0 tends to decrease. However, no significant correlation was observed between VFL, Jitter and CPPS; and VFDV, F0, Jitter and CPPS. The study found a significant relationship between VFL and CPPS in females, with longer vocal folds associated with lower voice quality. No significant relationships were found between other parameters of USG and acoustic parameters in females. These findings are in accordance with the previous investigation done by Rai et al. (2023) except for two findings: moderate negative correlation between VFL and CPPS; no correlation between VFL and F0 in females. The current study used CPPS measures whereas Rai et al. (2023) used CPP measures. This could have led to the difference in the above-mentioned findings. This could possibly be attributed to the difference in the instrumentation of USG and sample size employed between the studies.

The relationship between VFL and F0 has shown that changes in pitch are related to changes in vocal fold morphology. The pitch of the voice is determined by the complex interplay between vocal fold stiffness and tension, which cause the folds to shorten or lengthen, thereby altering the pitch. The frequency of vocal fold vibrations is primarily determined by three factors: length, mass and tension. Specifically, an increase in F0 is associated with increased tension, decreased length and decreased mass of the vocal folds (Zhang, 2016).

5.3 Comparison of acoustic parameters between gender

Significant difference was noted across males and females in F0 and CPPS with males having lower F0 and higher CPPS than females whereas there was no significant difference noted for jitter values. Toran et al. (2009) performed voice analysis with sustained phonation of vowel /i/ and found that females had higher F0 than males and found no significant difference in the jitter values. Spazzapan et al. (2022) reported sex differences (males > females) in the CPPS values for phonation of vowel /a/ in Brazilian Portuguese children and adolescents aged between 5 and 18 years. Gender effect was noted from 12 years of age with males having better CPPS values than females. Study done by Hippargekar et al. (2022) in Indian population revealed no significance effect of gender in jitter and that females tend to have higher F0 than males. The results of the present study support the findings of Sujitha & Pebbili (2022), where they reported higher CPPS values in Indian males whose age range was in 20-30 years. Since women have breathy voice compared to men (Hillenbrand et al., 1994) and voices that are perceived breathy had lower CPP measures on sustained phonation (Klatt & Klatt, 1990), gender differences are seen in the CPPS measures.

CHAPTER VI

SUMMARY AND CONCLUSIONS

A wide variety of techniques have been used to assess the laryngeal functions. Imaging modalities, like high-speed digital imaging, videokymography, and stroboscopy, directly visualizes the vocal folds but they have limitations like high imaging data occupies more space for storage and are more expensive. Computerized Tomography (CT) and Magnetic Resonance Imaging (MRI) are relatively longer procedures and presents with exposure of radiation. USG serves as a convenient and accessible alternate because it is non-invasive, patients can tolerate it better and it can be used safely. USG can provide highly specific characteristics such as vocal fold lengths and vocal fold displacement velocity. VFL is commonly used to investigate the vocal fold behavior and VFDV is an indicator of the physiological condition and stiffness of the vocal fold cover. Both these measurements, during active and passive maneuvers proved to be effective in detecting specific laryngeal and extra laryngeal diseases including thyroid gland carcinoma. Many studies were focussing on assessing the accuracy and feasibility of USG in evaluating the vocal fold mobility and compared with the results of CT and Videolaryngostroboscopy (VLS). But there is a dearth of Indian literature regarding the data for laryngeal parameters like vocal fold length, vocal fold width, horizontal length of thyroid cartilage and interarytenoid space in adults using USG. Hence, the study was carried out to bring to light the importance of utilization of USG in the diagnosis by healthcare professionals.

The study aimed at investigating the morphometric parameters of Larynx in young normophonic individuals using USG. The objectives of the study were to document the morphometric parameters of Larynx and to compare across tasks, laterality of VFs and gender; find if any correlations exist between USG and acoustic parameters and to find gender differences in the acoustic parameters. Thirty-seven phononormal individuals (19 males and 18 females) in the age range of 18-25 years were enrolled in the study. Participants with normal phonatory functions who scored ‘Zero’ on ‘G’ in GRBAS perceptual voice rating scale were included in the study and those who had history of voice problems or respiratory infections, pathologies surrounding the neck, reflux disorder, vocal tract irregularities, vocal fold pathologies, smoking, vocal abuse, high-dense or poorly visualised laryngeal structures during USG procedure were excluded from the study. The participants underwent USG examination and acoustic analysis which was done using two maneuvers (silent breathing and /a/ phonation). 3D/4D Mindray DC-80 Transcutaneous Ultrasound was used to acquire UGS parameters (VFDV, VFL, VFW, A-P TC length and IA space). The participants were made to lie down in supine position with their neck stretched out for better visualization of their laryngeal structures and examination was done in two different maneuvers- SB (VFDV, VFL, VFW, A-P TC length and IA space) and /a/ phonation (VFDV and VFL). Praat software was used to extract acoustic parameters (F0, jitter and CPPS) where participants sustained phonation of the vowel /a/ for about 5-8 seconds.

Summary of the study’s findings

- VFDV was higher in females than males during both /a/ phonation and SB, with higher displacement velocities during phonation of vowel /a/. Vocal fold length varied by task, with shorter lengths during SB and longer lengths during phonation task, and was longer in males than females. No significant differences were found in vocal fold width between genders or laterality of VFs during both the tasks. The antero-posterior thyroid cartilage length was longer in males than females and the IA space was larger in males.

- A marginal negative correlation was observed between the VFL and F0 in males, and moderate negative correlation was found between VFL and CPPS in females.
- Males had lower F0 and higher CPPS than females but no significant difference in jitter values.

The study concludes that USG can provide highly specific laryngeal morphometric characteristics such as vocal fold lengths and vocal fold displacement velocity which are used to investigate the vocal fold behavior, the physiological condition and stiffness of the vocal fold cover. Both these measurements, during active and passive maneuvers would probably suggest in detecting specific laryngeal diseases. Acoustic parameters provide an estimate of the vocal fold vibration patterns, vocal tract conformation, and laryngeal physiology. Present study found a marginal to moderate correlation of one USG parameter (VFL) between few acoustic parameters (F0 and CPPS). Thus, the study has highlighted the use of USG in the field of voice and laryngeal research.

Clinical Implications of the study

- The current study's findings would augment the voice researchers on Laryngeal metric details.
- Vocal fold palsy can also be detected using the Interarytenoid distance and vocal fold displacement velocity, which would enhance clinician's diagnostic skills.

- The study's research findings bring to light the importance of utilization of USG in the diagnosis of laryngeal diseases by healthcare professionals.
- As there is no data for certain USG parameters like vocal fold width, inter-arytenoid space and anteroposterior length of the thyroid cartilage in Indian scenario, the study’s findings can serve as a reference data in vocally normal individuals in the age range of 18 - 25 years.

Limitations of the study and Future directions

- Inter-rater reliability was not done. This would strengthen the outcome measures.
- Investigation was done in a smaller population, could be done on a larger sample size.
- The study was done only during silent breathing and further research during different maneuvers like deep breathing, Valsalva maneuver, phonation of other vowels like /i/, /e/, /o/ and /u/ vowels.
- The target population was only young adults. More research can be done on children and adolescents to document the pubertal changes.

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APPENDIX A

ALL INDIA INSTITUTE OF SPEECH AND HEARING, MYSURU-06
DEPARTMENT OF SPEECH-LANGUAGE SCIENCES

INFORMED CONSENT

Information to the participant

I, Ms. Sharon Esther S, II MSc (SLP) student, as a part of my postgraduate research program, am studying the "Morphometric parameters of Larynx in young normophonic adults using ultrasonography: Preliminary study", under the guidance of Dr. R Rajasudhakar, Associate Professor of Speech Sciences, Department of Speech Language Sciences, AIISH, Mysuru. This study aimed to investigate the size of certain laryngeal structures (voice box) and its function. The study involves Ultrasonography and Acoustic voice analysis. The participant will be taken to the Department of Radiology, KR Hospital, Devaraja Mohalla, Mysuru for performing the Ultrasound scan in the throat. This procedure is unharming and has only research benefits and the participants will not receive any financial benefits from it. There is no influence or pressure of any kind by the investigator or the investigating institute to your participation. Your kind co-operation in the study is needed in helping me to understand the structural dimensions and functions of Larynx (voice box) as it would benefit patients with voice disorders.

Consent for participation

I have been informed about the aims, objectives, and the procedure of the study. The possible risk-benefits of my participation as human subject in the study are clearly understood by me. I will also be given the opportunity to ask questions about the study. I understand that I have the right to refuse participation as participant or withdraw my consent at any time. I am also aware that by subjecting myself to this study, I will have to give adequate time for assessments done by the investigator. The specific needs for assessment and instructions have been explained to me. I have the freedom to write to the AIISH Ethical Committee chairman in case of any violation of these provisions without the danger of me being denied of any rights to avail the clinical services at this institute. I hereby give my full consent for enrolling in the laryngeal investigation program.

I, ____, the undersigned, give my consent to be a participant for this study

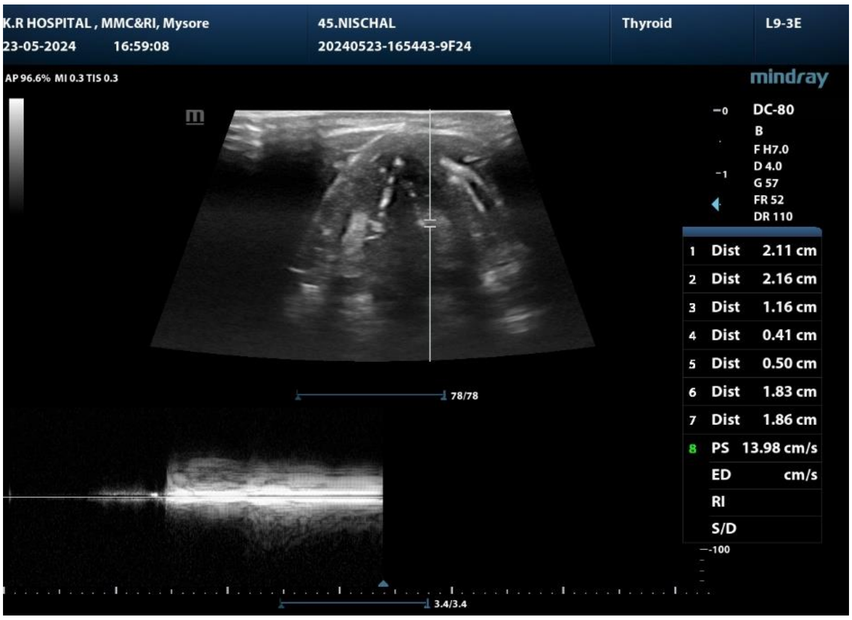
Signature of the participant   Signature of the investigator

Name, address, and phone number: Date:

APPENDIX – B

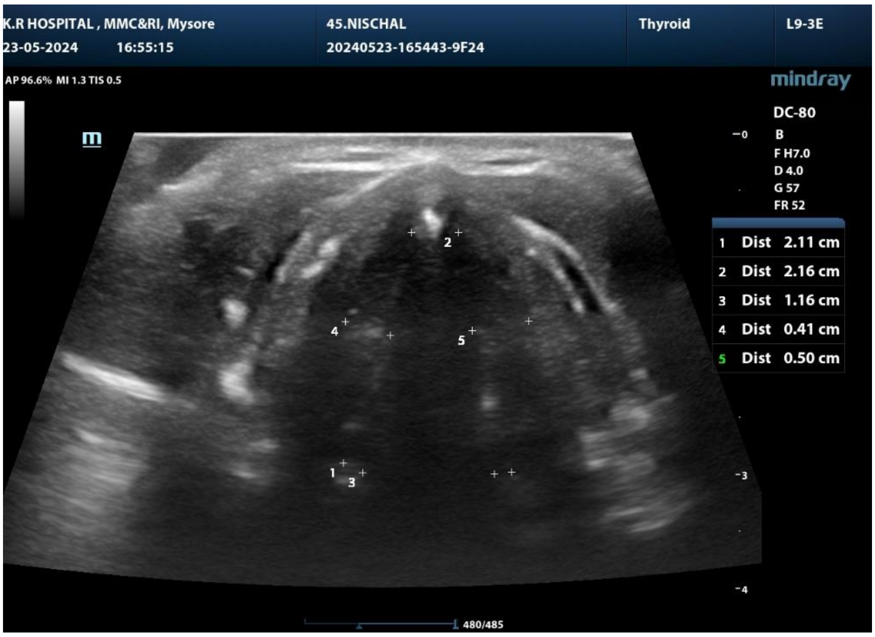
Ultrasonographic Images of the Morphometric Parameters

1) Vocal fold displacement velocity



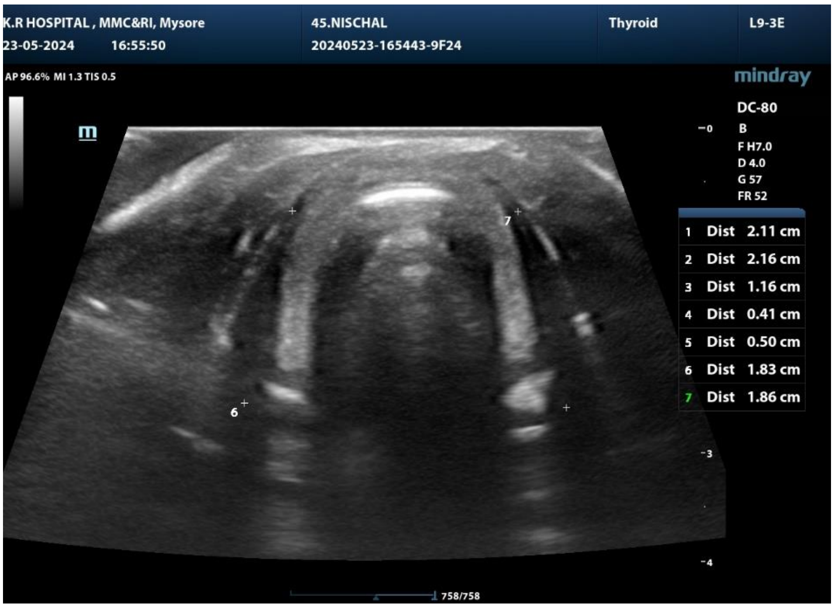
The doppler gate set at left vocal fold to extract the displacement velocity

2) Vocal fold length, vocal fold width and interarytenoid space



1 & 2- Right and left vocal fold length, 3- Interarytenoid space, 4 & 5- Right and left vocal fold width

3) Anteroposterior thyroid cartilage length



6 & 7- Right and left anteroposterior length of the thyroid cartilage