Role of Narrow Band Imaging in predicting the malignant potential of laryngeal structural lesions in subjects presenting with hoarseness for more than three weeks in comparison with histopathological examination in a tertiary care centre

A dissertation submitted in partial fulfilment of the rules and regulations for MS Otorhinolaryngology examination of the Tamil Nadu Dr.M.G.R Medical University, Chennai, to be held in May 2019

DECLARATION

This is to declare that this dissertation titled **— Role of Narrow Band Imaging in predicting the malignant potential of laryngeal structural lesions in subjects presenting with hoarseness for more than three weeks in comparison with histopathological examination in a tertiary care centre** is my original work done under the guidance and supervision of **Dr. (Prof) Rita Ruby Anbuselvi.A** It is submitted as a part of the fulfilment of requirement of the award of degree MS Otorhinolaryngology for 2019 examination to be held under **The Tamil Nadu Dr. M.G.R. University**. I have not submitted this thesis for the award of any degree or diploma from any other university.

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This is to certify that the dissertation entitled, “Role of Narrow Band Imaging in predicting the malignant potential of laryngeal structural lesions in subjects presenting with hoarseness for more than three weeks in comparison with histopathological examination in a tertiary care centre” is a bonafide work done by Dr. J.Justin Ebenezer Sargunaraj towards the partial fulfilment of rules and regulations for MS ENT Surgery degree examination of the Tamil Nadu Dr.M.G.R Medical University, to be conducted in May 2019.

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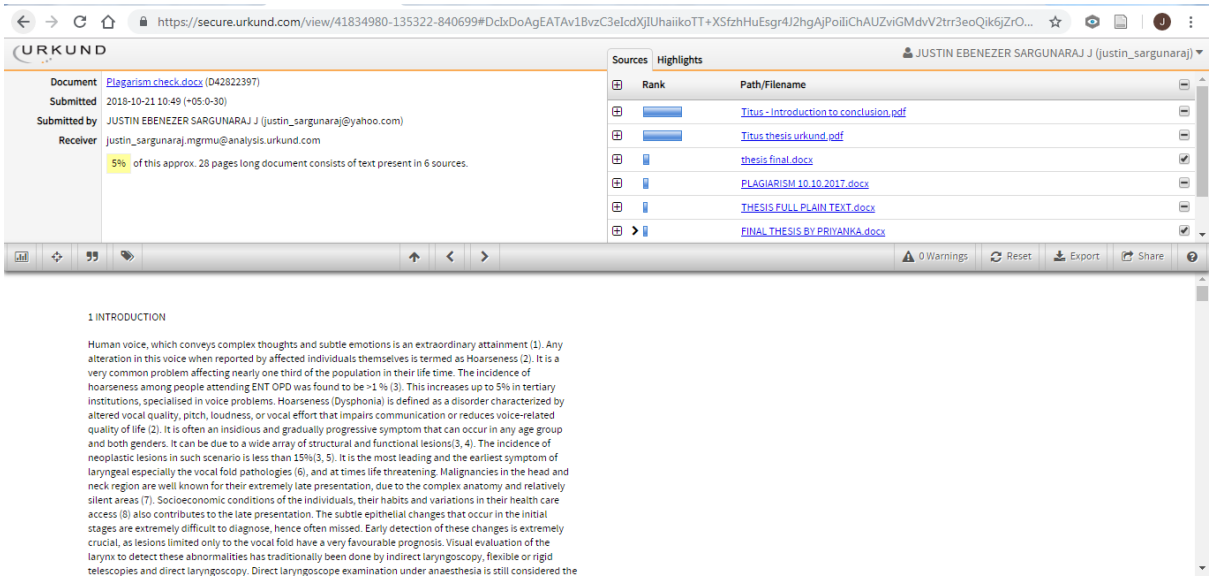

1 INTRODUCTION

Human voice, which conveys complex thoughts and subtle emotions is an extraordinary attainment (1). Any alteration in this voice when reported by affected individuals themselves is termed as Hoarseness (2). It is a very common problem affecting nearly one third of the population in their life time. The incidence of hoarseness among people attending ENT OPD was found to be >1 % (3). This increases up to 5% in tertiary institutions, specialised in voice problems. Hoarseness (Dysphonia) is defined as a disorder characterized by altered vocal quality, pitch, loudness, or vocal effort that impairs communication or reduces voice-related quality of life (2). It is often an insidious and gradually progressive symptom that can occur in any age group and both genders. It can be due to a wide array of structural and functional lesions(3, 4). The incidence of neoplastic lesions in such scenario is less than 15%(3, 5). It is the most leading and the earliest symptom of laryngeal especially the vocal fold pathologies (6), and at times life threatening. Malignancies in the head and neck region are well known for their extremely late presentation, due to the complex anatomy and relatively silent areas (7). Socioeconomic conditions of the individuals, their habits and variations in their health care access (8) also contributes to the late presentation. The subtle epithelial changes that occur in the initial stages are extremely difficult to diagnose, hence often missed. Early detection of these changes is extremely crucial, as lesions limited only to the vocal fold have a very favourable prognosis. Visual evaluation of the larynx to detect these abnormalities has traditionally been done by indirect laryngoscopy, flexible or rigid telescopies and direct laryngoscopy. Direct laryngoscope examination under anaesthesia is still considered the

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January 06, 2018

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Sub: **Fluid Research Grant: New Proposal:**
Role of Narrow Band Imaging in predicting the malignant potential of laryngeal structural lesions in subjects presenting with hoarseness for more than three weeks in comparison with histo pathological examination in a tertiary care centre.
Dr. J.Justin Ebenezer Sargunaraj, Employment Number: 34089, PG registrar / ENT /Unit 5, Dr. Rita Ruby Anbuselvi Employment Number: 10817, ENT, Dr. Suma Susan Matthews , 14423 , ENT, Dr. Rajiv Michael, 20085, Dr. Roshna Rose Paul, 28660, Dr. Meera Thomas 20116, Mrs. Mahasampath Gowri, 33418.

Ref: IRB Min. No. 10946 [DIAGNO] dated 07.11.2017

Dear Dr. J.Justin Ebenezer Sargunaraj,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Biju George, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Cc: Dr. Rita Ruby Anbuselvi, Dept. of ENT - 5 CMC, Vellore

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Dear Dr. J.Justin Ebenezer Sargunaraj,
The Institutional Review Board (**Blue**, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "Role of Narrow Band Imaging in predicting the malignant potential of laryngeal structural lesions in subjects presenting with hoarseness for more than three weeks in comparison with histo pathological examination in a tertiary care centre" on November 07th 2017.

The Committee reviewed the following documents:

1. IRB application format
2. Clinical Research Form
3. Patient information sheet and Consent form (English, Tamil, Hindi)
8. Cvs o Drs. Justin Ebenezer Sargunaraj, Rita Ruby Suma Susan Matthews , Rajiv Michael,. Roshna Rose Paul,. Meera Thomas
   Mrs. Mahasampath Gowri
5. No. of documents 1 - 4

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on November 07th 2017 in the BRTC Conference Hall, Biostatistics Building, Christian Medical College, Vellore 632 004. 2 of 4

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Director, Christian Counseling Center,
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Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Name	Qualification	Designation	Affiliation
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Dr. B. J. Prashantham	MA(Counseling Psychology), MA (Theology), Dr. Min (Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore	External, Social Scientist
Dr. Ratna Prabha	MBBS, MD (Pharma)	Associate Professor, Clinical Pharmacology, CMC, Vellore	Internal, Pharmacologist
Rev. Joseph Devaraj	BSc, BD	Chaplaincy Department, CMC, Vellore	Internal, Social Scientist
Dr. Sowmya Sathyendra	MBBS, MD (Gen. Medicine)	Professor, Medicine III, CMC, Vellore	Internal, Clinician
Dr. Visalakshi. J	MPH, PhD	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
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Mrs. Sheela Durai	MSc Nursing	Professor, Medical Surgical Nursing, CMC, Vellore	Internal, Nurse

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of Withdrawals for the study entitled: "Role of Narrow Band Imaging in predicting the malignant potential of laryngeal structural lesions in subjects presenting with hoarseness for more than three weeks in comparison with histo pathological examination in a tertiary care centre" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

Fluid Grant Allocation:

A sum of 50,000/- INR (Rupees Fifty Thousand Only) will be granted for 1 year.

Yours sincerely,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
M.D.S., M.D., D.M.
SECRETARY (ETHICS COMMITTEE)
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Christian Medical College, Vellore - 632 002.

IRB Min. No. 10946 [DIAGNO] dated 07.11.2017

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I thank God Almighty for giving me the grace and strength to complete this thesis work, All glory and honour to Him alone.

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Last but not the least I would like to thank my family members, especially my wife Dr.Asisha Janeela.M for her constant love and support.

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Human voice, which conveys complex thoughts and subtle emotions is an extraordinary attainment (1). Any alteration in this voice when reported by affected individuals themselves is termed as Hoarseness(2). It is a very common problem affecting nearly one third of the population in their life time. The incidence of hoarseness among people attending ENT OPD was found to be <1 %(3). This increases up to 5% in tertiary institutions, specialised in voice problems.

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Malignancies in the head and neck region are well known for their extremely late presentation, due to the complex anatomy and relatively silent areas(7). Socioeconomic conditions of the individuals, their habits and variations in their health care access(8) also contributes to the late presentation. The subtle epithelial changes that occur in the initial stages are extremely difficult to diagnose, hence often missed. Early detection of these changes is extremely crucial, as lesions limited only to the vocal fold have a very favourable prognosis.

Visual evaluation of the larynx to detect these abnormalities has traditionally been done by indirect laryngoscopy, flexible or rigid telescopies and direct laryngoscopy. Direct laryngoscope examination under anaesthesia is still considered the gold standard(9). With recent advancements like chip on tip cameras, providing better resolution, diagnostic accuracy has greatly improved(10) . Hence minute characteristics like the epithelial changes and their vasculature can be studied in great detail using them(11,12).

Biologic endoscopy (13) is the most recent advancement which by considering the biological behaviour of targeted lesions can provide more reliable distinction between benign, premalignant or malignant nature and can highlight a lesion that is actually invisible in white light endoscopy to become visible. The available biological tools are Toluidine blue, Lugol's iodine, Chemiluminescence, Contact endoscopy, Autofluorescense and Narrow band imaging.

With good understanding of the tumour characteristics like neoangiogenesis, and observation of the laryngeal structural changes, these evolving techniques can be used as a good predictor of malignant potentiality of the laryngeal lesions.

Narrow band Imaging has a distinct advantage over all the above mentioned tools as the process is quite simple, for it evaluates the neoangiogenetic pattern within and around a target lesion, hence the number of false positive predictions prior to histopathological examinations can be minimized. (14)

2 AIM OF THE STUDY

To assess the role of Narrow Band Imaging in predicting the malignant potential of laryngeal structural lesions in subjects presenting with hoarseness for more than three weeks in comparison with Histopathological examination in a tertiary care centre.

3 OBJECTIVES

Primary:

1. Categorize structural lesions of the vocal cord based on Narrow Band Imaging into either benign or malignant lesions.

2. Efficacy of Narrow Band Imaging to predict malignant potential in the structural laryngeal lesions with respect to Histopathological examination

Secondary:

1. To assess and validate the intraepithelial papillary capillary loop pattern [IPCL] in superficial laryngeal lesions in accordance with standard reference

2. To formulate a protocol for the management of early glottic malignancy.

4 REVIEW OF LITERATURE

4.1 EMBRYOLOGY OF LARYNX

Laryngeal development happens in three phases (15)

1. Embryonic phase during which organogenesis occurs [0-8 weeks]

2. Foetal phase, mainly 2nd trimester [13-26 weeks] during which maturation of the formed organs occurs

3. Post-natal phase during which the descent of the larynx occurs

The proper development through these phases is crucial for all its function later in life.

During the 4th week of intrauterine life from the tracheobronchial diverticulum arises in the ventral aspect of the primitive pharynx, just below the hypo branchial eminence. The edges of this groove form the tracheoesophageal septum. This fuses caudally and remains as a slit like aperture in the pharynx cranially. The end result is a tube lined with endoderm and the epithelial lining of the entire respiratory tract develops from it. Larynx develops from the cranial end of this tube.

On either side of the tracheobronchial diverticulum arises the arytenoid swelling, and as they enlarge the epithelial walls of the groove adhere to each other, thereby temporarily occluding the laryngeal inlet until the 3rd month, by which time the lumen is restored.

The arytenoid swellings enlarge upwards and also deepen to produce the aryepiglottic folds. The glottis forms just above the level of the primitive aperture , by the union of lateral furrows that develop on either side of the tracheobronchial primordium of the 6th arch(16). The thyroid cartilage develops from the ventral ends of the cartilages of the 4th pharyngeal arch. It appears as two lateral plates and each has two chondrification centres. The cricoid cartilage and the tracheal cartilages develop from the 6th arch during the 6th week. Each arch derivative receives its own afferent and efferent innervation. In the case of 4th arch it is the superior laryngeal nerve and for the 6th arch it is the recurrent laryngeal nerve. Thus most of the laryngeal components got derived mainly from the fourth and sixth branchial arch, exception being the epiglottis which develops from the hypo branchial eminence. (17) These embryological developments are significant as it determines the pattern of spread of laryngeal malignancies. The position of the larynx remains high up to 2 years of age, following which it descends and positional rearrangement occurs.

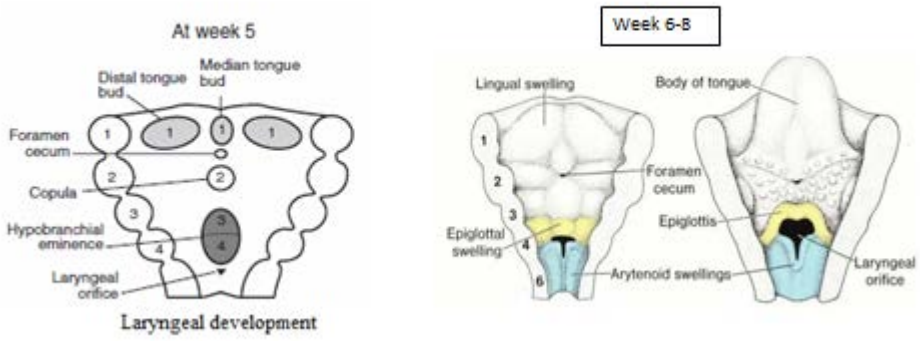

Figure 1: Laryngeal development (18,19)

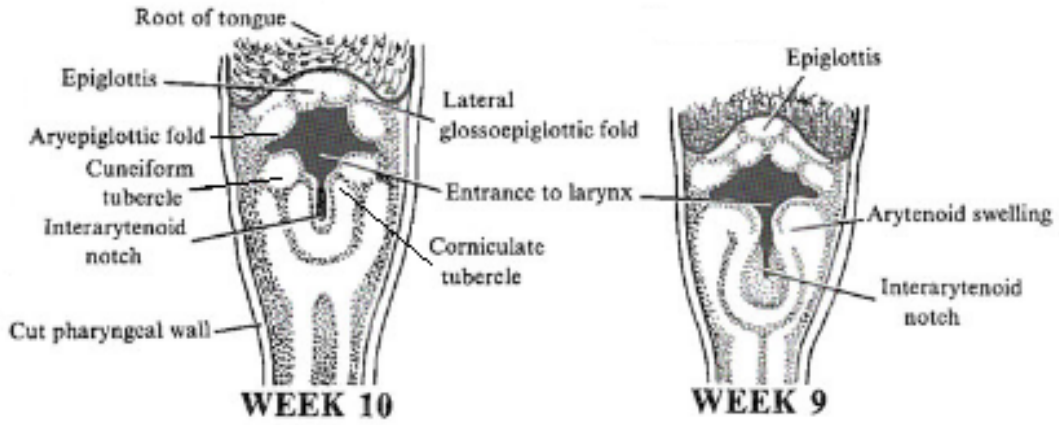

Figure 2 : Larynx development week 9 and 10

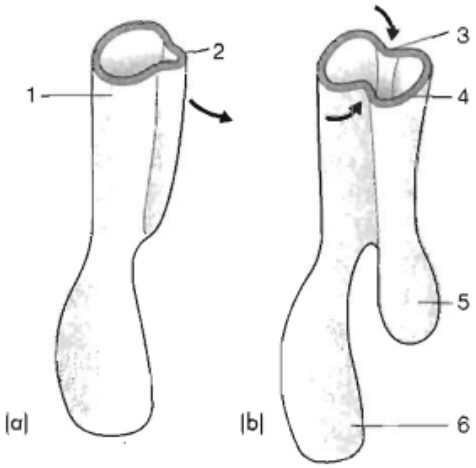

Figure 3 : Larynx development

4.2 ANATOMY OF THE LARYNX

Larynx or the voice box is a midline organ located in the anterior region of neck. It is the entry point to the respiratory system and serves important functions of the human body like phonation, protection of the lower airway.

It is primarily a cartilaginous skeleton. It is held together in its position by ligaments and membranes stretching across them. The laryngeal muscles attached to them forms the mobile component for phonation.

4.2.1 POSITION AND EXTENT

It is suspended from the hyoid bone spanning between the 3rd to 6th cervical vertebrae.

It extends from the laryngeal inlet at the level of epiglottis to the inferior border of cricoid cartilage.

It is placed at a slightly higher level in women and much higher in children, C1-C4 vertebrae.

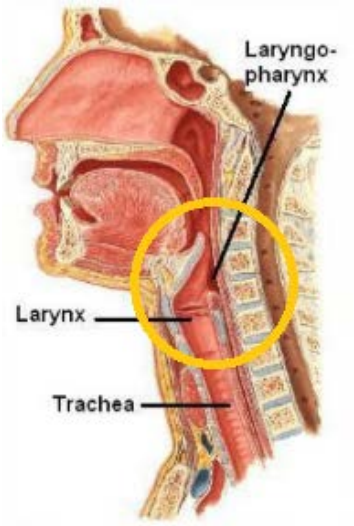

Figure 4:Larynx Anatomy

4.2.2 FRAMEWORK OF THE LARYNX

The laryngeal framework is made up of hyoid bone, three unpaired and three paired cartilages connected by membranes, ligaments and muscles to provide them the much needed stability.

The Hyoid bone:

It is a U shaped bone suspending the larynx in the neck. It also provides attachment to the laryngeal extrinsic muscles and ligaments. It includes the anteriorly placed body, laterally projecting greater cornua and superiorly placed lesser cornua.

Thyroid cartilage:

It is the largest and the most prominent structure in the anterior neck. It is composed of two laminae fused in the midline anteriorly to form the laryngeal prominence. This angle of fusion varies between men and women, being 90º and 120 º respectively. The posterior border of each thyroid laminae project superiorly and inferiorly to form the superior and inferior horns. The superior horn is long and thin, curved upwards, backwards and medially connecting via the lateral thyrohyoid ligament to the hyoid bone. The inferior horn is short and thick, curved downwards and medially and articulates with the cricoid cartilage.

Cricoid cartilage:

It is the only complete ring of cartilage in the airway. It consists of a broad laminae posteriorly and a narrower arch anteriorly, resembling a signet ring. It is attached to the inferior horn of the thyroid cartilage, by a synovial joint and the arytenoids.

Arytenoid cartilage:

Arytenoid cartilage is pyramidal in shape, having an apex, base, three surfaces and two processes.

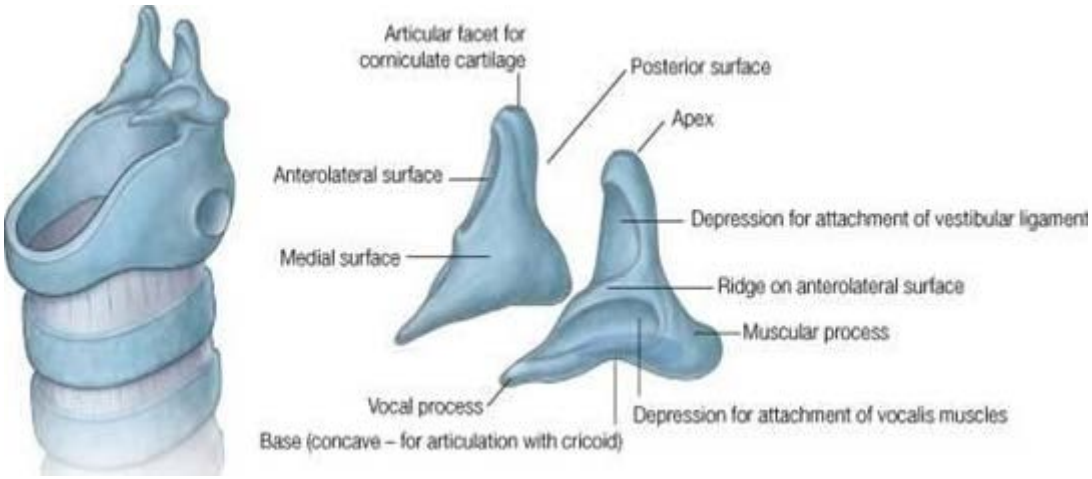

Figure 5: Arytenoid cartilage

Apex of the cartilage is curved medially and backwards and articulates with the corniculate cartilage. The base has a facet for articulation with the upper border of cricoid cartilage. This is a synovial joint which allows rotatory, medial and lateral gliding movements. Its anterior movement is prevented by posterior cricoarytenoid ligament. The two projections are the anterior projection, the vocal process and the lateral projection, the muscular process. The vocal process gives attachment to the vocal ligaments. The muscular process gives attachment to the posterior cricoarytenoid and the lateral cricothyroid muscle. The anterolateral surface between the two processes is divided by a crest into two fossae. The upper irregular triangular fossa gives attachment to the vestibular ligament while the lower fossa provides attachment to the vocalis muscle and the lateral cricoarytenoid muscle. The medial surface is lined by mucous membrane and forms the lateral boundary of the posterior glottis. The posterior surface is covered by transverse arytenoid muscle.

Corniculate and Cuneiform cartilages:

The corniculate cartilage are small conical nodules in the posterior part of the aryepiglottic fold articulating by means of synovial joint with the apex of arytenoid cartilage. They are fibro elastic in nature. The cuneiform cartilages are small elongated structure in the free margin of aryepiglottic fold.

Epiglottis:

The epiglottis is a thin, leaf shaped fibro elastic cartilage. This marks the entrance of the larynx. It mainly functions to protect the larynx during swallowing by becoming flat and closing it. It is attached to the thyroid cartilage inferiorly just beneath the thyroid notch by means of thyroepiglottic ligament. It is attached anteriorly to the hyoid bone by means of hyoepiglottic ligament. The preepiglottic space is the area between the two ligaments. The mucous membrane from the anterior surface gets reflected on to the base of tongue forming the medial and lateral glossoepiglottic fold.

Ligaments and membranes of the larynx:

Extrinsic ligaments:

These are the ligaments that connect the external framework of laryngeal cartilages to the hyoid and the trachea. Superiorly the thyroid cartilage is connected to the hyoid by the thyrohyoid membrane which is reinforced by fibrous tissue into median and lateral thyrohyoid ligaments. Inferiorly the cricoid is attached to the trachea below by the cricotracheal ligament.

Intrinsic ligaments:

The intrinsic ligaments which form a broad sheet of fibro elastic tissue connect the cartilages together beneath the mucous membrane forming the internal framework, holding them as one functional unit. It also serves to strengthen the intercartilagenous joints.

The laryngeal ventricle is the main divisive unit of the fibro elastic structure, dividing them into the upper quadrangular membrane and the lower conus elasticus.

The quadrangular membrane is attached between the lateral edge of epiglottis and the arytenoids. Its thickened upper margin is called the aryepiglottic fold and the lower margin is referred to as the vestibular ligament.

The thicker lower membrane called as the conus elasticus or cricovocal or cricothyroid ligament stretches between the upper border of cricoid cartilage and the midpoint of thyroid cartilage and the vocal process of arytenoid. The free upper border forms the framework of the vocal fold called the vocal ligament. The thickened anterior border forms the cricothyroid ligament connecting the thyroid and the cricoid in the midline.

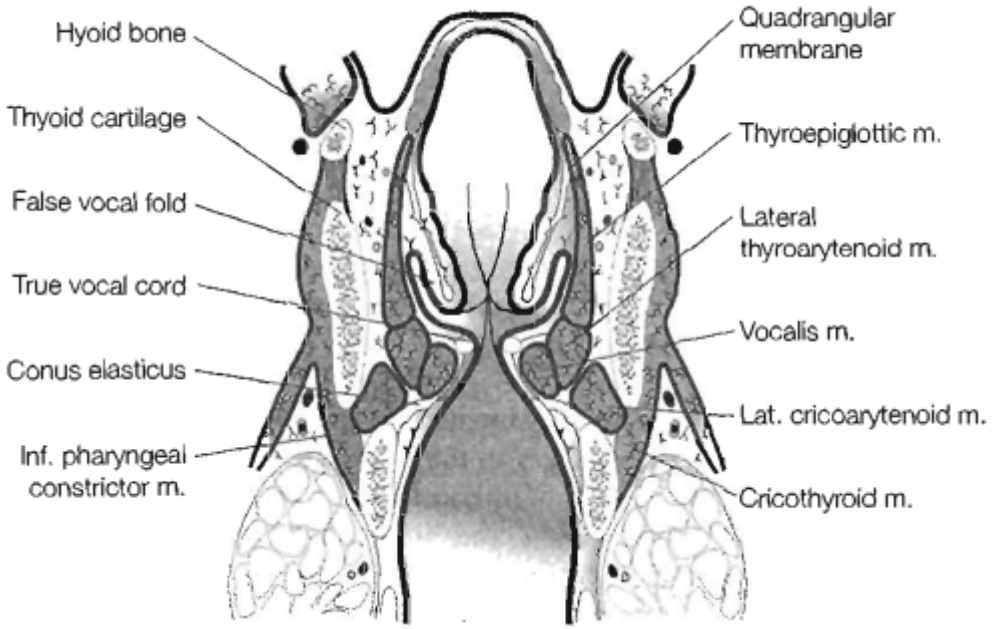

Figure 6: Ligaments and Membranes of the Larynx

Muscles of the larynx:

The laryngeal muscles are grouped as extrinsic and intrinsic:

Extrinsic muscle groups – namely the suprahyoid and the infrahyoid enable the larynx to be maintained in its position in the neck.

The suprahyoid group which generally helps in the laryngeal elevation includes the mylohyoid, geniohyoid, stylohyoid, digastric, stylopharyngeus, palatopharyngeus and salphingopharyngeus.

The infrahyoid group which generally depresses the larynx includes the sternohyoid, sternothyroid and thyrohyoid.

Intrinsic laryngeal muscles:

These are paired muscles which help in regulating the shape, position, tension and length of the vocal cords, thereby the mechanical properties of the larynx.

Based on their action, they are divided into adductors, abductors, relaxers, tensors.

Table 1 :Muscles of Larynx and their actions

Muscle	Origin	Insertion	Function
Posterior cricoarytenoid	Lower and medial surface of back of cricoid laminae	Back of muscular process of arytenoid	Abductor
Lateral cricoarytenoid	Superior border and lateral part of cricoid arch	Muscular process of arytenoid	Adductor
Transverse arytenoid	Posterior surface of muscular process and outer edge of arytenoids	Crosses over and attaches to the similar region on the other side arytenoid	Adductor
Oblique arytenoid	Posterior surface of muscular process, superficial to transverse arytenoid	Apex of the opposite arytenoid	
Thyroarytenoid(Thick lower part – vocalis muscle)	Back of thyroid prominence and cricothyroid ligament	Vocal process and anterolateral surface of body of arytenoid	Tensors

Cricothyroid	Lateral surface of cricoid arch	Lower border of posterior part of thyroid lamina and lower fibres to the inferior cornua of thyroid	Tensors
Aryepiglotticus	Posterior surface of muscular process of arytenoids	Crosses the apex of opposite arytenoid to attach to aryepiglottic fold	Weak sphincter of the laryngeal inlet
Thyroepiglotticus	Back of the thyroid prominence and the cricothyroid ligament	Attaches to aryepiglottic folds	Widens the laryngeal inlet

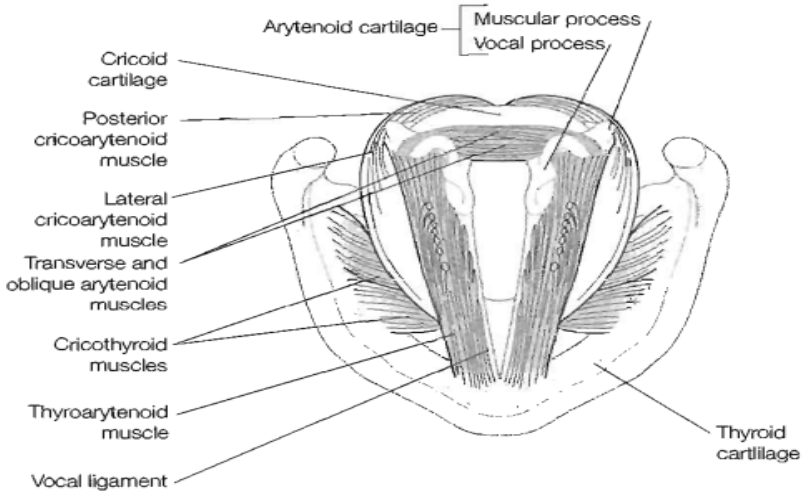

Figure 7: Intrinsic muscles of the larynx

Folds of the larynx:

These are two in number and are vital in phonation and protection of the airway.

True vocal cord – Vocal folds

False vocal cord – Vestibular folds

Vestibular folds:

These are two mucosa covered pink coloured fold over the vestibular ligament which is the lower free border of quadrangular membrane. They are fixed anteriorly at the angle of thyroid cartilage beneath the epiglottis attachment; extend posteriorly to the anterolateral surface of arytenoid above the vocal process. Their main role is in protecting the lower airway.

Vocal folds: [True cords]

The whitish structure which extends from the centre of the angle of the thyroid cartilage to the vocal process of the arytenoid cartilage with the upper border of conus elasticus lying underneath forms the vibrator of phonation. They are abducted, adducted, relaxed and tensed by the muscles of phonation to control the pitch of the sound generated.

The lateral space between the true cords and false cords constitutes the ventricle.

4.2.3 DIVISIONS OF THE LARYNX

The entire larynx is divided into Supraglottis, glottis and subglottis by demarcating lines.

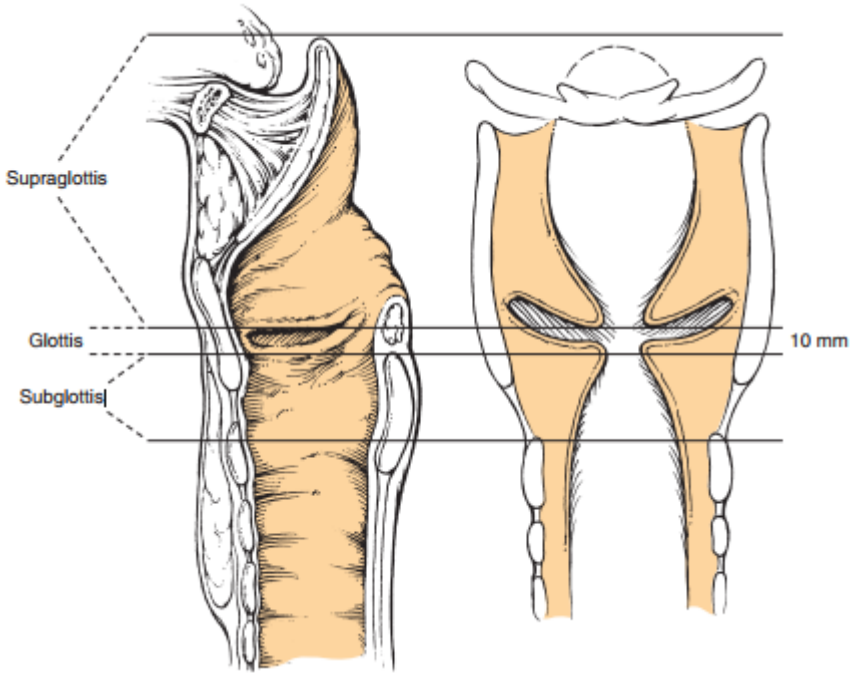

Figure 8: Divisions of larynx

Supraglottis extends from the suprahyoid epiglottis to the demarcating line which passes through the lateral margin of the ventricle at its junction with the superior surface of true cord.

Glottis extends further 1 cm down beneath the demarcating line.

Subglottis extends from inferior limit of glottis to inferior edge of cricoid cartilage.

This reminds about the embryological origin of the larynx and provides barriers to the spread of laryngeal malignancy.

The pyriform sinus lies posterolateral to the glottis and resembles an inverted pyramid. Aryepiglottic fold, arytenoid, and superior cricoid bound it medially and the thyrohyoid membrane and internal surface of the thyroid lamina bound it laterally. Superiorly, it begins at the pharyngoepiglottic fold. Inferiorly, the apex of the sinus blends with the esophageal inlet at about the superior border of the cricoid. There are

two important markings within the pyriform sinus; anteriorly in the floor of the sinus, a small fold can be seen, which marks the course of the superior laryngeal nerve. It is Possible to anesthetize the nerve topically in the piriform sinus. The second, more variable landmark is the protrusion made into the sinus from the superior cornu of the thyroid cartilage. This smooth protrusion, which usually presents in the elderly, should not be confused with neoplasm.

The pre-epiglottic Space (Of Boyer) is anterior to the epiglottis. Superiorly it is bound by the hyoepiglottic ligament, inferiorly by the thyroepiglottic ligament, anteriorly by the thyrohyoid membrane and the inner surfaces of the thyroid laminae, and laterally it opens in the paraglottic spaces. Cancer on the infrahyoid portion of the epiglottis can penetrate and gain access to the preepiglottic space.

The paraglottic space lies lateral as well as above and below the true and false vocal folds and is important in the transglottic and extralaryngeal spread of neoplasms. Medially it’s bound by the quadrangular membrane and lateral border of the aryepiglottic folds, ventricle and conus elasticus, laterally by the thyroid lamina and the cricothyroid membrane, anterosuperiorly it opens in the posterior portion of the preepiglottic space. The mucosa of the pyriform sinus forms the posterior boundary. Supraglottic cancer invading into this space may quickly extend extralaryngeally(18)

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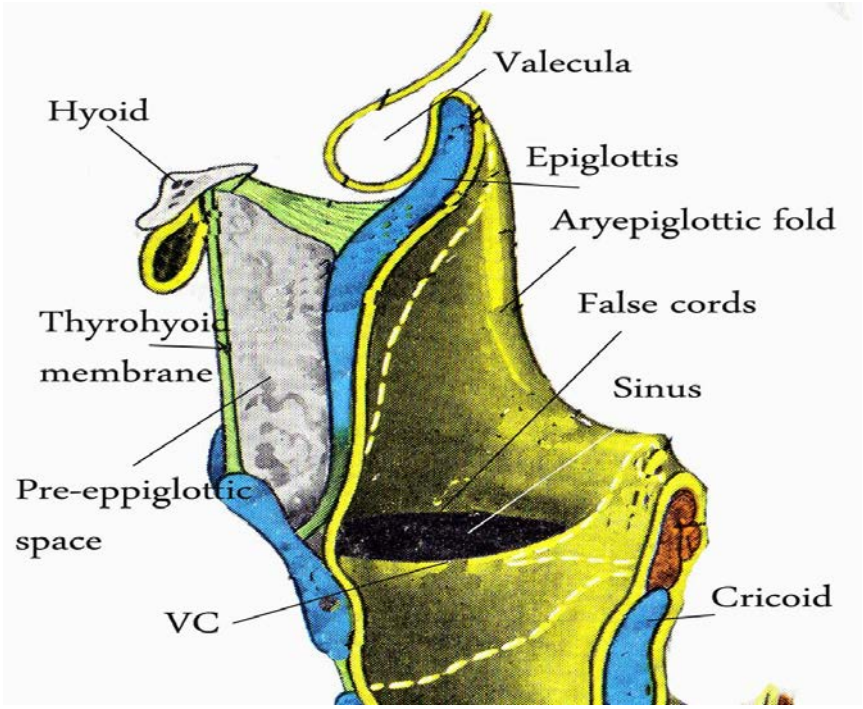

Figure 9: Pre epiglottic space

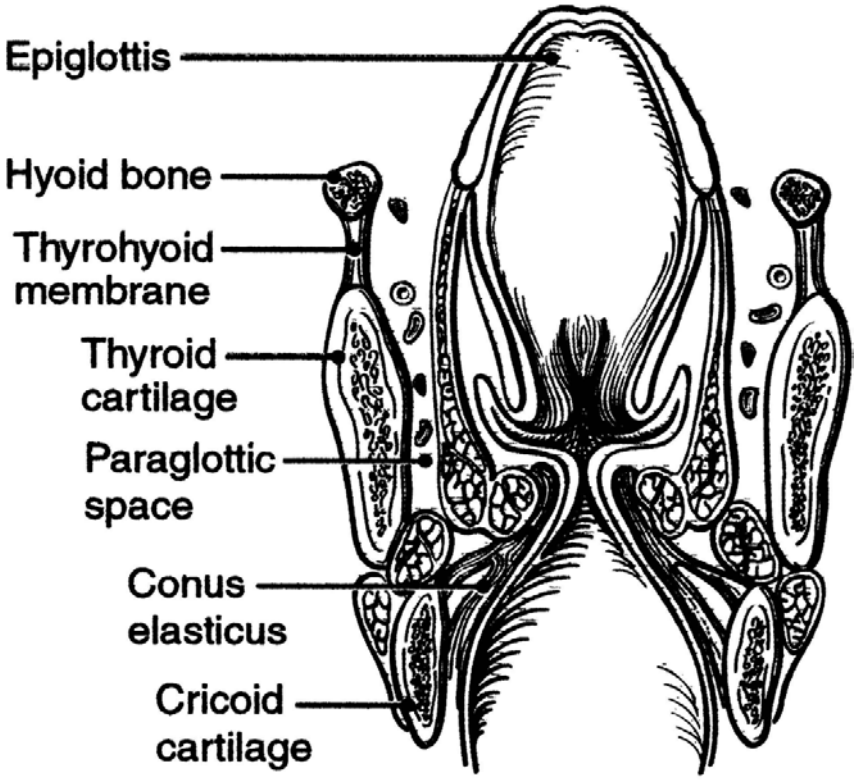

Figure 10: Paraglottic space

4.2.4 MUCOSAL LINING OF THE LARYNX

The mucous membrane lining the larynx is closely adherent to the posterior surface of epiglottis, corniculate and cuneiform cartilages and over the vocal ligament. These are loosely attached in the other regions and prone for edema.

Mucous glands are present all over the mucous lining of the larynx, which are increased in number over the posterior surface of epiglottis, lower part of aryepiglottic fold and the saccule.

The mucosal lining is predominantly pseudostratified ciliated columnar epithelium in the supraglottis, posterior part of glottis and in the subglotttis . It is found to be non keratinized stratified squamous epithelium at the upper part of posterior surface of epiglottis, free edge of aryepiglottic fold and the vocal fold.

4.2.5 NERVE SUPPLY OF LARYNX

Two branches of the vagus nerve (cranial nerve X), the superior and inferior laryngeal nerve are responsible for the entire laryngeal innervation. The superior laryngeal nerve leaves the trunk of the vagus at its nodose ganglion, curves downwards and forwards medial to the internal carotid artery, divides into 2 branches, about 1.5cm below the ganglion. The internal laryngeal nerve then runs between the lingual artery bifurcation from external carotid and the external laryngeal runs below the bifurcation along the superior thyroid artery. The external being the smaller branch runs with the superior thyroid artery deep to the upper pole of the thyroid gland in the

Joll's triangle. There it is laterally bound by the upper pole of the gland and superior thyroid vessels, superiorly by the strap muscles which attach to the hyoid and medially by the midline with the floor composed of the cricothyroid muscle. It gives a motor branch to the cricothyroid muscle and then becomes the communicating nerve. This then gives two branches, the first is presumed to be a sensory branch and the second terminates on the thyroarytenoid muscle or anastomoses with the recurrent laryngeal nerve and is motor. This motor branch is also shown to supply the inferior constrictor muscle in many cases (19). In about 15% of cases, the external laryngeal nerve runs with the superior thyroid vessels and leaves from close to the gland. The Cernea classification (20) divides this into-

Type I- where nerve crosses the superior thyroid pedicle more than 1 cm above the upper pole,

Type IIa- less than 1 cm and cranial to the upper pole (most common) and

Type IIb- less than 1cm but caudal to the upper pole (high risk of nerve injury during surgery). The internal laryngeal is the larger of the two and runs with the superior laryngeal artery and pierces the thyrohyoid membrane. In the larynx, it gives sensory and

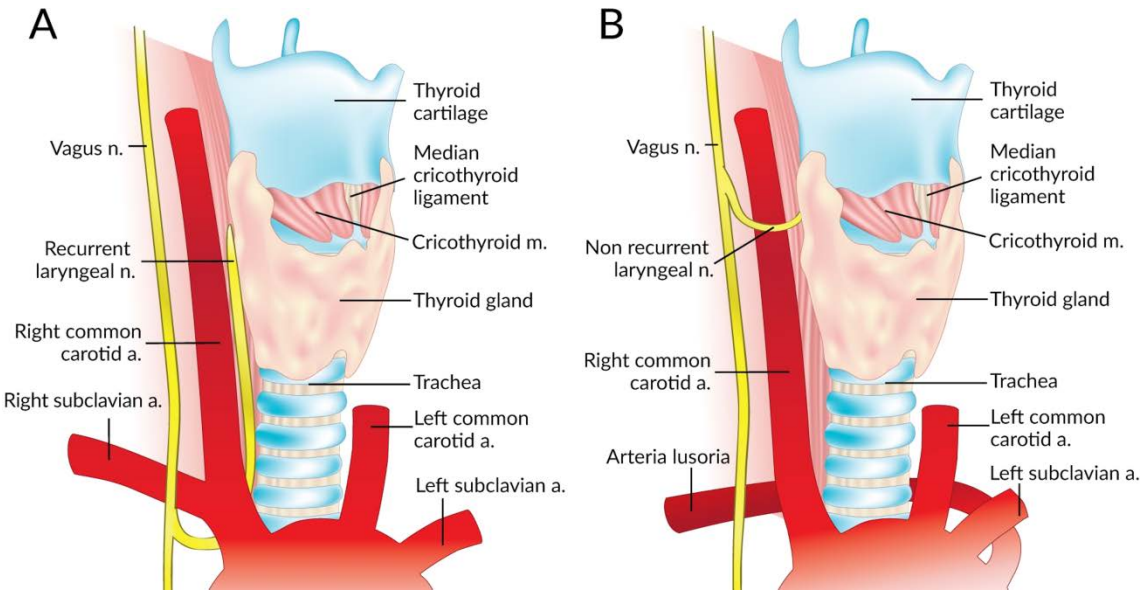

Figure 11: Nerve supply of larynx

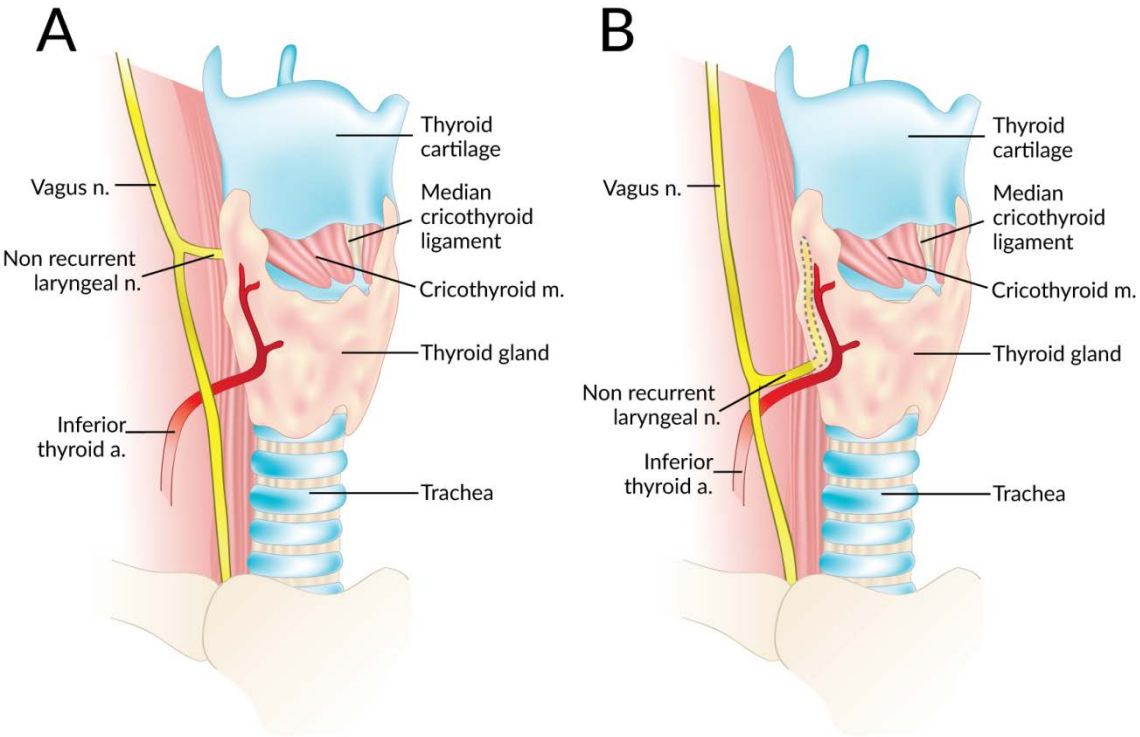

Figure 12: Non recurrent laryngeal nerve

secretomotor supply by anastomosing with the ascending branch of recurrent laryngeal nerve forming the Galen's anastomosis which is purely sensory.

The right recurrent laryngeal nerve (RLN) leaves the vagus in front of the subclavian artery (1st part). Left RLN originates from the vagus as it crosses the aortic arch. Both the nerves run up the neck towards the larynx in grooves between the esophagus and trachea, accompanied by inferior thyroid artery. Main trunk divides into 2 branches (motor/sensory) below the lower border of the inferior constrictor muscle and enters behind the cricothyroid joint. It is commonly sought in the tracheo oesophageal groove in between the branches of inferior thyroid artery. The most accurate and safe way of identifying the nerve is to seek it low down in the tracheo-esophageal(TE) groove in Beahr's triangle. This triangle is formed medially by the RLN and laterally by the common carotid and inferior thyroid arteries. Left RLN has a longer course and hugs the TE groove. Right side is more superficial and runs at a 45degree angle before reaching the TE groove. Also, extralaryngeal branching of the recurrent nerve in almost half of the cases must be taken into account(19). 2% of the population will have a non-recurrent nerve, mostly on the right side, which runs with inferior thyroid vessels. The left RLN is more prone to paralysis due to its longer intrathoracic course whereas the non recurrent and the right RLN is more prone to iatrogenic injuries. There exist different sets of anastomoses between the internal, external and recurrent laryngeal nerves. This variability in the prevalence of the different anastomoses could explain the variability in the vocal fold position after laryngeal paralysis and the challenges of laryngeal reinnervation research(19).

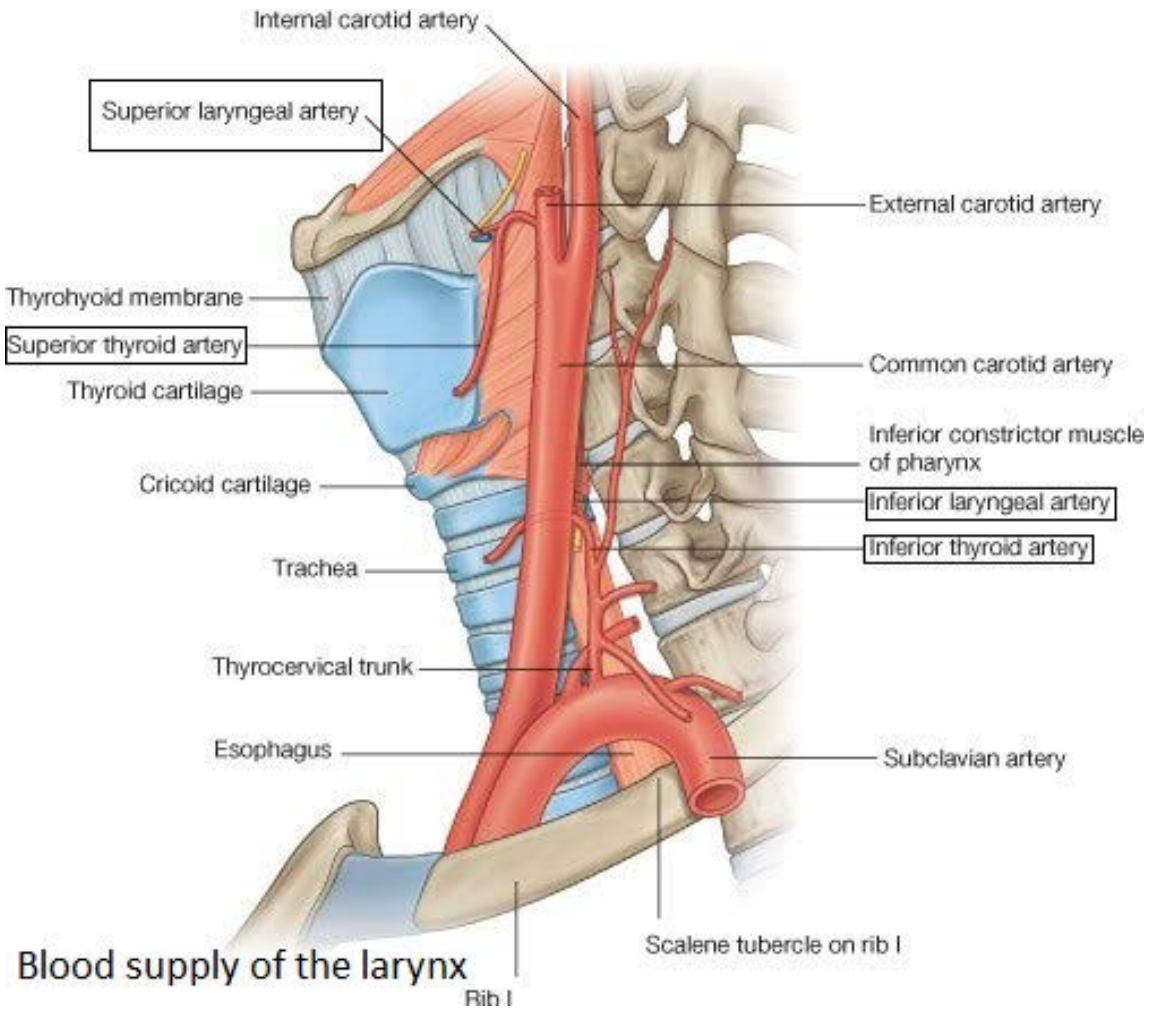

Figure 13: Blood supply of the larynx

4.2.6 VASCULAR SUPPLY OF THE LARYNX

The larynx receives arterial branches of the external carotid artery superiorly and the subclavian artery inferiorly. The superior laryngeal artery frequently originates from the superior thyroid artery, which mostly arises from the external carotid artery. In some rare cases it can arise from the lingual and/or facial artery (thyrolingual or thyrolinguofacial trunk). It can also originate, in about 20%, directly from the external carotid artery(19). It penetrates the larynx through the thyrohyoid membrane along with the internal branch of superior laryngeal nerve, the attending veins as well as lymphatic vessels.

The inferior thyroid artery arises on each side from the thyrocervical trunk of the subclavian artery, rises up, turns medially and crosses behind the deep cervical vessels and vagus nerve. Near the trachea it rises up again to approach the inferior pole of the thyroid gland. Here the artery has a very close relationship to the recurrent laryngeal nerve; its inferior laryngeal branch accompanies the nerve on its route to the larynx. The venous drainage passes superiorly via the superior thyroid vein to the internal jugular vein and inferiorly via the inferior thyroid vein to the brachiocephalic vein.

4.2.7 LYMPHATIC DRAINAGE

The lymphatics in the larynx have mucosal and a submucosal lymphatic vessels, both of which cross the midline, especially in the supraglottis. The highest density of lymphatic vessels is in the supraglottic region(19). Supraglottis drains into the upper deep cervical lymph nodes IIa-III. Subglottis drains into the prelaryngeal and pretracheal nodes which in turn drain into the deep cervical chain level III and IV, but

only on one side. Glottis is the watershed area with almost no drainage except the so-called Delphian node, located in the midline anteriorly to the cricothyroid membrane or trachea (Level VI), can be regularly observed in children up to the age of 10, whereas in only 50% over the age of 40(19).

4.2.8 STRUCTURE OF THE VOCAL FOLD

They are flat band, white in colour extending between the thyroid cartilage and the arytenoid. The length and thickness of it varies between men (1.75 – 2.5cm) and women (1.25 – 1.75cm), which is the reason behind difference in pitch.

It is divided by a line passing through the tip of the vocal process into anterior membranous portion which includes the anterior two third and posterior intercartilaginous portion which includes the posterior one third.

Hirano's contribution in 1974 is immense in the understanding of the laminar structure of human vocal fold.

The vocal fold is a five layered laminated structure. It is formed by the vocalis muscle which forms the main bulk covered by the mucosa which contains the epithelium and the lamina propria. The superficial layer of the mucous lining is formed by non keratinized stratified squamous epithelium and is devoid of mucous glands. These cells are homogeneous in their arrangement, with a spheroid nucleus, and a uniform nuclear cytoplasmic ratio. This epithelial lining is secured by the basement membrane zone to the underlying non cellular lamina propria which is

further subdivided into three layers – superficial, intermediate and deep based on their extracellular matrix content.

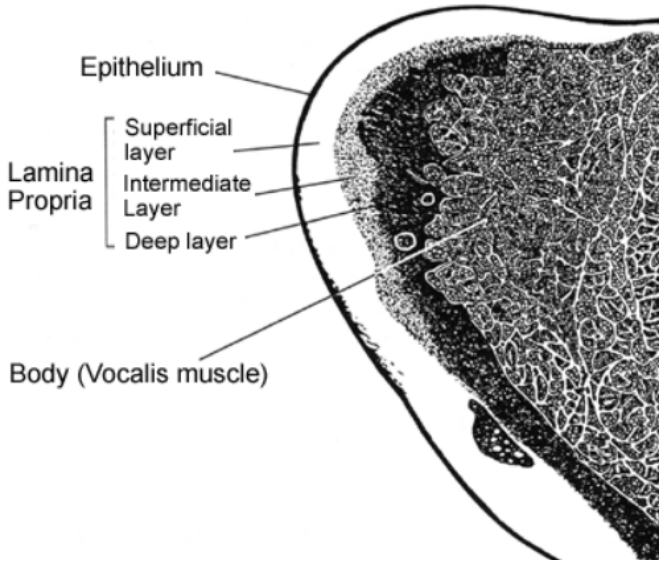

Figure 14: Microanatomy of the vocal fold

Mucociliary layer:

A mucociliary blanket layers lies over the epidermis.(21) It consists of mucinous layer and a serous layer. The mucinous layer is of thick, viscous, mucin, which helps in maintaining the hydration and protection of overlying layer, thereby the vocal fold healthy . The serous layer has less viscous and more watery, enabling the cilia to move freely. The cilia beats posteriorly and superiorly in a circular fashion in the lower airway and reaches the posterior glottis. This travels over the squamous epithelium of the glottis till it changes to the ciliated epithelium lateral to the free edge of vocal cord, from where again it is pushed posteriorly until it is swallowed.

Basement membrane zone:

The basement membrane zone is made of numerous protein and non proteinaceous substances like desmosomes, collagen IV and VII which helps in anchoring the epidermis to the lamina propria during intense vibration of the vocal folds. This zone itself constitutes three zones namely – lamina lucida, the lamina densa and the zona reticularis. This is the area where maximum shearing forces occur during vibration of the vocal folds and their collagen strength is genetically determined. This may be one of the reason behind predisposing only a few people with voice overuse for vocal cord pathology.

The Lamina propria: (21)

The lamina propria which are rich in elastin and collagen has been categorized into three layers, each are functionally significant. The superficial layer of lamina propria (SLP) also known as the Reinke's space contains fibrous substance similar to gelatin, hence they are extremely loose and pliable, facilitating vibration. The intermediate layers (ILP) are rich in elastin and the deep layers (DLP) predominantly contain collagen fibres. The intermediate and deep layers, together forms the vocal ligament.

The collagen fibrils, mainly type I provides support to the tissue to withstand the longitudinal stress and resist deformation. The elastin provides elasticity and enables the tissue to come back to its original form, if deformed. The extracellular matrix bundle is mixed with cells and interstitial molecules namely hyaluronic acid which fills the in between space, absorbs shearing force, promotes cell migration and facilitates wound healing.

Predominant cells within the lamina propria are the fibroblasts, macrophages and tissue macrophages. Fibroblasts are the cells present at uniform density in all layers maintaining the proteinaceous content and the integrity of lamina propria. Macrophages are present in the basement membrane zone and in the superficial layers of lamina propria and fight against the inflammatory agents at the level of surface epithelium. Myofibroblasts which are differentiated fibroblasts, the repair cells is located predominantly in the superficial lamina propria layer at all times, repairing the microscopic trauma that the vocal fold encounters. Hence if any persistent or violent injury to the vocal fold occurs, it is beyond repair and becomes pathological.

The five layers in the vocal fold are not uniform in their thickness. Near the anterior commissure lies a mass of collagen fibres connected to the inner perichondrium of thyroid cartilage called the Broyle's tendon. In the anterior and posterior end of membranous portion of the vocal folds lie bundles of elastic fibres in continuity with the intermediate layer called the anterior and posterior macula(22). These thickened structures act as cushions protecting the vocal fold ends from shearing force produced by vibration.

The vocal fold becomes thin near the anterior commissure due to the sloping up of the inferior edge, where it forms the apex with the fixed part of epiglottis. Hence tumors involving the anterior commissure also involves the subglottis.

Maturation of the vocal fold(23,24):

According to Boseley and Hartnick, the lamina propria is a single layer, which becomes two layered by 2 months and three layered by 11 months. Then the

maturation starts and the adult type can be noticed by around 13 years of age. The strength of these layers depends on the functionality of vocal cords. Studies have shown that the children whose vocal cords never vibrated or in older people following neurological insult, the vocal ligament architecture is altered.

Vascular arrangement of the vocal fold:

The normal vasculature of the vocal cord consists of capillaries in the superficial lamina propria, with arterioles and venules in the deeper layers. There exists direct communication between the arterioles and venules. This arrangement is well separated from the muscular layer. The vessels on the vocal cord enter from the anterior or posterior aspect and run along the long axis. (12,25) The branching and the anastomosis in the form of curving and meandering is noted markedly at the arteriolar level. These vessels can become prominent, increase in number, density, branching and anastomosis based on reactive processes.

4.3 PHYSIOLOGY OF PHONATION

Voice is generated by the vibration of the free edges of the vocal fold, in response to the excitation by the exhaled air from the lungs. They are then modified as it passes through the supralaryngeal resonators to produce a recognizable sound. However the articulators namely the lips, tongue and the soft palate shape the sound source into an audible speech. Thus the vocal fold acts like an energy transducer during phonation converting the aerodynamic energy generated by the chest, diaphragm and abdominal muscles into acoustic energy, heard as voice.

Mechanisms of the phonatory system:

Myoelastic – Aerodynamic theory: Van Der Berg,1958:

The essence of this theory is that the vocal fold vibration occurs due to interaction between the aerodynamic and the vocal fold muscular forces.

This theory is based on two principles:

The myoelastic part of this theory explains the neuromuscular control of the vocal fold tension and elasticity during phonation. Acccording to Wyke, vocal folds abduct just before phonation to take in air, termed as the prephonatory inspiratory phase. Then the vocal folds go in tensed, contracted and adducted state, due to lateral cricoarytenoid and interarytenoids to regulate the elasticity of the vocal fold. Configuration of the glottis aperture is determined by the neuromuscular control over the vocal folds. The difference in the pressure above and below the glottis is determined by the three dimensional shape of the glottis, which is key to the driving force of phonation. The subglottic pressure keeps on increasing below the level of closed vocal folds, until the pressure is more than the resistance between the vocal folds, forcing the vocal fold to give way from inferiorly causing them to oscillate resulting in phonation . This pressure that is required to begin voicing is called as phonation threshold pressure. Phonatory output is regulated mainly by the coordination between the subglottic pressure and the vocal fold elasticity.

The aero dynamic part of the theory explains the role of fluid dynamics in setting the vocal folds into vibration when they are in adducted position.

Physiology of phonation

The normal vibratory-phonatory cycle is regulated by several principles that include adequate breath support, approximation of vocal folds, favorable vibratory properties, favorable vocal fold shape and control of length and tension of vocal folds.(26) Voice is achieved by a complex repeating cycle in which glottal opening and closing modulates the transglottic air-stream at anywhere from 50 to 1000 cycles per second.

Each cycle begins with the subglottic pressure pushing against the under surface of the closed vocal folds. The medial closing force holding the vocal folds together is called as phonatory threshold pressure. The pressure from the lungs eventually overcomes the phonatory threshold pressure, and pushes the vocal folds apart. The inferior-most part of the folds opens first, and the tissue is progressively compressed as an “air bubble” rises to the superior surface of the folds. This compression from inferior to superior is called a traveling wave. When the “bubble” or wave reaches the superior portion of the folds, they start to unzip, and pressurized air begins to escape from the folds as a jet. This mucosal wave thus starts in the infero medial part of the vocal cords and proceeds in a rostral direction.(27) This jet can attain velocities of 50 or more meters per second. The vocal folds open first anteriorly. At the superior portion of the folds, the tissue wave propagates out laterally. While this lateral propagation of the wave occurs, the inferior most edges of the vocal folds begin to approximate together. There are many factors involved in this medial movement of the vocal folds which include fall in subglottic pressure due to escape of air, recoiling force because of the elasticity of the vocal folds and the Bernoulli effect which is the negative pressure created by high velocity of the escaping air. The vocal folds approximate

inferiorly, and this closure then progresses superiorly and anteriorly until the vocal folds close completely, leading to repetition of the cycle. Sound is produced during fold oscillation by the inertial interaction of the pulsating jet against the air columns above and below the folds creating an air pressure wave that then interacts with the resonators of the vocal tract before exiting the lips.

The simplest models of phonation are based on the principle that pitch depends on the frequency of vocal fold vibration, which is related to the vocal fold length, whereas loudness is the result of subglottic pressure and the amplitude of vibration of the vocal folds.

The three main types of vibratory patterns that exist are modal, falsetto, and glottal fry. In the modal register, the vocal folds exhibit a normal vibratory topography as the mucosa vibrates independently of the muscle. In falsetto, glottal closure is incomplete, and only the uppermost free edges of the folds are involved in vibration, creating a high-pitched voice. Glottal fry is characterized by an excessively low-pitched voice with the vocal folds tightly approximated for a longer than normal duration during the vibratory cycle.

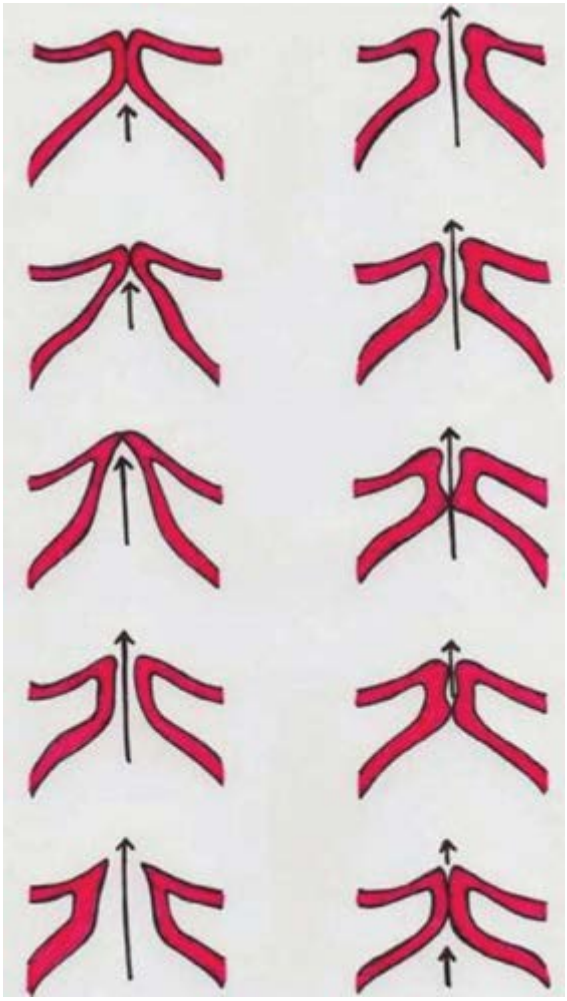

Figure 15. Vibratory - Phonatory cycle

Central connections of phonation:

Hence the quality of the sound generated depends mainly on the nature of the vocal fold approximation during phonation and the regularity of the mucosal waves of the lamina propria.

Any changes in the vocal fold or in any other extra laryngeal structures involved in phonation can lead to abnormal voice quality, termed as hoarseness.(28)

Yet the voice disorders cannot be always due to anatomical or functional changes in the voice box alone, breath control, articulatory tract and the oropharyngeal movements too will have their influence over the perceived voice. (29)

Mechanism of hoarseness(30,31):

1) Incomplete adduction of the vocal folds – Vocal fold paralysis

2) Any irregularities in the medial edge of the vocal fold, dampening the propagation of mucosal waves – Benign and Malignant conditions

3) Abnormal usage of extra laryngeal musculature – Muscle tension dysphonia

4) Pathology in the resonance chamber – nasal or pharyngeal pathology

This can occur in people of all ages and sexes, but significantly found in people with increased vocal demands and in old age. It is often caused by self-limiting or benign laryngeal conditions but can also harbour more serious or progressive condition requiring prompt diagnosis and management. Hence when dysphonia fails to improve or resolve within 4 weeks or if any serious underlying cause is suspected, patient needs complete laryngeal examination.(28)

4.4 VISUALISATION OF THE LARYNX

The oldest and the simplest method of laryngeal examination is indirect laryngoscopy using a laryngeal mirror. It is advantageous for it gives an undistorted image, yet can be hindered by an overhanging epiglottis or a hyperactive gag reflex. An adequate view of the vallecula, epiglottis, aryepiglottic folds, pyriform sinuses,

false vocal cords, true vocal cords and immediate subglottis can be obtained.(9,10,31,32)

Failure to examine all these structures warrants further examination using rigid rod lens telescope, transnasal fiberoptic laryngoscopy or flexible distal chip scopes. Fiberoptic laryngoscopic examination is superior than the rigid endoscopes for it facilitates dynamic assessment, especially in neurolaryngological cases.

Abnormalities in vocal fold vibration not appreciated by light laryngoscopy can be diagnosed using synchronized flashing light onto the vibrating vocal fold, called as Videostroboscopy. By synchronizing the strobe light to the frequency of repeatedly vibrating vocal fold, the vibration can be perceived at a much slower rate than the actual one. This helps in accurately diagnosing benign mid membranous vocal fold lesions, vibratory asymmetry, loss of vibration and also the glottal gap. Also when it is applied to suspicious malignant lesions or hyperkeratosis, where the amplitude of vibration or the mucosal wave can be reduced, Colden and his colleagues (33) found that the stroboscopy do not reliably predict the presence of malignancy or the depth of invasion into the laminae propriae. However the presence of mucosal waves, can predict that the lesion has not invaded the vocal ligament. Hence it can be confounded that stroboscopy, by detecting the mucosal wave abnormalities cannot be diagnostic of malignancy, but can raise doubts about suspicious lesions.

High speed imaging,(34) overcomes the limitation of videostroboscopy by accurately picking the vibratory patterns caused by aperiodic vibration like subtle vocal cord paresis or vocal cord scar. It can capture upto 10 to 20 frames per vibratory

cycle based on the fundamental frequency. This too can only aid but not directly diagnose malignant conditions.

Videokymography,(35)another modality to assess the vibratory patterns, actually visualizes the motion of the mucosal wave, periodicity, left to right symmetry , displays the open and closed phases, phase difference, and amplitude. The advantage being all these patterns can be depicted in a single image, thus adding precision to the stroboscopy.

Autofluorecence endoscopy(AFE),(36) which utilises the emission of natural fluorescence from tissue flurophores namely the nicotine amide adenine dinucleotide with hydrogen (NADH) and flavin adenine dinucleotide (FAD) following exposure and activation by a particular wavelength of radiation, usually a blue light of 440 nm. The normal tissues usually appear as green while the malignant tissues appear as reddish brown. This technology is based on the finding that the concentration of endogenous flurophores are extremely low in malignant conditions (37)and also thickened epithelial tissue prevents the light penetration into the submucosal tissues (38). This too could be a complementary method, but its sensitivity was found to be low in the oral lesions (39) where it is applied more frequently than in the larynx. In the larynx, high false negativity is reported with hyperkeratosis(40), a premalignant condition.

With more and more understanding of the biological characteristics of malignant lesions and technical innovations, even invisible lesions can be picked up. They have

been grouped together as Biologic endoscopy, enhancing the outcome of conventional endoscopies.

First in this group, is Toluidine blue(41) a metachromatic dye which found its application in the larynx since 1974. It stains the abnormally proliferating tissue blue by adhering to the phosphate group of the nucleic acid, on topical application using a swab, after clearing the mucous lining over the epithelium using 10 % hydrogen peroxide and 1 % acetic acid. The lesions are suspected to be malignant if the nuclear uptake is high. This helps mainly in lesions which are visible and easily accessible as lot of adjunctive tissue manipulation is needed, making it highly impractical for laryngeal lesions.

This was superseded by Lugol's iodine(13), an aqueous solution made with 1.25% iodine and 2.5 % potassium iodide. It is a glycophilic substance that adheres to the glycogen containing intermediate and superficial layers of the parakeratinized squamous epithelium, giving them mahogany brown colour. Rapidly differentiating or proliferating cells gives a mustard yellow or saffron colour after staining. This too needs prestaining mucosal preparation using mucolytic like 5 %carbocysteine syrup and wash off the excess iodine after the normal tissues are adequately stained brown to black. These has helped in picking the dysplastic lesions in oralcavity, esophageal and cervical transition zones. It has a low specificity and is also limited by its marked ulcer forming ability, though it can resolve in one week.(42) In this vital staining technique also hyperkeratotic lesions do not take the stain and pose a significant diagnostic challenge.

Chemiluminescense, is a chemical based technique, predominantly in the oral lesions wherein artificial luminescence is produced when a capsule containing separate containers of acetyl salicylic acid and hydrogen peroxide is activated. This produces an intense blue light in the bandwidth of 490nm to 510nm , which persists for 10 minutes approximately. These are absorbed by the normal mucosal cells giving a dark blue hue and reflected by abnormal cells with increased nuclear to cytoplasmic ratio, thus appearing pale. This is its main limitation for it cannot differentiate hyperplasia, dysplasia from carcinoma(43). It is technically more safe and less time consuming but is limited by low diagnostic accuracy.

Contact endoscopy(13,32,44) is another novel non invasive technique introduced in 1995 by Andrea et al(45) which allows in vivo evaluation of cellular architecture by placing magnifying straight or angled rigid endoscopes directly over the mucosal surface thereby obtaining a 60 to 150 times magnified image. Contrast enhancement of the superficial layers of the vocal cord is provided by methylene blue which on application over the surface stains the nuclei dark blue and the cytoplasm of normal cells lightly, with rapidly proliferating cells taking much stronger stain. This helps in direct evaluation of individual cells and their nuclear characteristics It also stains the subepithelial blood vessels due to the presence of haemoglobin considered as a natural dye, facilitating detection of angiogenesis and invasion(45,46). It also helps in measuring the severity of dysplasia by highlighting the extent of atypia within the cells. Hence in vivo diagnosis of dysplasia and invasive malignancy can be made out. Though the sensitivity and specificity were found to be more than 90 % by Warnecke et al with good expertise(47), its major limitation lies in its inability to differentiate

between carcinoma in situ and frank invasive carcinoma highlighted by Cikojevic et al (48) , as the methylene blue don't penetrate into the deeper layers, also the breach in basement membrane zone, the diagnostic criteria for invasive malignancies cannot be delineated due to higher magnification and glare effect from out of focus cells.

This shortcoming seems to be addressed in Confocal endomicroscopy (CEM), which with appropriate magnification and elimination of the out of focus light, facilitates three dimensional examination of the basement membrane zone upto depth of 0.5mm. Hence it is also called as Virtual biopsy. Their sensitivity has been enhanced further by the addition of both surface staining using Acriflavine hydrochloride which stains the nuclei of the epithelial cells and intravenous fluorescein which highlights the blood vessels. They were proven to have diagnostic accuracy of more than 98% with respect to colorectal malignancies in gastroenterology(49), yet in laryngeal malignancies it is still in the evolutionary phase(50).

Optical Coherence Tomography(OCT), (51)is a non invasive high resolution cross sectional imaging technology, using backscattered infrared light which provides details about the subsurface of tissues upto a depth of 3mm, thereby the epithelial thickness, basement membrane integrity and the lamina propria thickness can be analysed. With the addition of polarised light to OCT (PS-OCT), since collagen fibres in the lamina propria layers are birefringent, tissue structure can be characterised very well. This holds the potential to detect the extent of invasion, since basement membrane breech can be identified(52). But both benign and malignant lesions can disrupt the vocal fold mucosal layer, hence differentiating an early malignancy before invasion from benign conditions can still be a problem.

Then comes the Raman Spectroscopy. This is a non invasive molecular analysis based on the principle that intramolecular bonds which exist between biological tissues like proteins and nucleic acids have a specific spectrum and can scatter light rays in a predictable manner when stimulated. These emitted spectral peaks are identified, measured to lie in the range of 850 - 950 cm^{-1} and 1,200 - 1,350 cm^{-1}. These have been found to be directly related to the tissue protein architecture and the C-H bond that stretches between the nucleic acid bases. It has been reported that the intensity of these peak increases as the proliferation worsens towards the invasive nature of malignancy (53),hence can be applied to detect early glottic lesions. This is still in the laboratory analysis level, and yet to be applied in vivo.

4.5 NARROW BAND IMAGING [NBI]

Narrow band Imaging is the most recent of all the biological endoscopic techniques and is quite distinct as it focuses only on the microvascularization pattern of the lesions rather than the disease(13). Thus it overcomes most of the limitations faced by the above mentioned techniques. Its principles got refined and founds its application initially in 2004 with lower oesophageal, colorectal and gastric malignancies (54–59). Its application in the larynx was incidental as it enabled to identify metachronous hypopharyngeal lesions in people with oesophageal malignancies (60). Recently it also applied in the field of respiratory medicine, in diagnosing Angiogenic squamous dysplasia, a precursor for lung malignancy.(61)

In order to provide nutrition and other requirements to the proliferating tissue, tumour bed generally recruit the nearby blood vessels and the endothelial cells. Thus

new blood vessels arise from the pre-existing ones, called as neovascularisation by angiogenesis is one of the hallmarks of malignancy. These vessels will have variable characteristics like more tortuous, dilated, excessive branching, chaotic blood flow, widened junctions, discontinuous or absent basement membrane zone(62). The more tortuous the vessels are, more slower the blood flow through them, hence the red blood cells with haemoglobin within them conglomerate

If the blood vessels on the superior surface of true cord runs in transverse direction, as against the usual longitudinal pattern it is considered as the feeding vessel for various tissue changes like the cysts, polyps, malignancies that can occur on the medial or superior surface or in response to healing.

If they becomes more convoluted it suggests an advanced pathology. The changes in the vascular pattern due to carcinogenetic stimulus will be spiralling in the form of intraepithelial papillary capillary loop (IPCL). These IPCL will be in the form of wide angled turning points in recurrent respiratory papillomatosis, symmetrically arranged dot like loops with narrow angled turning point in precancerous conditions and irregularly arranged in cancerous conditions. (12,63)

Notable challenge occurs in the diagnosis of hyperkeratotic lesions as with every other biological techniques, as they can obscure the underlying vascular pattern. But by carefully analysing the vascular pattern occurring around such lesions called as the umbrella effect (64), even they cannot get missed. Thus it evaluates the neoangiogenetic pattern within and around a target lesion.

Principles of NBI:(7,11,65–68)

The idea of NBI as an optical method of imaging using endoscopy was conceived in 1999, by Kazuhiro Gono. It was officially launched for clinical use in 2004. Light has an unique characteristics called as the Wave – Particle duality. When considered as a wave, it has a wavelength ie the distance between two peaks in each wave. Light's penetration into different depths of various tissues depends on the wavelength. Its scattering and the absorption characteristics too have their influence on it.

The visible spectrum ranges between the wavelengths, 400 – 700 nm. The different wavelengths are perceived as different colours visually. The light appears white when having a broad bandwidth.

They are also found to behave differently in biological tissues, depending on their wavelengths. When light strikes any surface some of them get reflected while others diffuse into it. As it gets into the tissue and comes in contact with small particles like proteins and nucleic acids, its gets scattered in multiple directions and propagate three dimensionally. The blood also absorbs part of the scattered light.

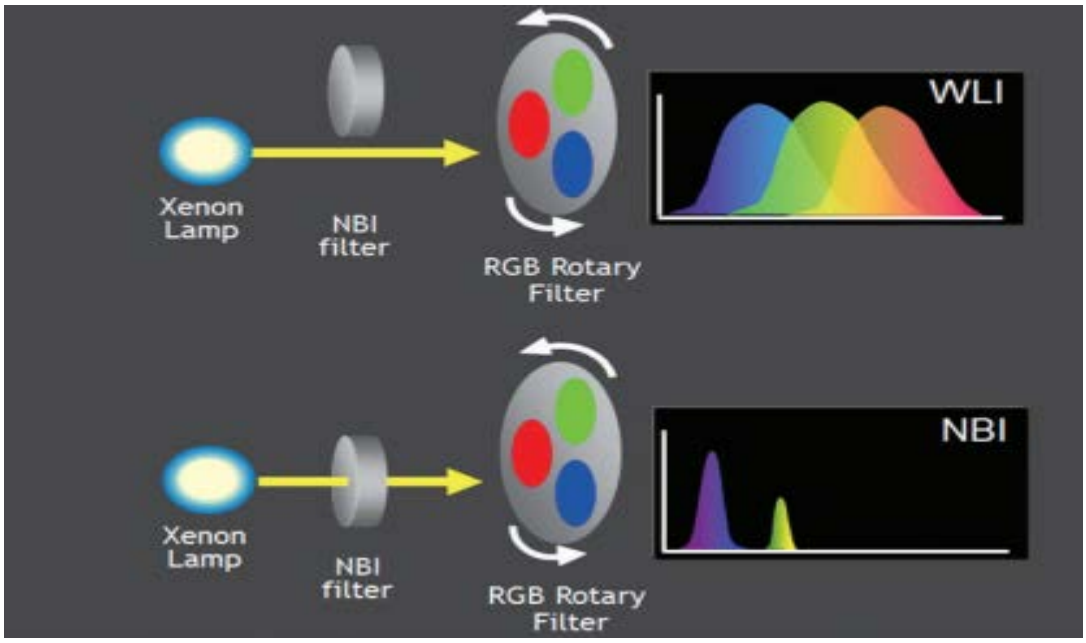

Figure 16: Principles of NBI

The spectrum is narrowed from broad band blue, green and red using optical filter which by moving in and out of the optical axis, absorbs the transmission of all except two bands, one between 400 – 430nm and the other between 525 – 555 nm. In view of NBI's unique rules regarding colour allocation, the final colour produces a different colour than the conventional one.

The first band that is centered at 415nm is the blue filter. It has been allotted the blue and green channel of the observational display, which penetrates a shorter distance into the superficial mucosa and shows the capillaries within the superficial laminal layer as brown.

The other band centered around 540nm is the green filter, which allocated to the red channel of the observational display penetrates the submucosal layer and highlights the thick prominent vessels as cyan.

These vascular patterns, termed as intraepithelial papillary capillary loop pattern observed in the larynx were studied in great detail by Ni et al. They proposed a classification, categorizing the patterns into five types, which ranges from normal to carcinomatous changes(63). The extent of destruction of these IPCL pattern helps in analysing the depth of infiltration by the tumour.(69)

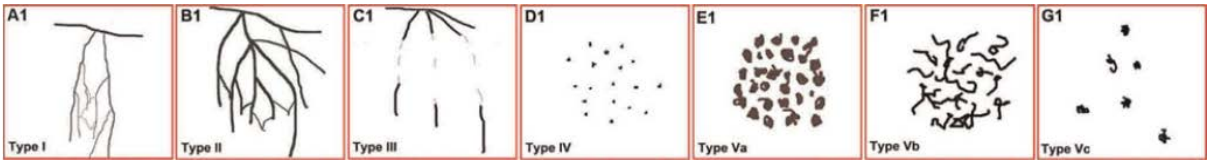

Figure 17. Ni et al IPCL patterns

With the available evaluation tools, pathological changes that occur in the vocal fold epithelium and in the lamina propria layer can be visualised clinically. Though the diagnosis seem evident at times, it is difficult sometimes even histologically, since most of them have subepithelial edema.

Based on the layered structure of the vocal fold, various classification systems have been proposed for the anatomical abnormalities, causing hoarseness. (70)

The epithelial abnormalities include papilloma, leukoplakia, and malignancies.

Those within the lamina propria could be diffuse like Reinke's edema or focal lesions like nodules, polyp, cyst, scar, web, varices and ectasias. Granuloma is the most common benign lesion in the arytenoid.

Hoarseness can also be due to movement disorders caused by neuromuscular control abnormalities such as vocal fold paralysis, paradoxical vocal cord motion, spasmodic dysphonia and essential tremors.

Disorders in the lamina propria that cause hoarseness:

Vocal nodules :

These are bilaterally symmetrical lesions that occur in the mid part of the membranous segment of the vocal cord involving the basement membrane zone and the superficial layer of the lamina propria. A characteristic hour glass shaped glottal closure is seen with minimal influence on the wave pattern. They usually respond to conservative measures like voice therapy.

Vocal fold Cyst:

This is due to focal abnormality of the superficial laminal proprial layer. These are usually unilateral and extend sometimes slightly deep into the lamina propria. The cyst when ruptures can result in vocal cord scarring or a sulcus. They can have a coexistent reactive changes on the contralateral vocal cord and can exist permanently in view of different histopathological changes, hence needs surgical intervention.

Vocal cord polyp:

Polyps can occur in various shapes, sizes and locations. They can be unilateral or bilateral, sessile or pedunculated and haemorrhagic or non haemorrhagic. They are mostly associated with voice overuse, misuse and abuse, especially in speech associated with hard glottal stops. Because of this, they are alleged to form more frequently in men. They can reach extremely large sizes, some being large enough to almost completely obscure the rima glottidis and flop in and out during respiration and phonation.

The pathophysiology involves capillary injury in the superficial lamina propria (SLP), escape of blood and local reaction to this insult leading to edema and reorganization of stromal architecture. Varices and large vessels can lead to formation of hemorrhagic polyps. Immunohistochemical studies have shown increase in levels of various inflammatory markers that promote neovascularization and reorganization of matrix structure. Small polyps maybe difficult to differentiate form nodules since both involve a fibrovascular change however it is important to note that nodules commonly arise from the basement membrane and polyps and cysts from the underlying lamina propria.

Examination may reveal feeding vessels, varices, ectasias and old healing hemorrhage in surrounding areas. The polyps may cause contact changes in contralateral vocal cord due to the striking forces. Often there is a decrease in mucosal waves in the surrounding region and the polyp may impede closure and present with anterior and posterior glottic chinks.

Management ranges from conservative, steroid injections, voice therapy and rest for small lesions to surgery for big polyps. Surgery should ideally be done using phonosurgical techniques with microflap elevation, with or without laser assisted feeding vessel ablation. Voice rest, therapy and adherence to vocal hygiene are essential to avoid recurrence. Adjuvant behaviour therapy and relaxation techniques are advocated in a few cases(71)

Reinke’s edema:

Chronic accumulation of gelatinous, mucinous material within the Reinke’s space leads to Reinke’s edema which is usually associated with tobacco usage, laryngopharyngeal reflux disease and voice overuse.

Vocal fold granuloma:

This usually arises from the vocal process of arytenoid cartilage and is due to the perichondritis, that occurs either due to endotracheal intubation or laryngopharyngeal reflux disease.

Disorders of the epithelium:

The epithelial abnormalities commonly include papillomas, leukoplakia and carcinomas. Among these malignancies are the most significant and maximum research has happened in this area highlighting their risk factors, significance of early detection and appropriate management.

85% to 95% of all laryngeal malignancies is due to squamous cell carcinoma. It arises from the stratified squamous epithelium that has undergone squamous metaplasia. Hence premalignant lesions as classified by WHO pathologically which includes hyperplasia, keratosis, mild, moderate or severe dysplasia and carcinoma in situ(CIS) is of prime importance(72). Visual appearance, does not predict their histological nature. Very early lesions demonstrate hyperkeratosis or parakeratosis without cellular atypia or dysplasia.

Squamous cell dysplasia is characterized by cellular atypia and loss of normal maturation and stratification. The cellular abnormalities of mild dysplasia are limited to the basal one third of the epithelium, moderate dysplasia includes upto two thirds of the epithelial thickness and severe dysplasia includes more than two thirds of the epithelial thickness. Carcinoma in situ (CIS) is an intraepithelial neoplasm where the full thickness of the epithelium shows features suggestive of carcinoma without violation of the basement membrane and stromal violation. Though severe dysplasia and CIS are two different lesions, they behave in a similar fashion, hence can be considered as one(73).

The risk of malignant transformation in mild dysplasia is upto 11% and the severe dysplasia's risk is upto 30%.(74) The mean time interval for such transformation has been found to be between three to four years, which extends up to even ten years(75).

Though biopsy is the gold standard for the diagnosis of such lesions, insufficient biopsy can lead to sampling error**(76).** Multiple adjuvant techniques as discussed before have been developed as a guiding tool to increase the diagnostic accuracy of premalignant conditions yet their ability to distinguish premalignant from malignant conditions is quite a challenge.

5 MATERIALS AND METHODS

This prospective cross sectional study was approved by the Institutional Review Board of Christian Medical College in February 2018 [IRB Min No.10946 [DIAGNO] dated 07/11/2017]

5.1 STUDY DESIGN

Cross Sectional Diagnostic Study

5.2 STUDY PERIOD

February 2018 – September 2018

5.3 SETTING

The study was conducted in the Department of Otorhinolaryngology, Department of Head and Neck surgery and the Department of Pathology, Christian Medical College, Vellore.

5.4 PARTICIPANTS

Every adult patient who came to the otorhinolaryngology outpatient department with hoarseness for more than three weeks and fulfilled the inclusion criteria was recruited into the study after taking an informed consent.

i) Inclusion criteria:

- Adults above 18 years and less than 75 years with hoarseness for more than 3 weeks.

ii) Exclusion criteria:

- Children and subjects less than 18 years
- Pregnant women
- Subjects with breathing difficulty
- Subjects with previous history of neck / laryngeal surgeries
- Subjects who received radiation therapy before
- Subjects with history of neck trauma
- Subjects allergic to lignocaine
- Subjects not cooperative for Narrow Band Imaging.

5.5 SAMPLE SIZE CALCULATION:

The main objective of this study is to assess the ability of Narrow Band Imaging in predicting the malignant potential of the laryngeal structural lesions. Hence the sample size was calculated based on the study done on the endoscopic diagnosis of laryngeal cancer and precancerous lesions by narrow band imaging in 2010 by Ni et al.

Sensitivity of NBI with HPE = 90%

Specificity of NBI with HPE = 90%

$n = 4\ p\ q\ /\ d^2$

p = sensitivity / specificity

q = 1- sensitivity / 1- specificity

d = precision = 10 %

n = (4 x 90 x 10) /100 = 36[40]

Hence we needed 40 in the malignancy arm and 40 in the control arm.

Based on Baitha et al. 2002 – Clinical profile of Hoarseness of voice, the incidence of malignant lesions was around 15 %.

In CMC, the incidence of malignancy among those with hoarseness for more than three weeks requiring micro laryngoscopy and biopsy in ENT, Unit V is around 25% based on the data available from January 2017 to September 2017.

Hence our screening sample was calculated to be 200.

6 STATISTICAL ANALYSIS

Data were summarized using mean(SD)/median(IQR) for continuous variables depending on the normality. the categorical variables were expressed as frequency along with percentage. The diagnostic accuracy of NBI and white-light corresponding to each observer with histology was presented with 95% CI.

The NBI results based on IPCL patterns was presented as frequency (%).

The relation between NBI vascular patterns and biopsy results were studied and validated between four observers.

The agreement between the observers were assessed using kappa and presented with S.E.

All the statistical analysis were performed using STATA /IC 15.0 software.

7METHODOLOGY

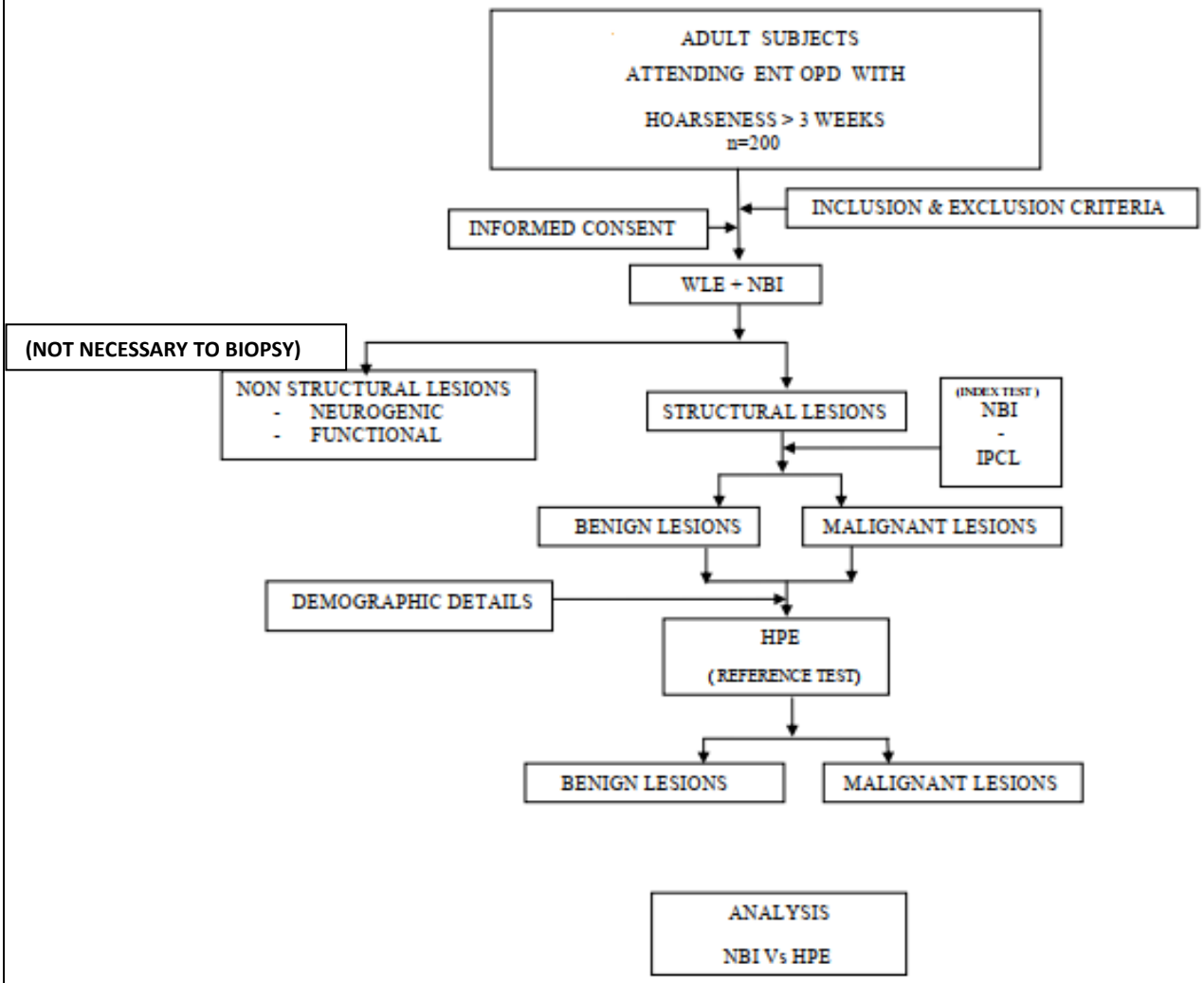

Figure 18: Methodology

Adult subjects who came to the ENT OPD with a change in voice for more than three weeks, fulfilling the inclusion and exclusion criteria, were recruited into the study after taking an informed written consent by the primary investigator.

Elaborate history was taken for each subject and documented in the proforma by the principal investigator.

The subject was seated comfortably and the mucosal surface of the nasal cavity was anaesthetized using 4% Lignocaine spray. The flexible endoscope was introduced and passed through the nasal cavity evaluating the nasopharynx, oropharynx, hypopharynx and the glottis in sequence initially with white light followed by the Narrow Band Imaging mode.

The clinical diagnosis were tabulated and categorized into functional (not necessary to biopsy) and structural lesions. Those subjects with structural lesions were explained the need for micro laryngoscopy and excision/biopsy under general anaesthesia either for complete cure or for obtaining diagnosis, to decide on definite treatment.

Histopathological examination of the excised lesion was carried out using the standard operating protocol in the general pathology department. The excised tissue was fixed using 10 % formalin within one hour of surgery. They were embedded in paraffin blocks, sections were made and subsequently stained with haematoxylin and eosin. The exact nature of the lesion was reported as benign or malignant based on WHO 2010 classification.

Each observer independently reported on the IPCL patterns of the recorded Narrow band images in accordance with the Ni et al types which based on the size of the visualized vessel, their prominence, their regularity and the intraepithelial papillary capillary loop [IPCL] pattern.

IPCL types –Ni et al(63)

Table 2:IPCL Types

Types	IPCL	Vessels	Pathological Correlation
I	Invisible	Prominent	Normal
II	Invisible	Oblique and Arborescence increased	Inflammatory
III	Obscured by white mucosa		Low grade dysplasia
IV	Small dots	Regular arrangement/ low density / capillaries are bifurcated / slightly dilated	Non-invasive High grade dysplasia
Va Shape Regularity Distribution	Solid / Hollow Brownish speckled Pattern		Malignant – micro Invasive carcinoma
Vb	Irregular tortuous Line like		Invasive carcinoma
Vc	Irregular		Invasive carcinoma

Their correlation were obtained statistically and presented below.

8 FUNDING AND APPROVAL

SOURCE OF FUNDING

A FLUID research grant was approved from the institution for the purpose of this study. The funds were used for Narrow Band Imaging charges.

INSTITUTIONAL RESEARCH BOARD APPROVAL AND ETHICAL CONSIDERATIONS

The research proposal was discussed by the Institutional Review Board in 2017 and approval was obtained [IRB Min No: 10946[DIAGNO] dated 07.11.2017]. There were no ethical issues related to this study. Institutional review board approval was obtained for the procedures.

9 RESULTS

200 patients with hoarseness were enrolled into this study.

The following observations were noted

9.1 AGE AND GENDER DISTRIBUTION

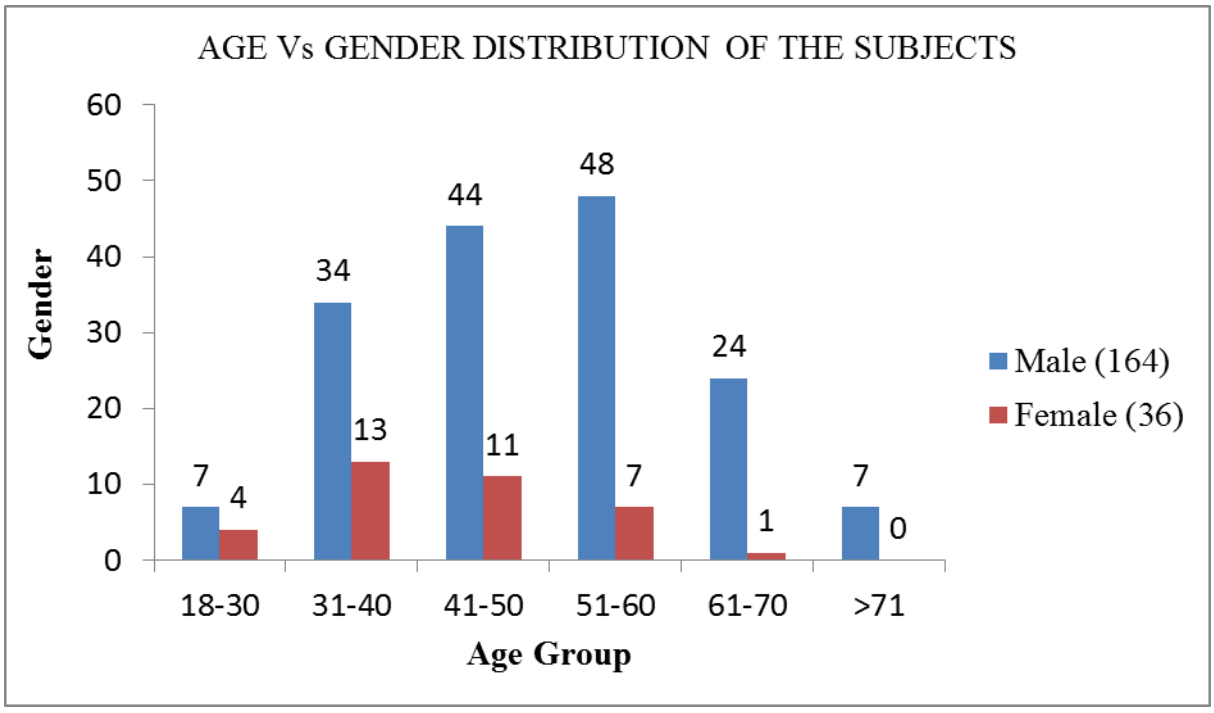

Figure 19: Age and gender distribution

The study participants ranged from 2nd to 7th decade with a mean age of 48 years and most of them [55%] were in the 5th and 6th decade.

Among the gender distribution, 82% were males [164] with females comprising only 18% [36].

9.2 SPECTRA OF LESIONS

In our study, there was a total of 202 lesions documented among the 200 patients based on WLE. These lesions were categorised as structural 168 [83.2%] and 34[16.8%] lesions not necessary to biopsy respectively. Two subjects have more than one lesion each ie; sulcus vocalis in addition to base of tongue growth and keratosis respectively.

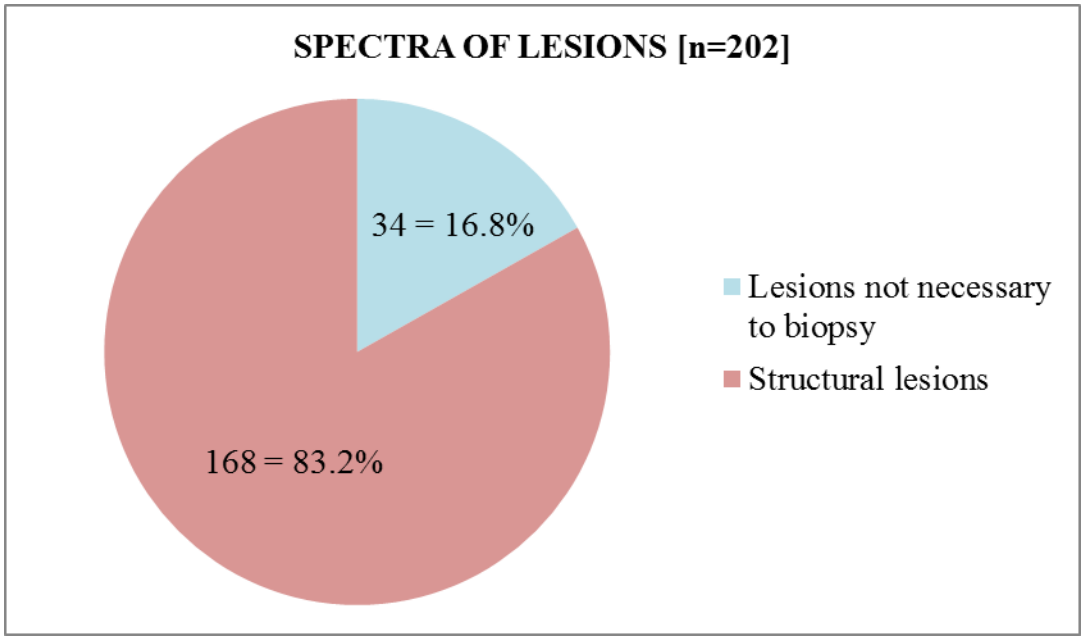

Figure 20: Lesions distribution

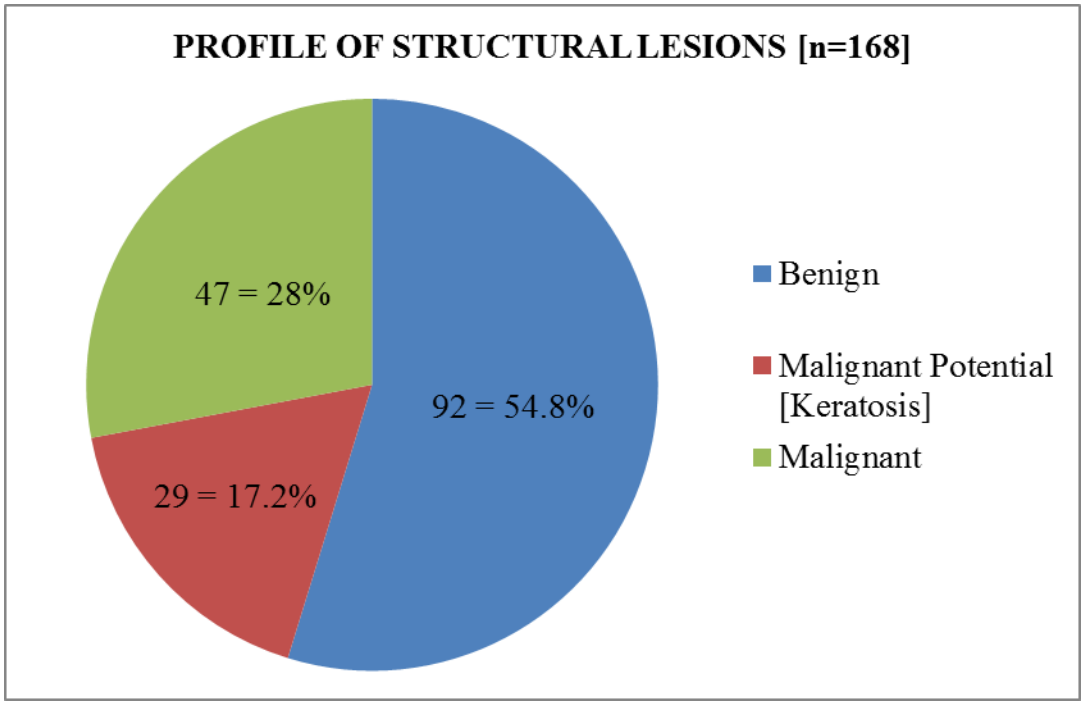

Figure 21: Profile of structural lesions

The structural lesions were classified as benign, ones with malignant potential and malignant. The benign lesions [92 = 54.8%] include vocal cord polyps, nodules, cysts, fungal infections and arytenoid granuloma. The group with malignant potential comprises the keratotic lesions [29 = 17.2%]. The malignant group includes the suspicious and obviously proliferative lesions [47 = 28%]. Further analysis was made by retaining the structural lesions into the above mentioned three groups.

Table 3: Lesions not requiring biopsy

LESIONS NOT REQUIRING BIOPSY	MALE	FEMALE	TOTAL
LARYNGOPHARYNGEAL REFLUX	10	6	16
VOCAL CORD PALSY	5	0	5
MUSCLE TENSION DYSPHONIA	5	0	5
SULCUS VOCALIS	3	0	3
FUNCTIONAL DYSPHONIA	0	1	1
TELENGECTASIA	0	1	1
CHRONIC LARYNGITIS	2	1	3
TOTAL	25	9	34

Among the lesions not necessitating biopsy we came across in our study, laryngopharyngeal reflux accounts for 47%. One subject while undergoing cross sectional imaging for the vocal cord palsy was diagnosed to have lung malignancy. The remaining subjects had no other comorbid conditions, hence managed conservatively and were on follow up.

9.3 POPULATION DISTRIBUTION

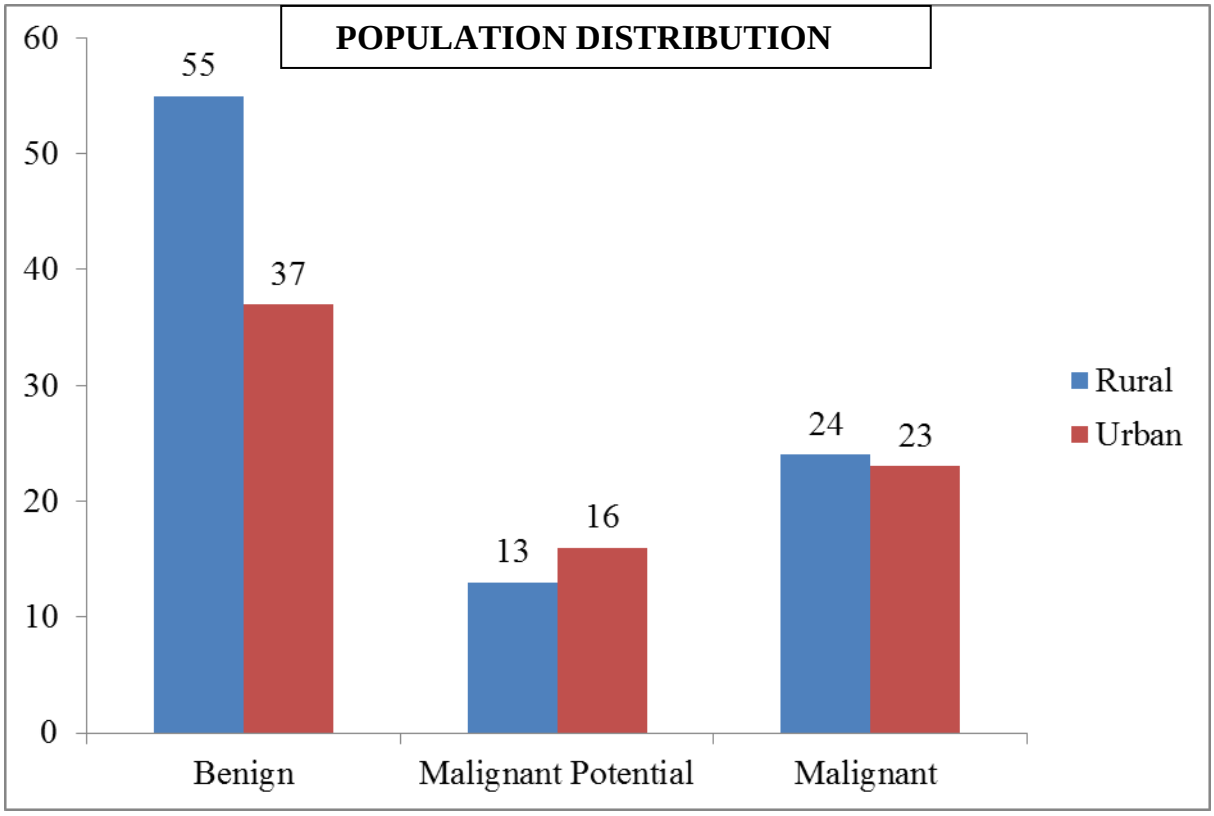

Figure 22: Population distribution

Among the subjects in the structural lesions group, 54% of them belonged to the rural population. The remaining 46% were from the urban areas giving a rural: urban ratio of 1.2:1.

9.4 DISTRIBUTION OF LESIONS BASED ON VOICE USER'S LEVEL

The study subjects were classified based on their level of voice usage into four grades.

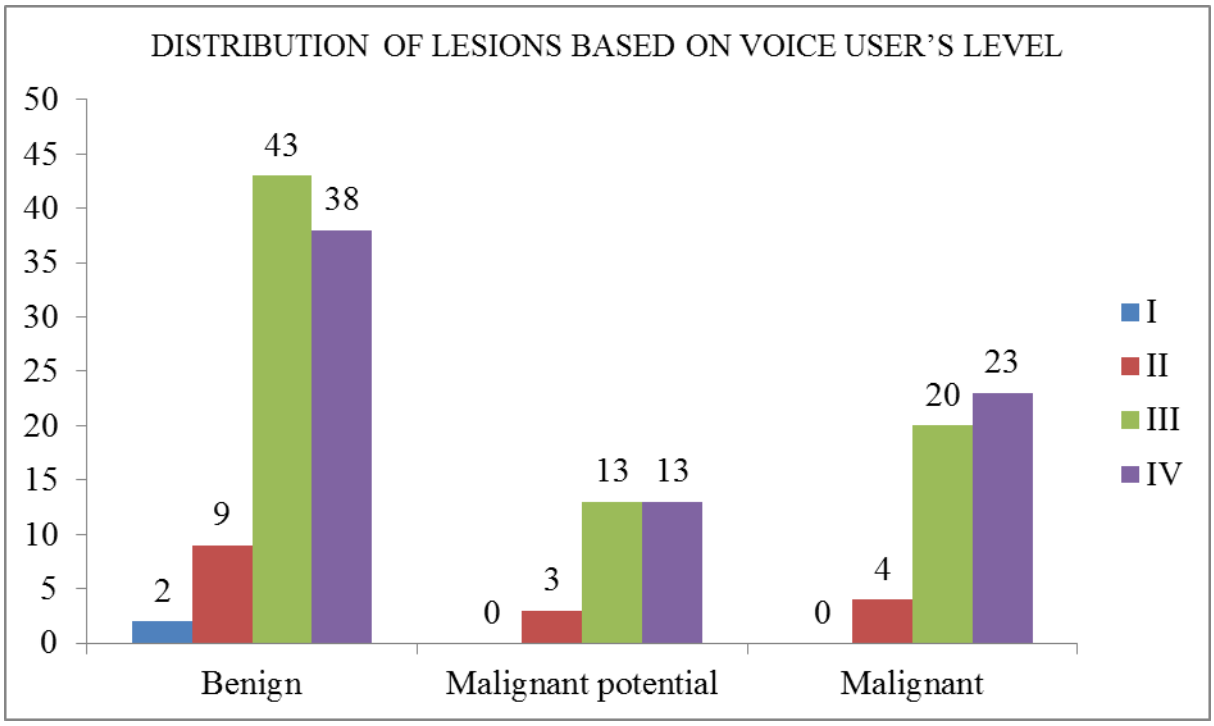

Figure 23: Distribution of lesions based on voice user's level

The subjects recruited in this study, belonged predominantly [89%] to the level III and level IV voice users. Among those with benign lesions, 88% were semi-skilled and unskilled workers, who probably had a lot of voice abuse.

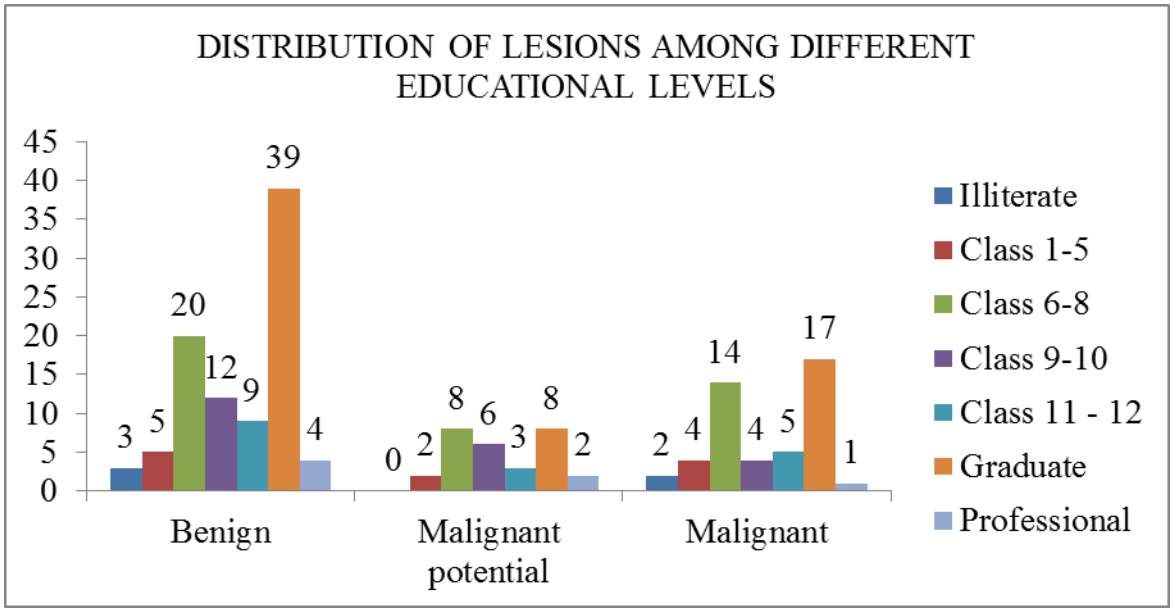

Figure 24: Distribution of lesions among different education levels

On comparing the distribution of structural lesions among subjects and their education levels, we observed that the benign lesions were found predominant among the graduates whereas malignant lesions and lesions with malignant potential were more among the illiterate subjects.

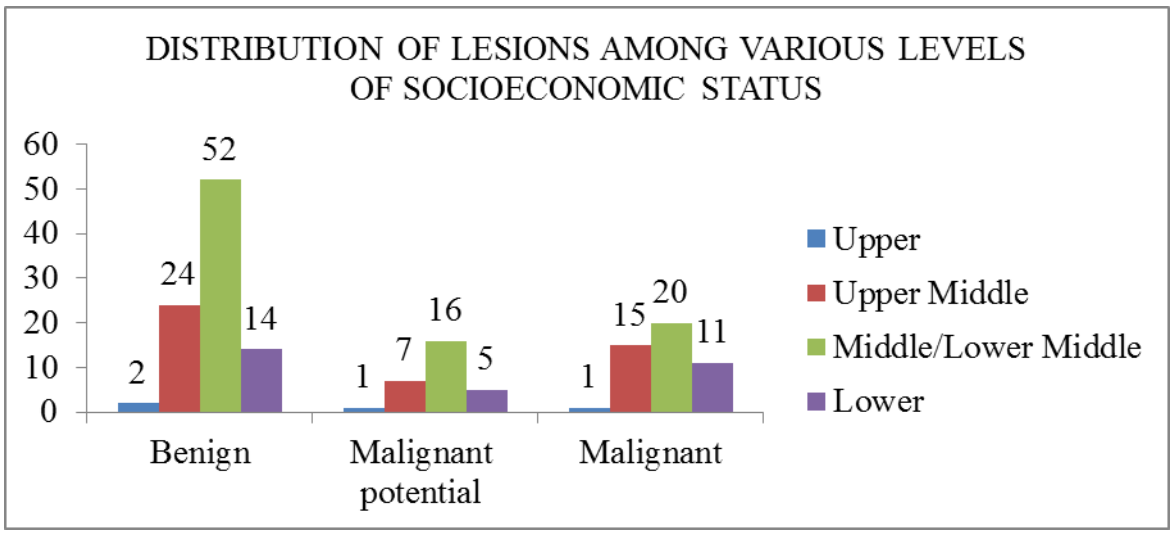

Figure 25: Distribution of lesions among various levels of socioeconomic status

It was interesting to observe that irrespective of type of the lesion, 75 to 80 % of them belonged to the middle class group.

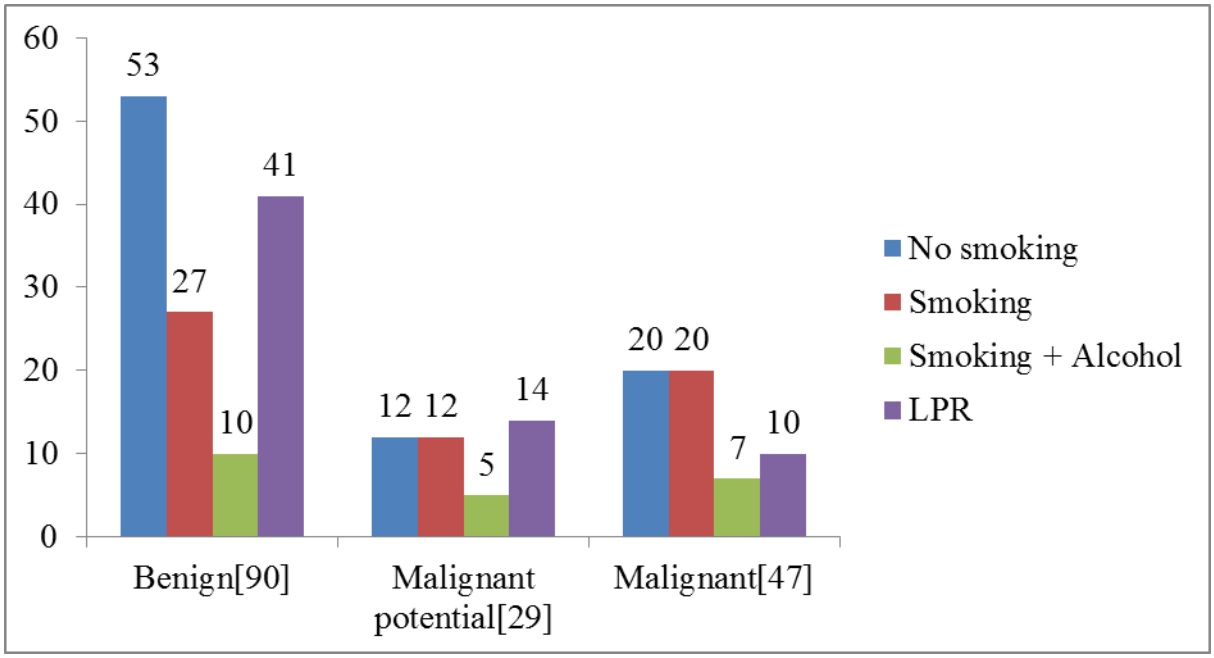

Figure 26: Risk factor analysis

Among the risk factors namely smoking, alcohol consumption and laryngopharyngeal reflux disease associated with laryngeal malignancies, it is conforming to the multifactorial pattern. Laryngopharyngeal reflux disease was predominantly found to exist in subjects with benign pathology.

9.5 DISTRIBUTION OF MALIGNANT LESIONS

All suspected malignant lesions in our study were grouped according to their site of origin. 85 % of lesions were noted in the supraglottic and glottis regions. Of the 47 subjects, 35 subjects consented for biopsy. 8 of the other 12 subjects were more than 60 years of age and refused further management when the possibility of malignancy

was discussed. Two subjects were sputum acid fast bacilli positive and started on anti-tuberculous therapy. Hence the lesion on the vocal cord was considered as laryngeal tuberculosis and not biopsied. One of them who turned for follow up had complete resolution. The two other subjects were enrolled close to the end of this study though planned for biopsy, could not be included for final analysis.

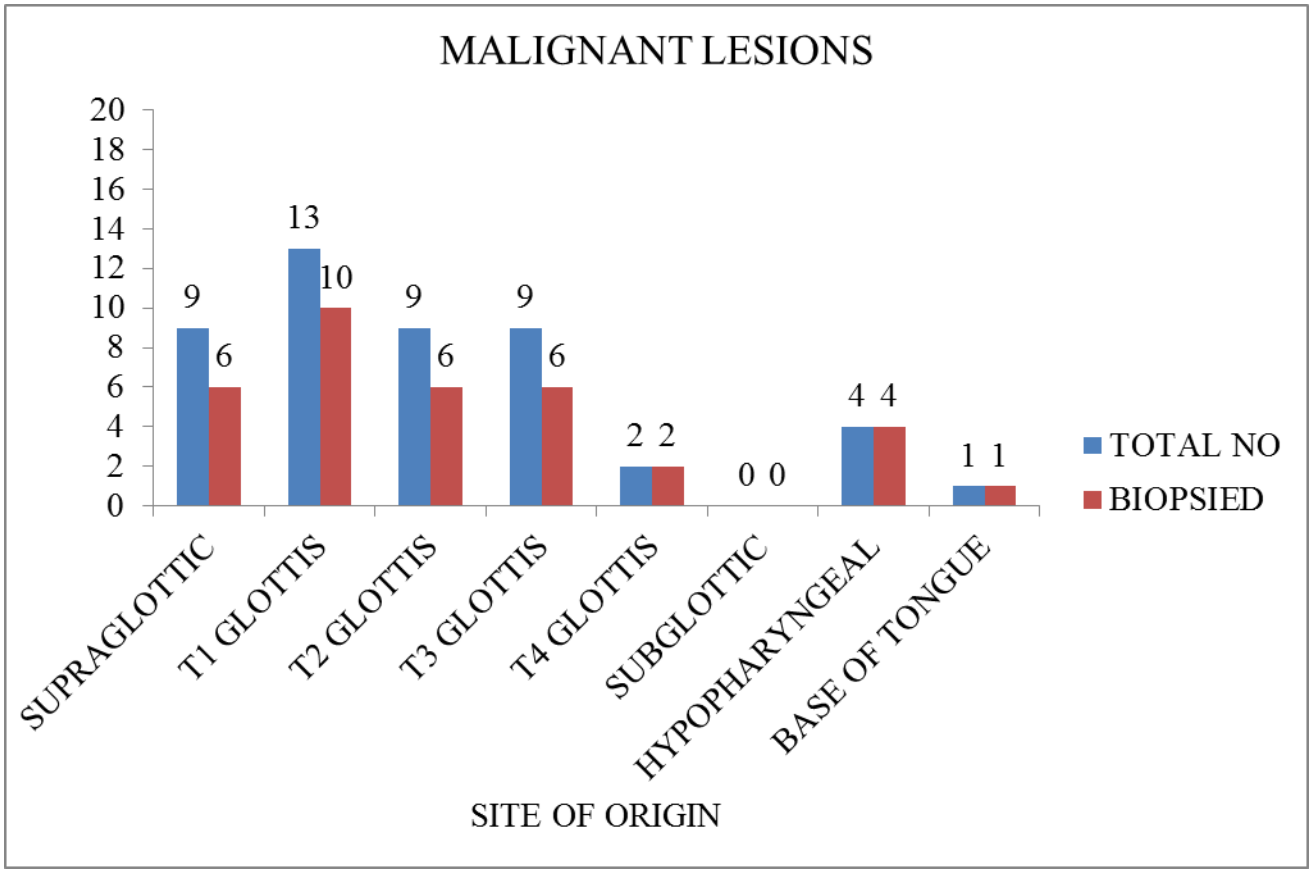

Figure 27: Malignant lesions and their site of origin

The NBI pictures of the lesions of all subjects who underwent biopsy, was observed and interpreted independently by all four observers. Their IPCL pattern has been documented in accordance with Ni et al classification.

How precise our four observers are, pertaining to agreement between them was observed using Kappa statistics. [0 – Chance agreement ; 1- perfect agreement].

Table 4: Inter observer variability

INTEROBSERVER VARIABILITY				
[IPCL]	EXPECTED AGGREMENT	OBSERVED AGREEMENT	KAPPA[S.E]	Degree of agreement
1 Vs 2	50.01%	82.76%	0.6551[0.0986]	Substantial agreement
1 Vs 3	47.00%	76.14%	0.5497[0.0890]	Moderate agreement
1 Vs 4	49.79%	75.00%	0.5021[0.1023]	Moderate agreement
2 Vs 3	47.13%	80.46%	0.6304[0.0889]	Substantial agreement
2 vs 4	49.58%	78.16%	0.5668[0.1004]	Moderate agreement
3 Vs 4	46.18%	68.18%	0.4088[0.0891]	Fair agreement

The inter observer variability among the four observers was calculated using Kappa and found that their agreement was fair to substantial.

Table 5: Observer 1 - NBI versus HPE

OBSERVER1	HISTOLOGICAL DIAGNOSIS												
NBI LESION		BENIGN									MALIGNANT		
		3	4	33	1	2	6	2	2	0	1	30	84
IPCL PATTERN		NODULE	CYST	POLYP	AMYLOID	FUNGAL	EPITH HYPER	DYSPLASIA			CIS	INVAS CARCI	
								LOW	MOD	HIGH			
I	Invisible	2		4							1		
II	Invisible		1	11			1					1	
III	Obscured	1	2	9		2	2					3	
IV	Small dots		1	4			2					3	
Va	Speckled			1					1			6	
Vb	Tortuous			1			1	2				12	
Vc	Speckled/ Tortuous			1	1				1			5	

Not typed				2									
		3	4	33	1	2	6	2	2	0	1	30	84

Table 6: Observer 2 - NBI versus HPE

OBSERVER2		HISTOLOGICAL DIAGNOSIS											
NBI LESION		BENIGN									MALIGNANT		
		3	4	33	1	2	6	2	2	0	1	30	84
IPCL PATTERN		NODULE	CYST	POLYP	AMYLOID	FUNGAL	EPIT HYPER	DYSPLASIA			CIS	INVAS CARCI	
								LOW	MOD	HIGH			
I	Invisible	2	1	2									5
II	Invisible		2	18	1		1					3	25
III	Obscured	1		6		1	3	1	2		1	4	19
IV	Small dots			2								1	3
Va	Speckled		1	1				1				7	10
Vb	Tortuous			2		1	2					13	18
Vc	Speckled/ Tortuous											1	1
Not typed				2									2
		3	4	33	1	2	6	2	2	0	1	29	83

Table 7: Observer 3 - NBI versus HPE

OBSERVER 3		HISTOLOGICAL DIAGNOSIS											
NBI LESION		BENIGN									MALIGNANT		
		3	4	33	1	2	6	2	2	0	1	30	84
IPCL PATTERN		NODULE	CYST	POLYP	AMYLOID	FUNGAL	EPIT HYPER	DYSPLASIA			CIS	INVAS CARCI	
								LOW	MOD	HIGH			
I	Invisible	2	2	5								2	11
II	Invisible	1	1	12			1	1				3	19
III	Obscured			2		1	3		2		1	4	13
IV	Small dots		1	2			1					3	7
Va	Speckled			2								6	8
Vb	Tortuous			1	1	1	1					8	12
Vc	Speckled/ Tortuous			1				1				3	5
Not typed				8								1	9
		3	4	33	1	2	6	2	2	0	1	30	84

Table 8: Observer 4 - NBI versus HPE

OBSERVER 4		HISTOLOGICAL DIAGNOSIS											
NBI LESION		BENIGN									MALIGNANT		
		3	4	33	1	2	6	2	2	0	1	30	84
IPCL PATTERN		NODULE	CYST	POLYP	AMYLOID	FUNGAL	EPIT HYPER	DYSPLASIA			CIS	INVAS CARCI	
								LOW	MOD	HIGH			
I	Invisible		2	1	1							1	5
II	Invisible	2		17		1	1					3	34
III	Obscured		1	6			1		1			3	12
IV	Small dots	1		1			1					2	5
Va	Speckled			1								5	6
Vb	Tortuous		1	7		1	2	2			1	12	26
Vc	Speckled/ Tortuous						1		1			4	6
Not typed													
		3	4	33	1	2	6	2	2	0	1	30	84

The NBI pattern which was reported individually by all four observers was tabulated against the histological diagnosis and was analysed.

Table 9: IPCL versus HPE results(a)

IPCL VS HPE	SENSITIVITY [95% CI]	SPECIFICITY [95% CI]	PPV [95% CI]	NPV [95% CI]
OBSERVER 1	69% [49.2 - 84.7]	83.3% [69.8 - 92.5]	71.4% [51.3 - 86.8]	81.6% [68 - 91.2]
OBSERVER 2	64.3% [44.1 - 81.4]	85.4% [72.2 - 93.9]	72% [50.6 - 87.9]	80.4% [66.9 - 90.2]
OBSERVER 3	53.6% [33.9 - 72.5]	83.3% [68.6 - 93]	68.2% [45.1 - 86.1]	72.9% [58.2 - 84.7]
OBSERVER 4	72.4% [52.8 - 87.3]	72% [57.5 - 83.8]	60% [42.1 - 76.1]	81.8% [67.3 - 91.8]

Table 10: IPCL versus HPE results (b)

IPCL VS HPE	LIKELIHOOD RATIO [LR-POSITIVE]	LIKELIHOOD RATIO [LR-NEGATIVE]	DIAGNOSTIC ODDS RATIO [DOR]
OBSERVER1	4.14 (2.1-8.15)	0.372 (0.213-0.65)	11.1 (3.78-32.7)
OBSERVER2	4.41 (2.11-9.22)	0.418 (0.251-0.697)	10.5 (3.52-31.6)
OBSERVER3	3.21 (1.5-6.87)	0.557 (0.366-0.848)	5.77 (1.95-17)
OBSERVER 4	2.59 (1.57-4.26)	0.383 (0.207-0.708)	6.75 (2.46-18.5)

The sensitivity, specificity, negative predictive value, positive predictive values were obtained for NBI against Histological examination. In view of being a diagnostic study in this tertiary centre, likelihood and odds ratio were also calculated to remove the influence of prevalence over the results.

The Receiver Operating Characteristics curve was plotted for IPCL pattern to validate the Ni et al classification.

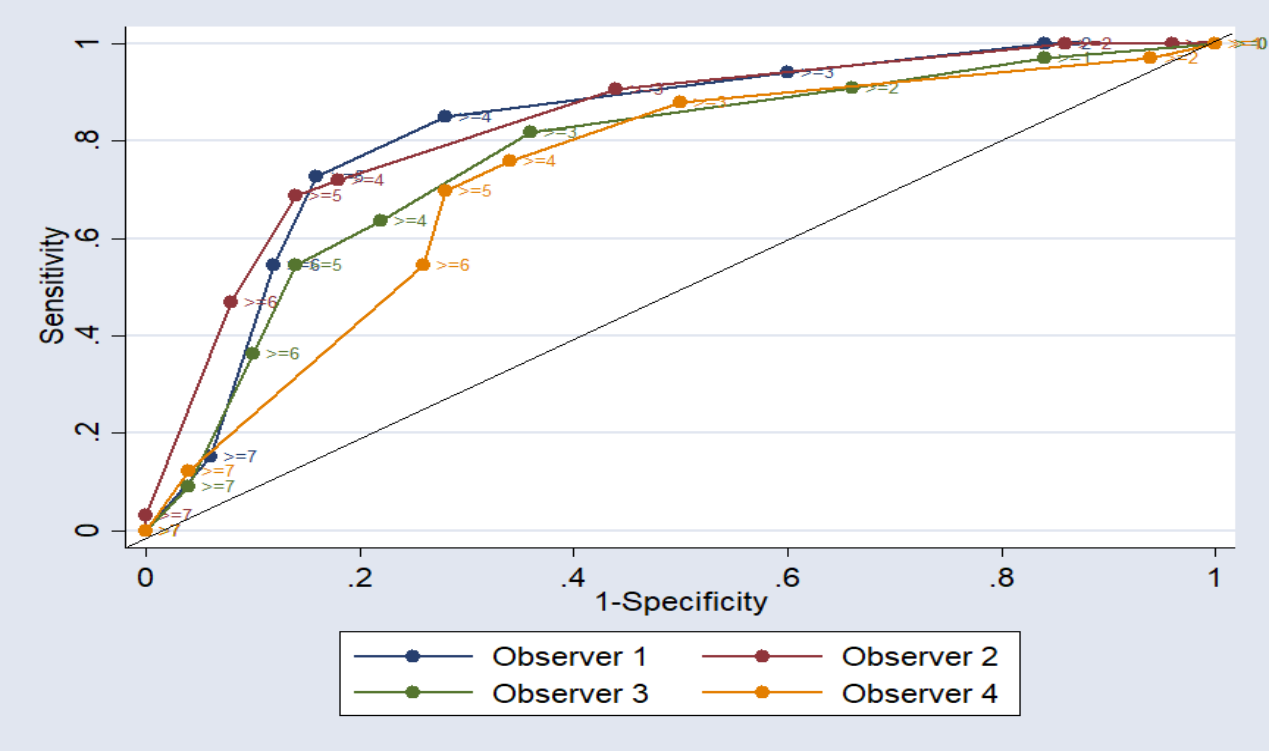

Figure 28: ROC - IPCL

OBSERVERS	AUC
OBSERVER 1	0.8216
OBSERVER 2	0.8344
OBSERVER 3	0.7772
OBSERVER 4	0.7247

Table 11: AUC - IPCL

The sensitivity and specificity for each IPCL pattern is tabulated below.

Table 12: Sensitivity of IPCL

NBI	SENSITIVITY			
IPCL Cut off points	OBSERVER 1	OBSERVER 2	OBSERVER 3	OBSERVER 4
I	100%	100%	96.97%	100%
II	100%	100%	90.91%	96.97%
III	93.94%	90.63%	81.82%	87.88%
IV	84.85%	71.88%	63.64%	75.66%
Va	72.73%	68.75%	54.55%	69.70%
Vb	54.55%	46.88%	36.36%	54.55%
Vc	15.15%	3.13%	9.09%	12.12%
Not typed	100%	100%	100%	NIL

This table highlights the good sensitivity of NBI with type I-III IPCL patterns.

Table 13: Specificity of IPCL

NBI	SPECIFICITY			
IPCL	OBSERVER 1	OBSERVER 2	OBSERVER 3	OBSERVER 4
I	4%	45	16%	0%
II	16%	14%	34%	6%
III	40%	56%	64%	50%
IV	72%	82%	78%	66%
Va	84%	86%	86%	72%
Vb	88%	92%	90%	74%
Vc	94%	100%	96%	NIL
Not typed	0%	0%	0%	0%

This table highlights the good specificity of NBI with Type V IPCL pattern.

10 DISCUSSION

Narrow Band Imaging is a recent optical technology conceived in 1999 by Kazuhiro Gono, which reduces the bandwidth of white light endoscopy into a narrow band comprising only 415 and 540nm. It contrasts and highlights the sub epithelial capillary pattern with respect to the surrounding mucosa. This helps in vivo analysis of the microvascular architectural transformation that occurs in malignant conditions. The narrow band light falls within the absorption spectrum of haemoglobin, enhancing the terminal micro vessels located on the epithelium. These are referred to as intraepithelial papillary capillary loops, which are the prime determinants in the clinical diagnosis of epithelial lesions.

About 85-95 % of laryngeal malignancies are squamous cell carcinomas. Premalignant changes that occur in the epithelium include a wide array of pathological changes. They start with epithelial hyperplasia, progress to dysplasia of various grades, than carcinoma in situ before becoming frank invasive carcinoma.

We studied 200 patients with hoarseness and most of them were male 164(82%) as seen in other studies. (3,5) This could be because the known risk factors for hoarseness like voice abuse, smoking, tobacco chewing and alcohol are more prevalent among the male population in India(77,78).

The most common age of presentation was between the 4^{th} and 5^{th} decade (55%) which is in concurrence with other Indian studies(3,5).

Most of our subjects were from West Bengal (103/200 – 51.5%) followed by Bangladesh (32/200 – 16%), as ours is a tertiary referral centre to which most of the subjects come from the above mentioned regions.

Though our study population was predominantly graduates from middle socioeconomic status, there was no major difference in the number of subjects who came from urban (114/200-57%) or rural (86/200-43%) place . This is probably because of better awareness, health seeking behaviour and access to healthcare among the educated group. There is a close association between a person's profession, his voice usage and laryngeal pathologies as noted in various studies(3,79). However, in this study most of them belonged to the levels III and IV voice users (176/200 – 88%).

The average duration of hoarseness was 5 months with the earliest presentation time being 6 weeks and the latest 14 years, however the majority presented within one year (131/200, 65%) as has also been reported by Chopra and Kapoor (80,81). Since hoarseness is one of the early symptoms of laryngeal pathology, there is a need for creating awareness to facilitate early diagnosis.

Smoking (87/200, 43.50%), alcohol(26/200, 13%) and laryngopharyngeal reflux(81/200, 40.50%) were studied to be the risk factors associated with laryngeal lesions in our study. Baitha et al and Raja et al report 25.45% and 19% smokers respectively(3,82). However we found no significant correlation between the above analysed risk factors and malignancies.

The main objectives of the study included categorizing lesions and predicting their malignant potential. This was done using the proposed IPCL pattern by Ni et al.(63)

Four observers separately classified the lesions in the above given IPCL patterns and their results were calculated using appropriate statistical tools.

The overall **sensitivity** of NBI in predicting malignancy with histopathological examination as gold standard was between **53.6% – 72.4%** for four independent observers. In comparison multiple other studies have found the sensitivity to be between 88% - 97% as tabulated below in Table 14.

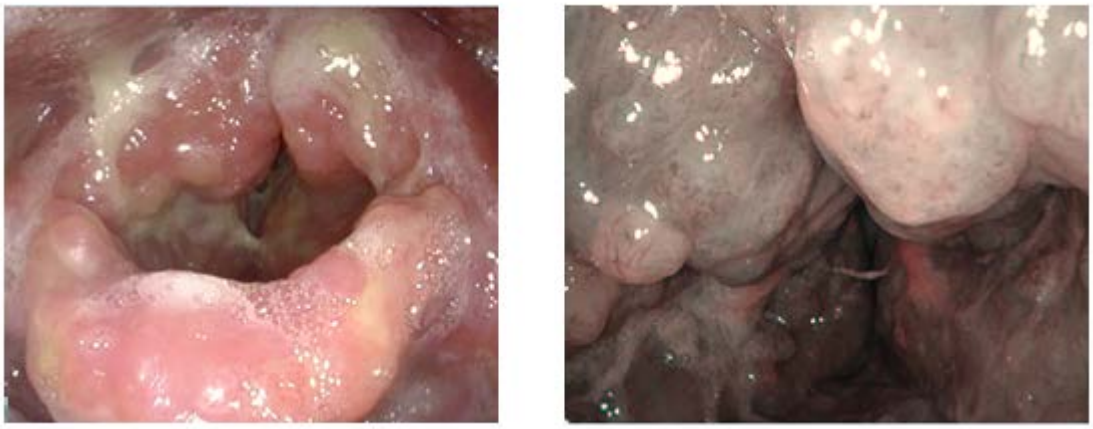

Figure 29: WLE and NBI

The above image suggestive of irregular growth in WLE, IPCL too suggestive off malignant pattern, but biopsy came as benign pathology - Histoplasmosis.

The **specificity** of NBI with histopathological examination as gold standard **varied between 72% - 85.4%.** Other similar studies have found the specificity to be between 92% to 96%, shown in Table 14.

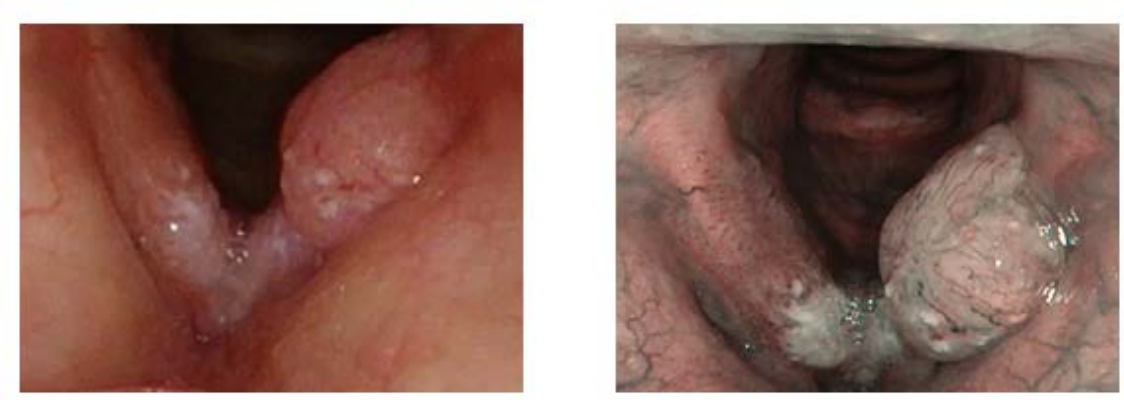

Figure 30: WLE and NBI - Malignancy

The above NBI image highlights the Right vocal cord involvement which is not seen with the WLE.

Table 14: Comparison with other studies

REFERENCES	SENSITIVITY (95% CI)	SPECIFICITY (95% CI)	PLR	NLR	ODS
Wantanabe et al(83)	91% (72 - 99)	92% (62 -100)	10.96 (1.67-71.86)	0.09 (0.02-0.36)	115.50 (9.40-1419.60)
Ni et al(63)	89% (76 - 96)	93% (84-98)	13.11 (5.06-33.96)	0.12 (0.05-0.27)	110.00 (27.77-435.68)
Stanikova et al(84)	88% (0.69-0.97)	95% (82-99)	16.72 (4.31-64.93)	0.13 (0.04-0.37)	132.00 (20.42-853.17)
Kraft et al(85)	97% (90-100)	96% (92 - 99)	26.78 (11.31-63.37)	0.03 (0.01-0.12)	864.50 (163.32-4576.05)

Meta analysis(86)	94% (91 – 96%)	89% (85-92)	10.98 (4.29-28.14)	0.08 (0.05-0.14)	140.09 (46.44-422.60)
Our study	53.6% – 72.4% (33.9 – 87.3)	72% - 85.4% (57.5%-93.9%)	2.59 – 4.41 (1.5-9.22)	0.372 – 0.557 (0.207 – 0.848)	5.7 – 11.1 (1.95- 32.7)

The Likelihood ratio positive[**LRP**] of NBI with histopathological examination was between 2.59 – 4.4, other studies quoted have the LRP between 10.96 – 26.78

The Negative Likelihood ratio **[LRN]** of NBI with histopathological examination was between 0.372 – 0.557, other studies quoted have the NPV between (0.03 – 0.13)

The Diagnostic odd's ratio [**DOR**] of NBI with histopathological examination was between 5.7 – 11.1 in comparison other studies has (110-864.50).

In order to assess the best cut-off points of the Ni et al score we created an receiver operating characteristics curve (ROC – Figure 28). Each independent observer is represented by a separate line. As analysed there was no statistically significant difference between the four observers. The area under the curve (AUC) varied between 0.72 to 0.83, suggesting reasonable differentiating ability of the score. (Table11)

Scores 1-3 have a good sensitivity (Table12) to rule out malignancy while score of 5a to 5c have good specificity (Table 13) to rule in malignancy. Scores in-between need additional diagnostic tests or clinical expertise to make a management decision.

11 LIMITATIONS OF THIS STUDY

1. The number of malignant lesions included in this study was not large enough to come to a significant conclusion.

2. Majority of the malignant lesions were advanced at presentation, hence NBI's influence on small, superficial mucosal lesions could not be ascertained.

3. The IPCL pattern reporting was based on recorded images, however real time assessment probably would have helped in analysing them better.

4. There was ambiguity in reporting the lesions obscured by hyperkeratosis, which needs better assessment.

12 CONCLUSION

1. Narrow Band Imaging is a novel diagnostic tool which enabled us to differentiate benign from malignant lesions.

2. The potentially malignant laryngeal lesions can be effectively diagnosed using this modality.

3. NBI has a significant role in the diagnosis of superficial laryngeal lesions on the basis of IPCL pattern.

4. To formulate a protocol for the management of early glottic malignancy incorporating NBI needs a much larger study.

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14 ANNEXURE

14.1 ANNEXURE 1 - CONSENT FORM

Informed Consent form to participate in a research study

Study Title:

Role of Narrow Band Imaging in predicting the malignant potential of laryngeal structural lesions in subjects presenting with hoarseness for more than three weeks with respect to Histopathological examination in a tertiary care centre.

Study Number: 10946 [DIAGNOSTIC]

Subject's Initials: _____ Subject's Name: _____

Date of Birth / Age: _____

(Subject)

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that the Sponsor of the clinical trial, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). [ ]

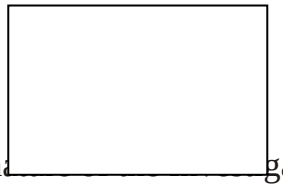
(v) I agree to take part in the above study. [ ]

Signature (or Thumb impression) of the Subject/Legally Acceptable

Date: _____/_____/_____

Signatory's Name: _____ Signature:

Or



Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

Signature or thumb impression of the Witness: _____

Date: _____/_____/_____

Name & Address of the Witness: _____

14.2 ANNEXURE 2 – PATIENT INFORMATION SHEE

INFORMED CONSENT- INFORMATION SHEET

DEPARTMENT OF OTORHINOLARYNGOLOGY

CHRISTIAN MEDICAL COLLEGE, VELLORE.

You are being requested to participate in a study which involves subjects with voice change for more than three weeks. Please read this information form carefully. Take time to understand and clear your doubts if any. The study personnel will explain to you about any of the information which you do not clearly understand.

Study title:

"Prediction of cancer risk in voice box lesions based on Narrow Band Imaging"

[NBI] – A diagnostic study.

Description of the study:

Voice change persisting for more than three weeks, could be due to changes in the voice producing structures. This could be due to a mass or nerve weakness which does not allow the voice box to close well during speaking. The mass could be either cancerous or non cancerous.

Normally, subjects with persistent voice change will be evaluated by passing a tube with a camera attached to its tip through the nose to examine the voice box. And subsequently, subjects who are found to have a mass lesion will undergo surgical procedure under sedation, where few pieces of the mass will be removed and examined under the microscopy to confirm its exact nature as cancer or not.

In this study, a newer technique named Narrow band imaging is employed. It also looks like a normal endoscope with high quality camera at its tip, but with few modifications of the light passing through it using filters. This will highlight the blood vessel arrangement in the voice box. Based on the alterations in the blood vessel arrangement pattern, as happens early in cancer, it would be possible to predict whether the lesion is cancerous or not.

Following this, the subjects with voice box lesions will undergo a surgical procedure under anaesthesia, where the lesion will be removed and examined under the microscopy to confirm its exact nature.

This would help in the early initiation of treatment for better voice and survival outcome.

You will be asked a few questions in relation to your symptoms and life style, as part of this study.

Benefits of the study:

This new technique will help us to predict the cancer risk associated with voice box lesions.

Risks or discomfort to the subject:

The side effects if any, are rarely bleeding from the nose or throat.

Confidentiality:

Your identity will be strictly kept confidential and only the results of this study will be published in a scientific journal. No one other than the treating doctors and the
investigators of this study shall have access to your medical records.

Participation:

Your participation in this study is voluntary and you are free to withdraw at any time, without giving any reason. Refusal to participate in the research study will not involve any penalty or loss of benefits to which you are otherwise entitled.

Cost:

There is absolutely no additional cost to you as a result of participation in this study.

Contact person:

Dr. J.Justin Ebenezer Sargunaraj

Dept of ENT 5,

CMC Vellore.

Mobile: 9940919262.

14.3 ANEXXURE 3 - DATA COLLECTION FORM

STUDY TITLE: Role of Narrow Band Imaging in predicting the malignant potential of laryngeal structural lesions in subjects presenting with hoarseness for more than three weeks in comparison to Histopathological examination in a tertiary care centre.

Study Number: 10946 [DIAGNOSTIC]

Investigator: DR. J.JUSTIN EBENEZER SARGUNARAJ

Site of Collection / Institution: DEPARTMENT OF ENT, CMC VELLORE.

Subject No : ☐☐☐

Name: ☐☐☐☐☐☐ ☐☐☐☐☐☐ ☐☐☐☐☐☐ ☐

Hospital No:☐☐☐☐☐☐ Date of entry : ☐☐☐☐☐☐ ☐

Address and Contact details:

Mobile no : ☐☐☐☐☐☐ ☐ ☐☐☐

☐☐☐☐☐☐ ☐ ☐☐☐

Email id:

DEMOGRAPHIC DETAILS:

Age: ☐ ☐ Years Date of Birth: ☐☐☐☐☐☐ Gender:☐Male ☐Female

Marital status: Single 0 Married 0 Religion:

Residence : Rural ☐ Urban: ☐

Profession: Voice user's :Level I ☐ II ☐ III ☐ IV ☐

Education Level: Illiterate ☐ School (Class): 1-5 ☐ 6-8 ☐ 9-10☐11- 12☐

☐ Graduate ☐ Professional ☐

Socioeconomic status : ☐

[Kuppuswamy's scale]

Family history of Malignancy: Yes ☐ No ☐

Clinical Symptoms:

HOARSENESS : Yes ☐ No ☐ Duration : ☐Weeks ☐ ☐ Months ☐ ☐ Years

Associated symptoms:

Throat pain : Yes ☐ No ☐

Foreign body sensation in the throat: Yes ☐ No ☐

Heart burn / Reflux / Acidity: Yes ☐ No ☐

Difficulty in breathing : Yes ☐ No ☐

Difficulty in swallowing – Solids / Liquids : Yes ☐ No ☐

Cough – Nocturnal / Periodicity : Yes ☐ No ☐

Swelling in the neck: Yes ☐ No ☐

Risk factor assessment:

Smoking:

Non smoked tobacco:

Alcohol:

Medications: Proton pump inhibitors ☐ Steroids ☐ Thyroid medications ☐

Neck Trauma ☐ Surgery – Neck / Spine ☐ Endo tracheal intubation ☐

Neurological condition ☐ Radiation therapy ☐

Structural lesions:

Vocal nodule / Cyst / Polyp / Papilloma / Keratosis / Growth

Analysis:

White light endoscopy diagnosis:

NBI done by : Dr. ----------

Vascular pattern:

Longitudinal ☐ Perpendicular ☐

Vessel thickness – Thin☐ Prominent ☐ Oblique ☐

IPCL - Invisible ☐ Obscured ☐ Small dots ☐ Solid ☐ Irregular ☐

Inference : [In accordance with Ni et al classification] :

Observer 1 / Observer 2 / Observer 3 / Observer 4 :

Types I ☐` II ☐ III ☐ IV ☐ V ☐ a / b /c

Benign ☐ Malignant ☐

Histological diagnosis: Biopsy No ----------

Papillae Present ☐ Absent ☐

Hyperkeratosis Present ☐ Absent ☐

Epithelial hyperplasia Present ☐ Absent ☐

Mild dysplasia Present ☐ Absent ☐

Moderate dysplasia Present ☐ Absent ☐

Carcinoma in situ Present ☐ Absent ☐

Invasive carcinoma Present ☐ Absent ☐

Inflammation Present ☐ Absent ☐

Pathological diagnosis : ----------

14.4 ANNEXURE 4 – DATA SHEET

1) NBI – SEPARATE ATTACHMENT

2) PATHOLOGY – SEPARATE ATTACHMENT.