UNIVERSITY OF THE WITWATERSRAND

A topographical and surgical anatomical description of the recurrent laryngeal nerve in adult and paediatric populations of South Africa

Dr Joshua Boanerges Lusamba Kiluba (MBBCh-Wits)

SUPERVISORS

Dr Pedzisai MAZENGENYA
School of Anatomical Sciences
University of the Witwatersrand

Professor Thifhelimbilu Emmanuel LUVHENGO
MBChB (Medunsa), FCS(SA)
Department of Surgery
University of the Witwatersrand

Dr Candice Small
School of Anatomical Sciences
University of the Witwatersrand

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DECLARATION

I, Joshua Boanerges Lusamba Kiluba (Student number: 320883) declare that this Thesis is my own, unaided work. It is being submitted for the Degree of Master of Science in Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

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(Signature of candidate)

On the 28th of October 2019 in Johannesburg, South Africa.

To Jesus Christ, the only Son of God in whom are hidden all the treasures of science and knowledge

To my beloved family

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CONFERENCES AND PRESENTATIONS

1. 45th Annual Conference of the Anatomical Society of the Southern Africa (ASSA) 23-26 April 2017, Club Mykonos, Langebaan, Western Cape, South Africa. Poster presentation entitled "Anomalous vertebral artery"
2. 46th Surgical Research Society (SRS) Meeting 21st – 22nd June 2018, Mthatha Health Resource Centre, Nelson Mandela Central Hospital, Mthatha, Eastern Cape, South Africa. Oral Presentation of the preliminary results of this study.
3. Multi-disciplinary Endocrine Society of Southern Africa (MESSA) Meeting 5th October 2018, Gateway Hotel, Durban, KwaZulu Natal, South Africa. Awarded Best Oral Presentation of the preliminary results of this study.

ABSTRACT

Background. The prevalence of post-surgical injury of the recurrent laryngeal nerve (RLN) globally is at most 11%. There is currently an overwhelming amount of literature on the anatomy of the RLN and the advent of intraoperative neural monitoring. Most of the available literature describes the normal anatomy of the RLN in adult cadavers, less in adult patients with neck masses and least in paediatric patients. Thyroid surgery in paediatric patients has not been thoroughly researched despite the notable increase in thyroid neoplasms in this population.

Aim: The current study was conducted to assess as well as compare the normal and variant anatomy of the recurrent laryngeal nerve in cadaveric cohorts (adults and paediatrics), and an adult cohort undergoing thyroidectomies.

Methods: A prospective study was undertaken on cadaveric collections in the School of Anatomical Sciences at the University of the Witwatersrand and the University of Pretoria. The neck region was dissected to expose the normal and variant anatomy of the RLN. A convenient sample of 50 adult (age range 42-77 years with a mean of 77.6±12.3 years) and 31 paediatric (age range 0-144 weeks with a mean of 39.70±51.73 postnatal weeks) cadavers were dissected and analysed following standard dissection procedures. Measurements of the diameter of the nerve on either side were made where the nerve grossly appeared widest. The relationship of the recurrent laryngeal to the inferior thyroid artery was assessed. Furthermore, the course of the nerve in relation to the tracheoesophageal groove was noted and the nerve was assessed for terminal branching. An additional 30 adult patients scheduled to undergo thyroidectomies were recruited from the University of the Witwatersrand teaching hospital complex (Charlotte Maxeke Academic Hospital, Chris Hani Baragwanath Academic Hospital and Helen Joseph Hospital) and the RLN in these patients was analysed as above. The data was managed and analysed on Microsoft Office Excel 2013. Shapiro-wilk test was used to test for normality. The student's t-test was performed on normally distributed data. Whitney Mann U test was performed on non-normally distributed data. Categorical data was analysed for associations using the Pearson Chi-square test. The level of significance was set at a p-value of less than 0.05. Ethical clearance for the cadaveric dissection in this study was covered under the Human Tissue Act No. 65 of 1983 and the National Health Act No. 61 of 2003 reference number (W-CJ-140604-1). Further ethical approval was obtained from the Human Research Ethics

Committee of the University of the Witwatersrand (Medical - M160664**)** for thyroidectomy patients to participate in this study.

Results: The total paediatric cadaver study sample comprised of 53.1% South African White, 45.9% South African Black and 0.9% South African Indian. The mean diameter of the RLN in the paediatric cadaveric population was 0.74±0.28mm and 0.72±0.37mm on the left and right respectively. In the adult cadaveric population, the mean diameters of the RLN were 1.75±0.59mm and 1.63±0.47mm on the left and the right respectively. 40.3% of the RLN irrespective of side, gender, age and demography coursed posteriorly to the inferior thyroid artery (ITA). Most of the RLN on the left side followed the tracheoesophageal groove (TEG) (i.e. 71% and 89% in adult and paediatric patients respectively). The nerves that deviated from the TEG were found within 0 - 30^{o} of the groove. There was 61% bifurcation rate of the RLN in adult specimens. Morphological branching of the RLN was better demonstrated in adult specimens compared to paediatric specimens due to size constraints. Intraoperative visualization rate of the RLN was 73% in patients undergoing thyroidectomies.

Conclusion: On the overall, irrespective of age, gender and side, the RLN coursed posterior to the ITA. The majority of RLNs on the left side coursed in the TEG while on the right side the nerve deviated from the TEG at an angle ranging between 0 - 30^{o}. Most of the RLNs showed two or more branches on both sides. The non-recurrent inferior laryngeal nerve was not observed and remains a rare finding. This information is vital to surgeons and clinicians to avoid intraoperative injuries of the RLN and diagnoses of the disorders of the neck, larynx and voice production.

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ABBREVIATIONS

RLN: Recurrent laryngeal nerve

ITA: Inferior thyroid artery

RET: Re-arranged during transfection

FNA: Fine needle aspiration

TERT: Telomerase reverse transcriptase

MNG: Multinodular goitre

NRLIN: Non-recurrent inferior laryngeal nerve

TEG: Tracheoesophageal groove

SCM: Sternocleidomastoid muscle

AA: Aortic arch

BCT: Brachiocephalic trunk

LSA: Left subclavian artery

RSA: Right subclavian artery

LCA: Left common carotid artery

RCA: Right common carotid artery

TRA: Trachea

OE: Oesophagus

TG: Thyroid gland

ThoA: Thoracic aorta

PA: Pulmonary artery

PDA: Patent ductus arteriosus

HRT: Heart

CMJAH: Charlotte Maxeke Johannesburg Academic Hospital

CHBAH: Chris Hani Baragwanath Academic Hospital

HJH: Helen Joseph Hospital

SD: Standard deviation

VAN: Vagus nerve

VA: Vertebral artery

AVA: Anomalous vertebral artery

POB: Point of bifurcation

PHN: Phrenic nerve

IONM: Intraoperative neural monitoring

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CHAPTER 1: INTRODUCTION

Malignant neoplasms of the thyroid gland are the most prevalent form of endocrine cancers that often require surgical intervention (Deveze *et al.,* 2003, Ling and Smoll, 2016). Injury to the recurrent laryngeal nerve (RLN) remains among the most severe complications associated with thyroidectomies and neck surgeries (Calo *et al.,* 2014; Chiang *et al.,* 2005; Chiang *et al.,* 2008; Dralle *et al.,* 2008; Schneider *et al.,* 2016; Sun *et al.,* 2001; Wagner and Seiler, 1994). The prevalence of post-surgical injury of the RLN is at most 11% globally. This prevalence has not changed over time despite the large amount of literature on the anatomy of the RLN and the advent of intraoperative nerve (neural) monitoring (IONM) (Calo *et al.,* 2016; Dralle *et al.,* 2008; Ling and Smoll, 2016). The isolated injuries to a normal RLN (that is without anatomical variations) have a prevalence of 2-5% compared to the overall 11% including situations with anatomical variations (Henry *et al.,* 2017a). Bilateral RLN palsy may occur in 0.2 - 1% of the patients; requiring the insertion of a permanent tracheostomy to avoid glottal and airway obstruction (Calo *et al.,* 2014; Chiang *et al.,* 2005; Henry *et al.,* 2016; Ling and Smoll, 2016, Périé *et al.,* 2013; Wagner and Seiler, 1994).

A thyroidectomy involves a transverse cervical incision to expose the thyroid gland, followed by ligation of thyroid vessels and dissection of the inferior thyroid artery (ITA) (Wagner and Seiler, 1994). This is followed by the exposure and identification of the RLN which some scholars advocate against, as it correlates positively with the occurrence of nerve injury (Deveze *et al.,* 2003; Wagner and Seiler, 1994). However, the gold standard in the prevention of intra-operative injury of the RLN is identification and visualisation of the nerve before ligation of the ITA (Dralle *et al.,* 2004; Dralle *et al.,* 2008; Sun *et al.,* 2001). This requires an understanding of the relations and variations of the RLN to the ITA (Ling and Smoll, 2016). Furthermore, one needs to locate the RLN as it enters the larynx either at the cricopharyngeus border of the inferior constrictor muscle, Berry's ligament or the inferior cornu of the thyroid cartilage (Cernea *et al.,* 2009; Dolezel *et al.,* 2015; Prades *et al.,* 2015).

Chiang et al. (2008) described Berry's ligament to be the most vulnerable place of injury to the RLN as a result of traction injury during a lobectomy, thermal injury when trying to achieve haemostasis and accidental severing of the anterior branch where it usually bifurcates. The branching patterns of the RLN as it enters the larynx varies from a bifurcation; trifurcation; or to even quadrification (up to four branches) (Cernea *et al.,* 2009). It is not uncommon to find an unbranched RLN (Cernea *et al.,* 2009). The implications of multiple branches may be accidental severing or ligation of the branches of the nerve along with the blood supply to the thyroid gland during a thyroidectomy (Cernea *et al.,* 2009). Most authors concluded that the terminal segment of the RLN on the right side compared to the left side is most likely to bifurcate (Cernea *et al.,* 2009). The exact distance from the branching point of the RLN to the point of entry into the larynx is still debated, however, the site is universally considered vulnerable to injury (Ardito *et al.,* 2004; Cernea *et al.,* 2009; Nemiroff and Katz, 1982; Yalcin *et al.,* 2008).

Transient damage to the nerve heals spontaneously but permanent damage will require intervention depending on the site, the extent of the injury and the severity of the symptoms (Chiang *et al.,* 2005; Ling and Smoll, 2016). For example, treatment of unilateral vocal cord paralysis includes injection laryngoplasty, medialisation thyroplasty and vocal cord re-innervation procedures (Prades *et al.,* 2015). Bilateral vocal cord paralysis may, however, require a permanent tracheostomy (Deveze *et al.,* 2003; Prades *et al.,* 2015). Re-innervation has a better prognosis than other modalities of treatment in the restoration of normal phonation (Prades *et al.,* 2015). It involves an end to end anastomosis of the ansa cervicalis to the RLN (Prades *et al.,* 2015).

Thyroidectomies in paediatric patients are rare but they are classified as high risk for RLN injury (White *et al.,* 2009). The main indication for surgery in paediatric patients is malignancy and its incidence is increasing (Ledbetter, 2014). The surgical procedure for thyroidectomies in paediatric patients is extrapolated from what is known in adult patients where thyroid disorders are more common (Akkari *et al.,* 2014). In addition, there is a paucity of information about the variant anatomy of the RLN in the paediatric population (Akkari *et al.,* 2014). The incidence and prevalence of a non-recurrent inferior laryngeal nerve (NRILN) irrespective of the side in paediatric populations has not been studied (Akkari *et al.,* 2014; Konschake *et al.,* 2016). Paediatric patients pose a high risk of RLN injury on account of anatomical challenges in the dimensions and proximity of structures in the neck (Ledbetter, 2014). Surgical procedures around the neck in this population may require the use of operating/dissection microscopes to reduce the chances of intraoperative injuries (Akkari *et al.,* 2014).

Studies on the RLN have been done in North America, Europe and Asia, there is however, a paucity of information on the RLN in African populations. Hence, the current study was undertaken to describe and assess the normal and variant anatomy of the RLN in cadaveric cohorts (adult and paediatric) in a South African population. Comparisons were made with patients who underwent thyroidectomies. A description of the normal and variant anatomy of the RLN in paediatric populations of different ages is invaluable towards the understanding of the anatomy of the RLN at different stages of development.

CHAPTER 2: LITERATURE REVIEW

2.1. Thyroid surgery and Epidemiology of thyroid neoplasms

2.1.1. Prevalence of thyroid disorders and neoplasms in adult populations

Thyroid gland neoplasms are the most common indication in endocrine surgery affecting more females than males (Sierra *et al.,* 2016). Neoplasms of the thyroid gland only constitute 1% of the total global cancer burden (Sierra *et al.,* 2016). Thyroidectomies represent about 12.72% and 11.3% of all surgical procedures in the Central and West Africa respectively (Nyeki *et al.,* 2015). A five year review study in a South African population revealed a 5.7% prevalence of multinodular goitre (MNG) in patients who underwent thyroidectomies in the review period (Bombil *et al.,* 2014).

A review study in a Taiwanese population between the years 2004 and 2010 showed an increase in the survival rate post thyroidectomy from 92.69% to 95.57% (Chiang *et al.,* 2005). Overall, mortality due to thyroid related neoplasms is rare ranging below 1% globally (Sierra *et al.,* 2016). The increase in the incidence of thyroid neoplasms is not correlated with mortality (Sierra *et al.,* 2016). This observed increase is related to improved diagnostic modalities that are able to detect sub-clinical disease states (Chiang *et al.,* 2005).

2.1.2. Prevalence of thyroid malignancy in paediatric population and its pathogenesis

There is an overall increase in the papillary subtype of thyroid neoplasms which carries a good prognosis compared to other subtypes (Jan *et al.,* 2019;Sierra *et al.,* 2016). In the paediatric population (children and adolescents), thyroid nodules present with a higher prevalence of malignancy of up to 20% compared to the adult population (Akkari *et al.,* 2014). Thyroid neoplasms observed in the paediatric population are associated with a hereditary RET (re-arranged during transfection) proto-oncogene (encodes for a tyrosine kinase receptor) mutation (Akkari *et al.,* 2014). Although, thyroid neoplasms are more prevalent in paediatric populations, the operative risk of RLN injury is the same as the adult population (Akkari *et al.,* 2014).

The observed increased prevalence and incidence of thyroid neoplasms in paediatric patients is also attributed to improved diagnostic modalities (Sierra *et al.,* 2016). For example, ultrasound-guided Fine Needle Aspiration (FNA) is able to target thyroid nodules that are less than 1 square centimetre in size (Sierra *et al.,* 2016). Approximately 7 - 20% of thyroid nodules are evident on palpation of the thyroid gland but ultrasound techniques help to identify up to

50 - 70% of the thyroid nodules. Of the observed thyroid neoplasms, 20% are classified as malignant neoplasms (Bojunga, 2018; Bombil *et al.,* 2014).

The increased pickup/identification rate of thyroid nodules can be correlated with increased number of thyroidectomies (Bojunga, 2018). Thyroidectomies carry parathyroid and RLN related complication rate of 2% and 11% respectively (Ling and Smoll, 2016). Bojunga (2018) advocated for combined thyroid scintigraphy and ultrasound guided FNA to establish both the neoplastic and hormone status of a thyroid nodule in order to successfully classify benign thyroid conditions and neoplasms. Benign conditions such as multinodular goitres (MNGs) affect only 5 – 7% of the population globally (Bombil *et al.,* 2014). MNGs have a very low malignancy rate ranging from 0.7 - 2.2% (Bombil *et al.,* 2014). The most common type of thyroid malignancy is the papillary carcinoma that affects about 80% per cent of individuals with MNG. The black population in South Africa and globally are mainly affected by the follicular variant of papillary carcinoma (Bombil *et al.,* 2014).

2.1.3. Indications for surgery

A thyroidectomy can be performed as a life preserving procedure in the case of localized thyroid neoplastic conditions or thyroid enlargement causing compressive symptoms on the neck structures (Akkari *et al.,* 2014; Chiang *et al.,* 2008; Feroci *et al.,* 2014). It can also be instituted in the case of benign hyper functioning of the thyroid gland that is resistant to anti-thyroid medical therapy (Feroci *et al.,* 2014). Aesthetic, fertility and local recurrence are also common indications for a thyroidectomy (Feroci *et al.,* 2014; Freschi *et al.,* 1994).

2.1.4. Intraoperative neuro-monitoring

Intraoperative neuro-monitoring (IONM) was introduced to mitigate the effects of recurrent laryngeal nerve (RLN) injury during thyroidectomies (Dionigi *et al.,* 2013; Julien *et al.,* 2017; Périé *et al.,* 2013). IONM involves placing electrodes directly or in close proximity to the vocal cords and sometimes over the vagus nerve in order to record the muscle electric activity (electro-myographical signal) arising from nerve stimulation (Dionigi *et al.,* 2013; Julien *et al.,* 2017; Périé *et al.,* 2013). Positive stimulation has been correlated with a good prognosis in excluding permanent RLN injury (Périé *et al.,* 2013). Furthermore, it can reliably detect pre-operative RLN palsy and is useful in the visualisation of vocal cord mobility (Périé *et al.,* 2013). Intraoperative neuro-monitoring (IONM) can either be done intermittently or continuously (Dionigi *et al.,* 2013). Continuous intraoperative neuro-monitoring is more beneficial and is associated with a reduction in traction related injury of the RLN when compared to intermittent intraoperative neuro-monitoring (Dionigi *et al.,* 2013).

The technique was not intended to prevent post-operative RLN palsy but rather to detect injury and limit further intraoperative damage of the RLN (Julien *et al.,* 2017; Périé *et al.,* 2013). It is now routinely used in more than half of all neck procedures that carry a risk of RLN injury in the United States of America (Julien *et al.,* 2017). It is also used as a guide to surgical dissection in difficult cases in the paediatric population (Akkari *et al.,* 2014). However, IONM has low sensitivity and is associated with multiple false positives (Julien *et al.,* 2017; Périé *et al.,* 2013). Furthermore, it comes with multiple technical challenges and there is no consensus on the standardization of the methodology and reporting of results (Calo *et al.,* 2016; Dionigi *et al.,* 2013; Nyeki *et al.,* 2015) and it is a costly modality that may limit both its acquisition and use in low resource settings (Calo *et al.,* 2014). Nonetheless, Périé et al. (2013) advocated for the use of both IONM and visualization of the RLN in order to improve the prognosis and the confidence of the surgeon in the safety of their dissection.

2.2 Anatomy of the RLN

2.2.1. Development of the RLN

During Carnegie's stage 16 of development at about 39 days of embryological development, the descent of the heart drags down the RLN along on the left side causing it to recur around the sixth pharyngeal arch (Deveze *et al.,* 2003; Dolezel *et al.,* 2015). The sixth pharyngeal arch forms the ductus arteriosus and later on becomes obliterated at birth to form the ligamentum arteriosum (Deveze *et al.,* 2003; Dolezel *et al.,* 2015; Prades *et al.,* 2012). On the right side, degeneration of the fifth and sixth pharyngeal arches leads to the recurrence of the RLN around the fourth pharyngeal arch that will later contribute to the formation of the subclavian artery (Deveze *et al.,* 2003; Dolezel *et al.,* 2015).

2.2.2. Development of the non-recurrent inferior laryngeal nerve (NRILN)

Non-recurrent inferior laryngeal nerve (NRILN) results from an embryological aberration arising due to the absence of both the fourth pharyngeal arch and sixth pharyngeal arch on the right and left sides respectively (Deveze *et al.,* 2003). The NRILN courses transversely to the right thyroid lobe with a frequency of 0.3 - 0.8% on the right and 0.004% on the left (Dolezel *et al.,* 2015; Sun *et al.,* 2001). The presence of NRILN on the left is associated with the presence of situs inversus and viscerum (positioning of the organs in thorax and abdomen in a mirror image reversal of their normal position) conditions that are extremely uncommon (Deveze *et al.,* 2003; Dolezel *et al.,* 2015).

The NRILN variation was first identified by Steadman in 1823 (Gao *et al.,* 2014; Morais *et al.,* 2015; Wang *et al.,* 2014). Embryologically, NRILN has been described as the non-descent of the RLN on the right side due to an anomalous right subclavian artery (also known as the arteria lusoria) which is found in 0.5 - 2% of the general population (Morais *et al.,* 2015). An anomalous right subclavian artery takes various courses including behind the oesophagus; between the trachea and oesophagus; and in front of the trachea at an 80%; 15% and 5% prevalence respectively (Morais *et al.,* 2015). The rate of operative injury to the NRILN is comparatively greater (12.9%) than that of a non-variant RLN (2 - 5%) (Gao *et al.,* 2014).

On radiography (X-ray), the NRILN presents as a special linear shadow on the anterior view and the oesophagograms show the bayonet's sign (notching) of the posterior oesophagus at the level of the 3rd thoracic vertebral level due to compression of the oesophagus by the NRILN (Morais *et al.,* 2015). The radiographic findings are only accompanied by clinical signs of dysphagia in 5% of the cases (Morais *et al.,* 2015).

IONM irrespective of the technical challenges of standardization has been reported to reliably identify a NRILN at a latency threshold of 3.5ms (Citton *et al.,* 2016; Morais *et al.,* 2015). Furthermore, a Doppler ultrasound of the neck has a 100% detection rate of the Y sign that denotes the absence of the brachiocephalic trunk which invariably comes with a NRILN (Citton *et al.,* 2016; Morais *et al.,* 2015; Wang *et al.,* 2014). Gao et al. (2014) advocated for a dual modality (computerized tomography or ultrasound and Intraoperative neuro-monitoring) to diagnose a NRILN and prevent intra-operative nerve injury.

A false NRILN can present when the inferior cervical ganglion anastomoses with the RLN forming a sympathetic inferior laryngeal communicating branch (Deveze *et al.,* 2003; Dolezel *et al.,* 2015). This normal variation should be excluded in patients who are thought to have a NRILN but do not have a concomitant vascular abnormality (Deveze *et al.,* 2003; Dolezel *et al.,* 2015). A duplex Doppler ultrasound suffices as a confirmatory modality to distinguish a true NRILN from a false NRILN by detecting the presence or absence of a concomitant vascular anomaly typically affecting the right side (Deveze *et al.,* 2003; Dolezel *et al.,* 2015). The relationship of the NRILN to the ITA can be classified using the Toniato and Hong's classification (**see Table 1**) (Dolezel *et al.,* 2015; Wang *et al.,* 2014; Lee *et al.,* 2009).

Table 1. Toniato and Hong's classification of the Non-recurrent inferior laryngeal nerve adopted from (Dolezel *et al.,* 2014).

Type I	Type IIA	Type IIB	Type III
Runs with the vessels of the superior thyroid peduncle	Runs over the trunk of the ITA*	Runs medially under the trunk or between the branches of the ITA*	Loop or V shape course crossing the ITA* inferiorly

***ITA: Inferior thyroid artery**

2.2.3 General description of the recurrent laryngeal on the right and left sides

The RLN has been referred to as a highly variable plexus due to its extra-laryngeal branching at its distal segment (Yalcin *et al.,* 2008). The vagus nerve gives off branches that recur around the subclavian artery and aortic arch on the right and the left sides respectively (Deveze *et al.,* 2003; Prades *et al.,* 2012). The larynx receives its motor and sensory supply from the superior laryngeal and RLN (Lee *et al.,* 2009). The RLN supplies the region inferior to the vocal folds (Dolezel *et al.,* 2015). The lengths of right and left RLNs are 4 - 9.5cm and 9 - 17.5cm respectively in both males and females (Prades *et al.,* 2012). The disparity in length is proven to not affect the speed of nerve conduction on either side (Prades *et al.,* 2012). Lee et al. (2009) demonstrated that the RLN can be found about three tenths (3/10) of the distance between the tip of the cricoid cartilage and the sternal notch, using the cricoid cartilage as the reference point. There is no literature or previous evidence of racial or tribal differences in the anatomy of the RLN.

2.2.4. Relations of the recurrent laryngeal nerve to the inferior thyroid artery

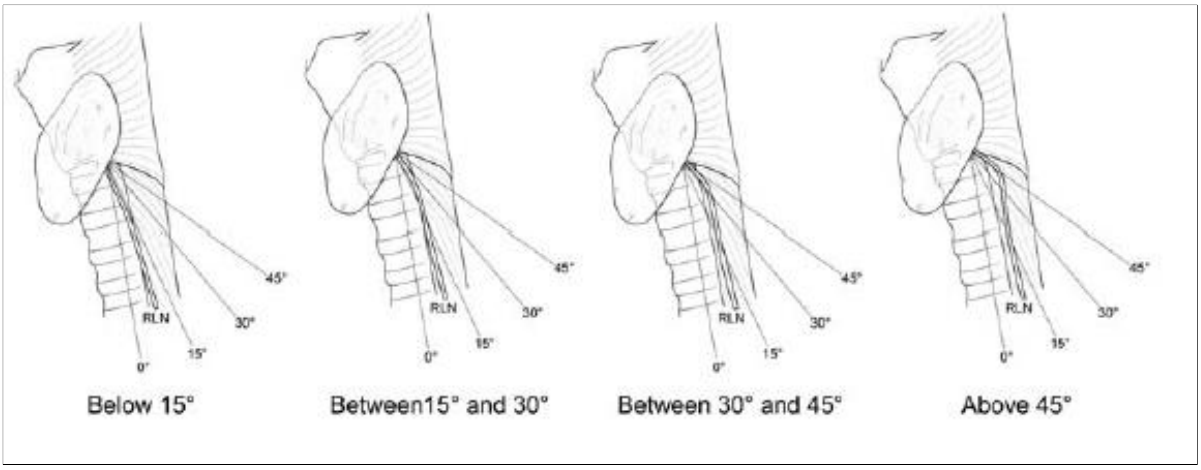
The left subclavian artery gives off the thyrocervical trunk that ascends cranially to become the ITA (Lee *et al.,* 2009; Sakamoto, 2013). The ITA, in turn, gives off branches that course side by side with the RLN as the nerve goes under the cricopharyngeus part of the inferior constrictor muscle (Henry *et al.,* 2017c; Sakamoto, 2013). The relationship of the RLN to the ITA is classified into three categories: anterior, posterior and in between the branches of the artery with a combined prevalence of 27.6%, 50.7% and 21.7% respectively (Ardito *et al.,* 2004; Lee *et al.,* 2009). It is generally accepted that the nerve courses posteriorly to the ITA and its branches on the left and anteriorly on the right side (Lee *et al.,* 2009; Prades *et al.,* 2012; Sun *et al.,* 2001). On the left side the RLN courses posteriorly to the ITA in 62.6% of the time whereas on the right side the RLN courses anteriorly in 66% of the time (Henry *et al.,* 2017a). The RLN was found to course in-between the branches of the ITA in 29% and 20.2% of the cases on the right and the left respectively (Henry *et al.,* 2017a). A symmetrical relationship was only observed in 36.6% of cadaveric studies (Henry *et al.,* 2017c). On the right side, the RLN is most vulnerable to traction injury when it courses anterior to the inferior thyroid artery (Henry *et al.,* 2017c).

2.2.5. Trajectory of the RLN

The course of the RLN is variable and has been found to follow considerably three normal trajectories that include the tracheoesophageal groove (50 - 77%), paratracheal margin (10 - 40%) and the paraoesophageal margin (4 - 6%) (Morais *et al.,* 2015). In the majority of individuals the RLN courses along or lateral to the tracheoesophageal groove (TEG) before entering the larynx at the inferior margin of the cricopharyngeus muscle (Ardito *et al.,* 2004; Lee *et al.,* 2009). At this point, it is related to the posteromedial aspect of the right and left lobes of the thyroid gland (Lee *et al.,* 2009). It is also closely associated with the ITA and the Berry's ligament (Lee *et al.,* 2009). Berry's ligament has proven to be a reliable landmark for the identification of the RLN which courses above this ligament in 78.2% of cases. This pattern displays 75.5% symmetry on both sides (Henry *et al.,* 2017c; Henry *et al.,* 2017d). The RLN may pierce Berry's ligament in 7% cases making it vulnerable to traction during surgery (Henry *et al.,* 2017c). (Chang *et al.,* 2010) demonstrated a 100% identification of RLN in the TEG laparoscopically.

Although the course varies, the RLN is consistently identified at the inferior horn of the thyroid cartilage, except when displaced by pathologies such as neck masses (Lee *et al.,* 2009; Sun *et al.,* 2001). Lee et al. (2009) defined an angle between the TEG and the RLN and classified the variations into four groups that include angle below 15°, between 15 - 30°, 30 - 45° and greater than 45°. The majority of RLN in the TEG will course in an angle between 15 - 30° and 30 - 45° in males and females respectively (Sun *et al.,* 2001). With regards to the side, the RLN courses between 0 - 30° and 15 - 45° on the left and right sides in the TEG respectively (Sun *et al.,* 2001; Prades *et al.,* 2012). 68.1% of RLNs were found coursing in the TEG with a 61.1% symmetry (Henry *et al.,* 2017c).

Figure 1. Schematic illustration of the angular course to the TEG adapted from (Henry *et al.,* 2017a)



2.2.6. Branching patterns of the recurrent laryngeal nerve

The RLN characteristically branches before entering the larynx, with over 80% of individuals presenting with two branches at the level of the cricothyroid articulation (Prades *et al.,* 2012; Sun *et al.,* 2001). The anterior branch further subdivides to supply the cricoarytenoid and internal arytenoid muscles, (Dolezel *et al.,* 2015; Nguyen *et al.,* 1989; Serpell, 2010). At this point, the nerve takes on the name of the muscle it innervates (Nguyen *et al.,* 1989). The nerve to the internal arytenoid muscle runs towards the superior half of the inferior cornu of the thyroid cartilage in 60% of cases (Dolezel *et al.,* 2015; Nguyen *et al.,* 1989). All intrinsic muscles of the larynx, except the cricothyroid muscle, are innervated by the anterior branch of the RLN; the exception is innervated by a lateral branch of the superior laryngeal nerve (Ardito *et al.,* 2004; Dolezel *et al.,* 2015). The posterior branch of the RLN innervates the mucosa of the larynx inferior to the vocal folds (Ardito *et al.,* 2004; Dolezel *et al.,* 2015; Nguyen *et al.,* 1989).

The RLN may branch outside the larynx to innervate other structures of the neck namely the oesophagus, trachea, thyroid gland and inferior constrictor muscle (Yalcin *et al.,* 2008). The inferior border of the cricoid cartilage has also been used as a landmark to determine how far from this point extra-laryngeal branching occurs (Nemiroff and Katz, 1982). A meta-analysis by Henry et al. (2016) showed that 60% of RLN present with extra-laryngeal branches and 90.2% branch 2cm from the cricothyroid joint with 36.5% symmetry (bilateral branching).

A bifurcation is the most common type of laryngeal branching with 51.1% prevalence and this pattern is not consistently replicated on both sides (Henry *et al.,* 2016; Nemiroff and Katz, 1982). Reed (1943) reported up to 6 terminal branches.

Table 2. Branching pattern of the terminal segment of the RLN described by Yalcin et al. (2008)

Type	Description
I	The laryngeal and extra-laryngeal branches arise from the same level
Ia	The origin of the branches is **just** below the inferior border of the cricopharyngeal part of the inferior constrictor muscle.
IIb	The origin of the branches is **15-35mm** below the inferior border of the cricopharyngeal part of the inferior constrictor muscle.
II	The branches arise from different points, laryngeal branches arise 3-5mm above the extra-laryngeal branches

MOTIVATION FOR THIS STUDY

The highly variable anatomy of the recurrent laryngeal nerve (RLN) continues to feature in surgical and anatomy research. Novel strategies (intraoperative neuro-monitoring) put in place to avoid the sometimes-serious complications of intraoperative RLN injury are still being developed and improved. This indicates the need for more research of this century old topic, especially in different population groups with the aim to make evidence-based comparisons that may inform the current practice of general, endocrine and ENT (ear, nose and throat) surgeons. This study aims to describe the normal and variant anatomy of the RLN in a South African population. .

AIMS

1. To study the normal anatomy and anatomical variations of the recurrent laryngeal nerve in a cohort of adult and paediatric cadavers, patient undergoing thyroidectomies and surgical candidates with neck pathologies.
2. To study the anatomy of the RLN in patients undergoing thyroidectomies
3. To compare the cadaveric findings to a cohort of candidates with neck pathologies.

OBJECTIVES

1. To determine the anatomy, course, relations and terminal branching of the recurrent laryngeal nerve.
2. To determine how the anatomy of the recurrent laryngeal nerve differs between adult and paediatric populations.
3. To determine the alteration of the anatomy, course, relations and terminal branching of the recurrent laryngeal nerve by neck masses.
4. To determine the prevalence of non-recurrent inferior laryngeal nerve on the left and the right sides.

CHAPTER 3: MATERIALS AND METHODS

3.1. Study sample

3.1.1. Cadaver population

This study sampled a total of 50 adult cadavers with an average age of 77.6 ± 12.3 years (age range: 42 - 77 years) donated for learning and research to the school of Anatomical Sciences at the University of the Witwatersrand. 25 male and 25 female cadavers were dissected and photographed. A total of 31 paediatric cadavers with mean age of 39.7 ± 51.7 postnatal weeks, were sourced from the Departments of Anatomical Sciences of the University of the Witwatersrand and the University of Pretoria, and similarly dissected and photographed.

3.1.2. Candidates for thyroidectomies

Thirty (30) adult patients (age range: 21 – 80 years and mean age 49.76±14.27 years), scheduled for thyroidectomy at Charlotte Maxeke Johannesburg Academic Hospital, Helen Joseph Hospital and Chris Hani Baragwanath Academic Hospital were included in the study. Indications for thyroidectomy included benign and malignant conditions of the thyroid. Re-do thyroidectomies were excluded. Eligible participants were recruited through a 3-step process comprising detailed information on the study, an open invitation to participate in the study and written informed consent that could be withdrawn at any point during the study.

The study focussed on patients operated upon by a single surgeon at each of the participating hospitals. Convenient sampling was used. All thyroidectomies were done through a collar incision ±2cm above the sternal notch. The incision was deepened beyond the platysma and inferior and superior flaps were raised to the sternal notch and summit of the thyroid cartilage, respectively. The strap muscles were separated in the midline. Each thyroid lobe was mobilized and retracted antero-medially after ligation of the ipsilateral middle thyroid vein and superior thyroid vessels. Routine identification of the recurrent laryngeal nerve (RLN) was performed in all cases and started where the nerve crosses inferior thyroid artery.

3.1.3. Standardization of photographs

All exposed tissues were photographed with a high-resolution Nikon® Camera and analysed with the open source software ImageJ® (National Institutes of Health in Bethesda, Maryland United States Of America). Photographs were taken 15 - 30cm from the specimen at a 45° angle and a standard 1mm scale bar was applied to all the pictures. The software program, ImageJ® calculated pixel differences between two selected points and translated them into accurate metric measurements. Measurements of the diameter of the nerve on either side were made where the nerve grossly appeared widest. The course and relations of the RLN were investigated in terms of the angle that the RLN made with the trachea-eosophageal groove. The angles were categorized as either being **X** (angle) < 15°; 15° < **X** < 30°; 30° < **X** < 45° and **X** > 45°.

3.1.4. Ethical clearance

Ethical clearance for the cadaveric dissection in this study was covered under the Human Tissue Act No. 65 of 1983 and the National Health Act No. 61 of 2003 reference number (W-CJ-140604-1) at the University of the Witwatersrand and at the University of Pretoria. Permission to conduct research on patients undergoing thyroidectomies was obtained from the hospital management of Charlotte Maxeke Academic Hospital, Helen Joseph Hospital and Chris Hani Baragwanath Hospital. Ethical clearance for the collection of data and measurement of the candidates undergoing thyroidectomies was granted by the Human Research Ethics Committee (Medical) at the University of the Witwatersrand: Ethics number M160664. The data collection procedures used in the current study did not interfere or delay any of the patient's therapeutic procedures.

3.1.5. Data Analysis

The collected data was managed in Microsoft Excel 2013 (Microsoft Corporation) and STATA® version 14 statistical software was used for analysis. Percentages were used to summarize categorical data including demographics. Measurements derived from the photographs of each specimen using ImageJ® software were compared using the student t-test for between measures. The topography of the RLN in males and females; the dissected side, its relationship with the inferior thyroid artery (ITA), its angular course in the trachea-oesophageal groove and branching pattern were subjected to a Chi-square statistical test to test for respective associations. Correlation and regression analysis were done to assess the regularity and the predictability of the multiple configurations of the RLN.

3.1.6. Dissection of the Adult cadavers

The dissection technique described by (Kieser and Allan, 2001) was followed during dissection of the recurrent laryngeal nerve in adult and paediatric cadavers. In order to confirm the recurrence of the nerve, the sternum was disarticulated from clavicle and ribs to expose the great vessels (the aorta and subclavian arteries) in preparation for neck dissection.

A midline superficial incision made from the mandible to the sternal notch. The skin was dissected superficially to expose the platysma. The investing layer of fascia was dissected to reveal the strap muscles (omohyoid, sternohyoid, thyrohyoid and sternothyoid muscles). The sternocleidomastoid muscle was reflected superiorly. The strap muscles were reflected to reveal the carotid sheath and its contents. The vagus nerve was identified in the carotid sheath on the right and traced caudally up to the point of recurrence around the right subclavian artery. The recurring branch of the vagus nerve was followed as it coursed in the TEG. The relation of the RLN to the ITA was documented. The internal jugular vein was removed to achieve better visibility. The terminal end of RLN was examined and the presence or absence of branches including the number of branches was counted and documented. The steps and findings during dissection are shown in figures 1-8.

3.1.7. Dissection of Paediatric cadavers

A midline superficial incision was made from the mandible to the sternal notch. The skin was dissected superficially to expose the platysma. The investing layer of fascia was dissected to reveal the strap muscles (omohyoid, sternohyoid, thyrohyoid and sternothyroid muscles). The strap muscles were reflected to reveal the carotid sheath and its contents. The vagus nerve was identified in the carotid sheath on the right and traced caudally up to the point of recurrence around the right subclavian artery. See figures 1 - 8 for steps followed during dissection and findings.

At this point the thorax was opened along the imaginary line traced in fig. 9 along the inferior border of the clavicle, on the right border of sternum and on the right and left side along the subcostal margin which led to exposure of the thoracic content shown in fig. 10. The point of recurrence of the RLN was observed and documented on the right (subclavian artery) and the left (aortic arch). Furthermore, the relationship of RLN to the ITA and TEG on either side of the neck was recorded (figures 11 – 12 and 14). The terminal end of RLN was examined and the presence or absence of branches including the number of branches was documented fig. 13.

3.1.7. Thyroidectomy surgical technique

All the patients recruited for this study underwent indirect laryngoscopy pre- and post-operatively and vocal fold mobility was assessed. After general anaesthesia and endotracheal intubation the patient was placed in a supine position with the head on a donut pillow. The neck was slightly extended, and the bed placed at 15 - 30^{o} in reverse Trendelenburg position. A 3 - 4cm collar cervical incision was made 1cm below the cricoid cartilage. The platysma was retracted exposing the strap muscles. After medially dissecting the superior pole of the thyroid gland, space was created medial to the superior thyroid artery to reveal the external superior laryngeal nerve. The upper parathyroid gland was separated from the thyroid gland. A mid - thyroid dissection was carried out to visualise the RLN.

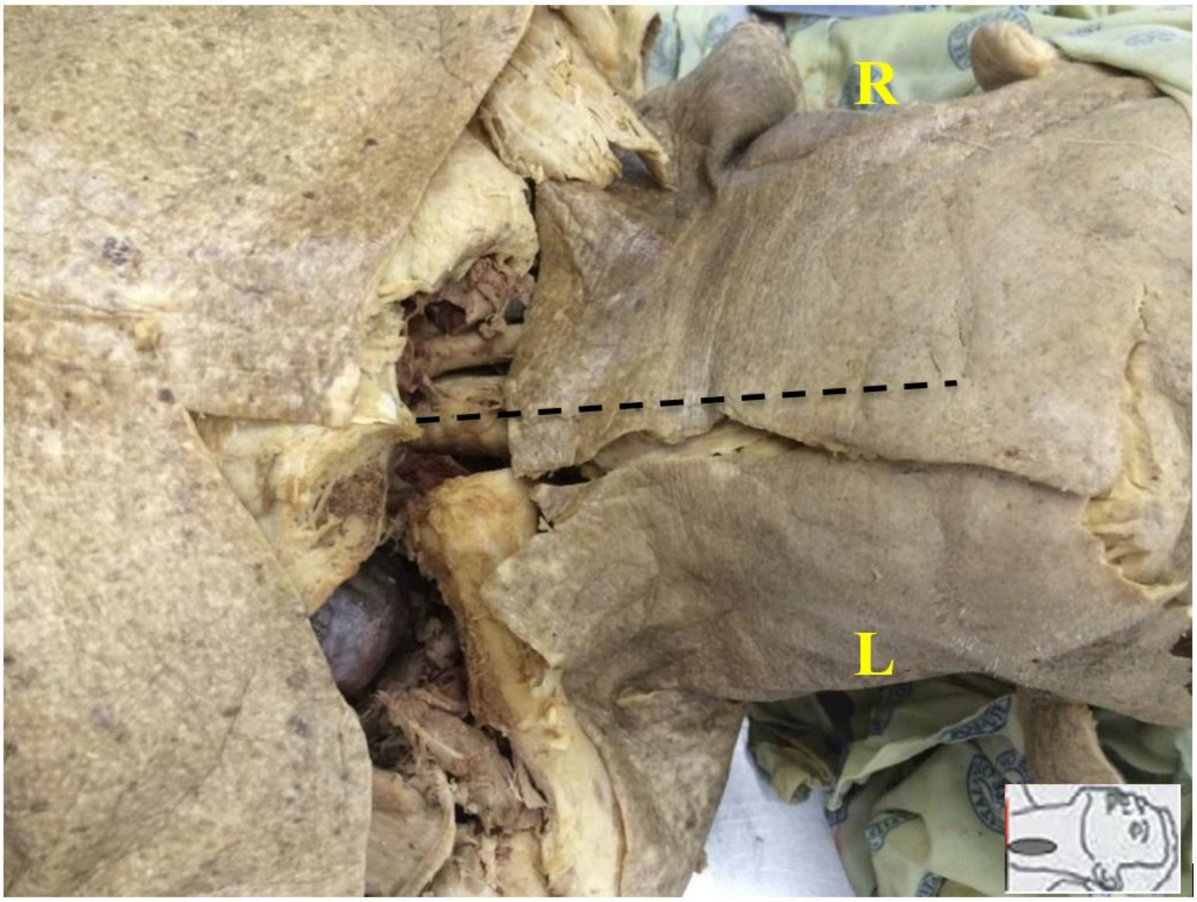


Figure 2. Midline incision from the mandible to the sternal notch.

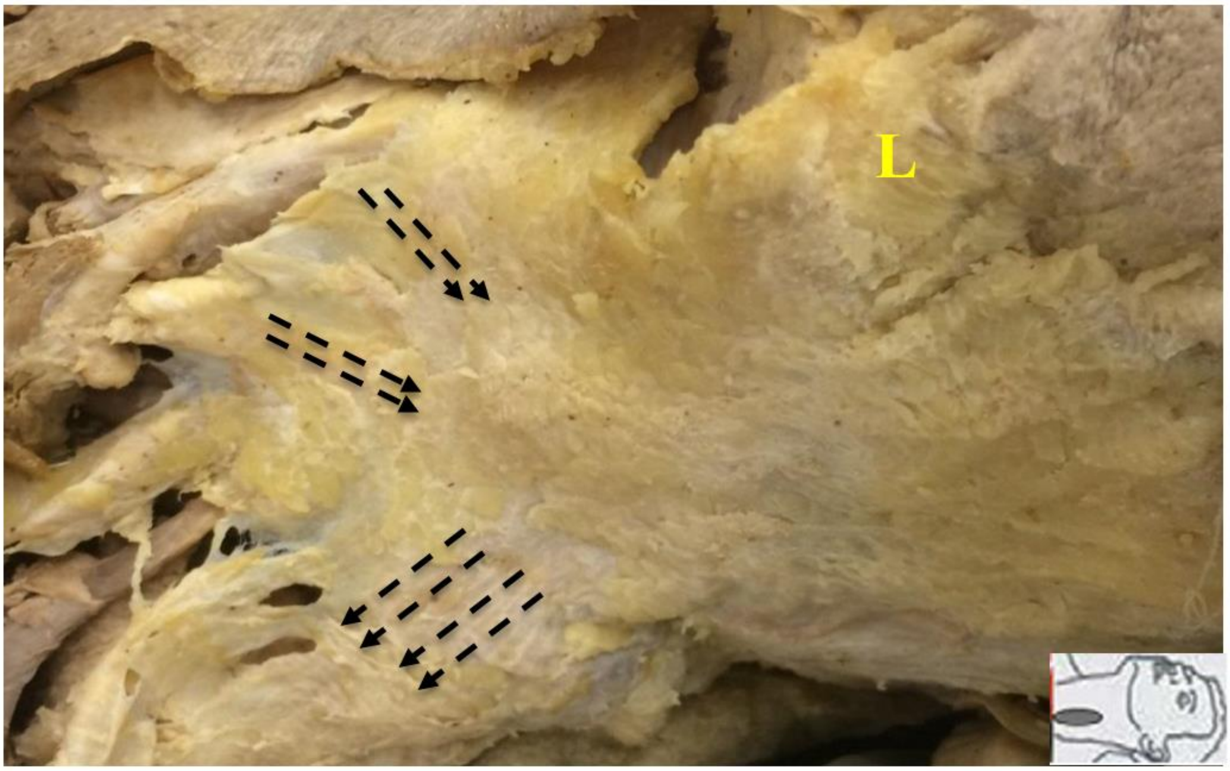


Figure 3. Photograph showing the platysma muscle (broken arrows) embedded in cervical fascia.

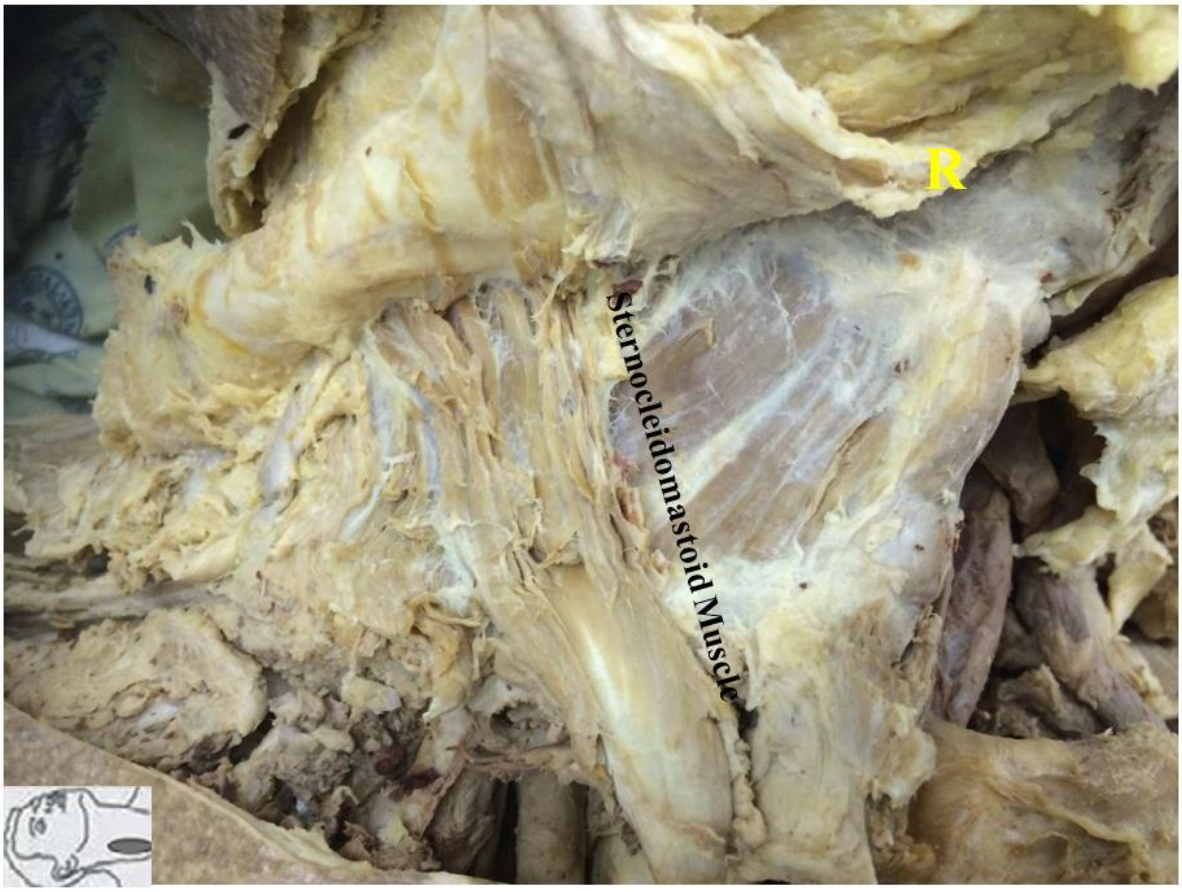


Figure 4. Photograph showing the reflection of platysma muscle and revealing of the sternocleidomastoid muscle (SCM).

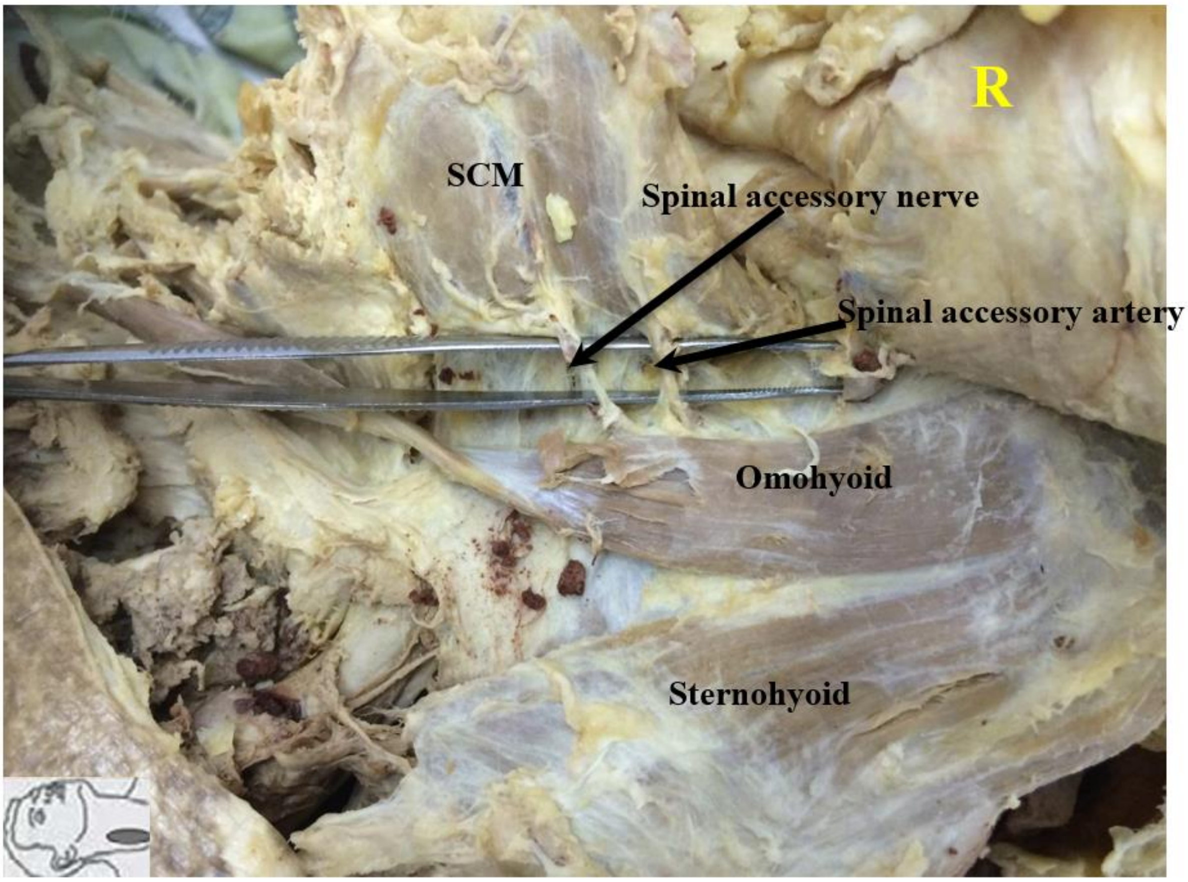


Figure 5. Photograph showing the reflection of the SCM to reveal strap muscles and the spinal accessory neurovascular bundle.

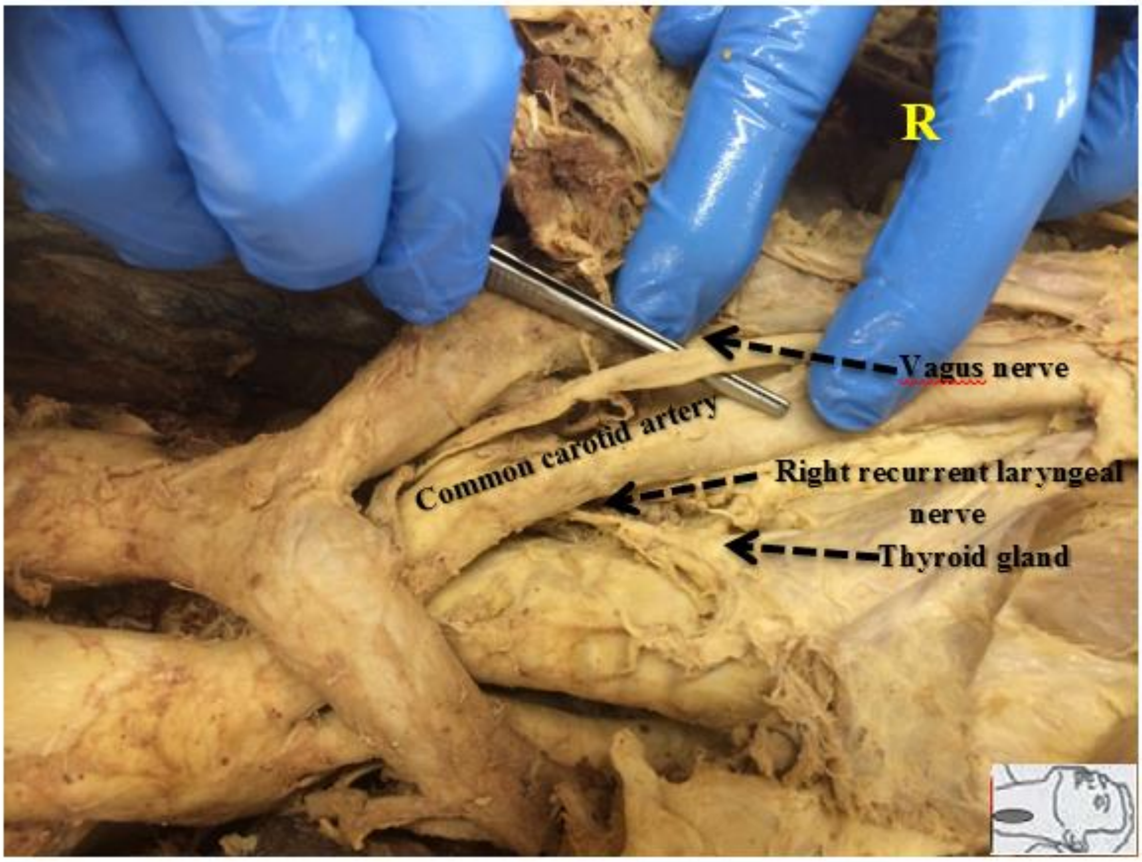


Figure 6. Photograph showing the contents of the carotid sheath after reflection of strap muscles.

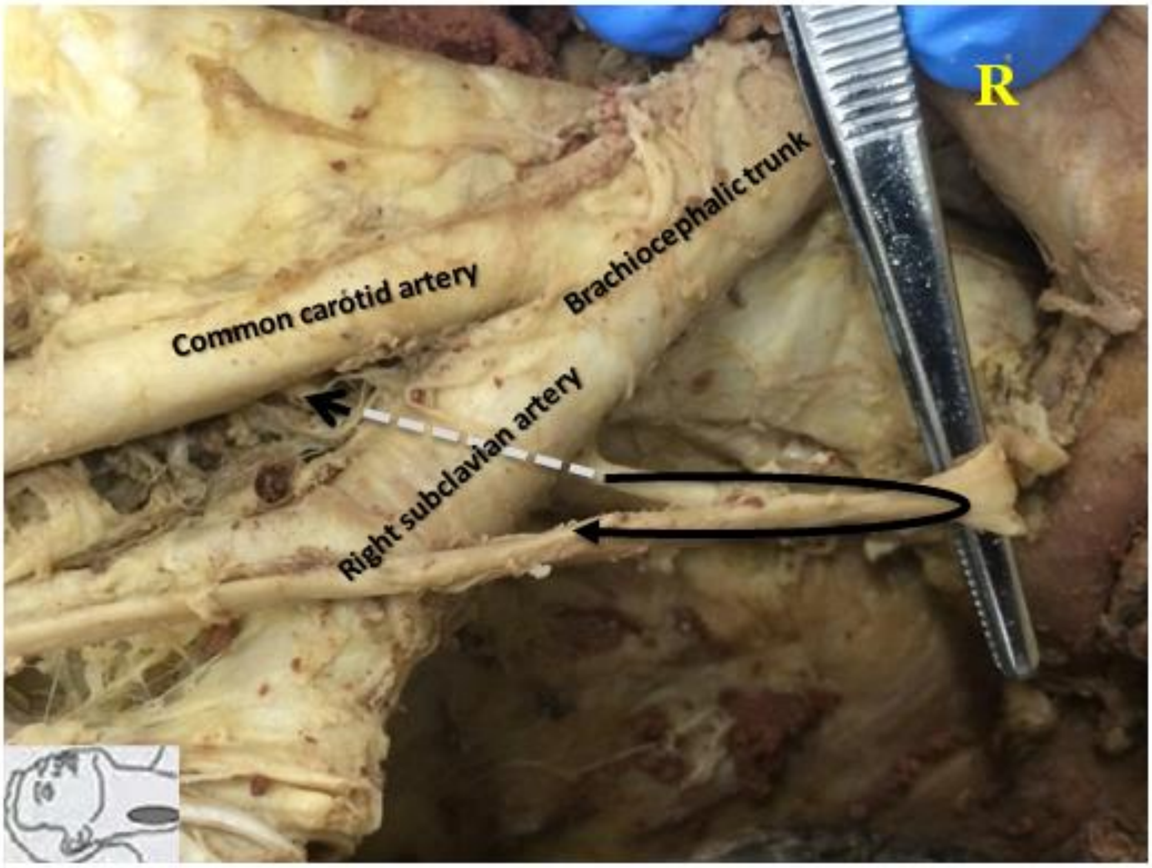


Figure 7. Photograph showing the recurrent branch of the vagus nerve around the right subclavian artery.

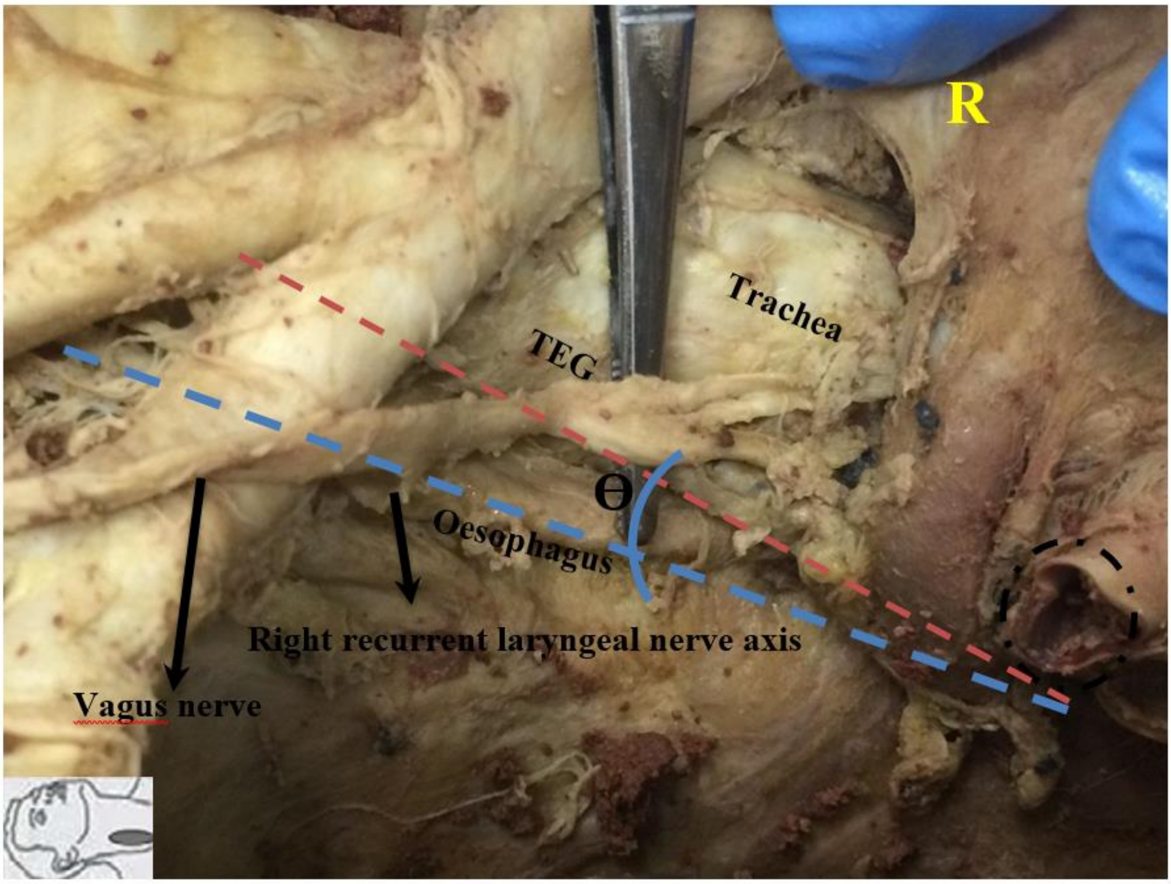


Figure 8. Photograph showing the tracheo-oesophageal groove (TEG)

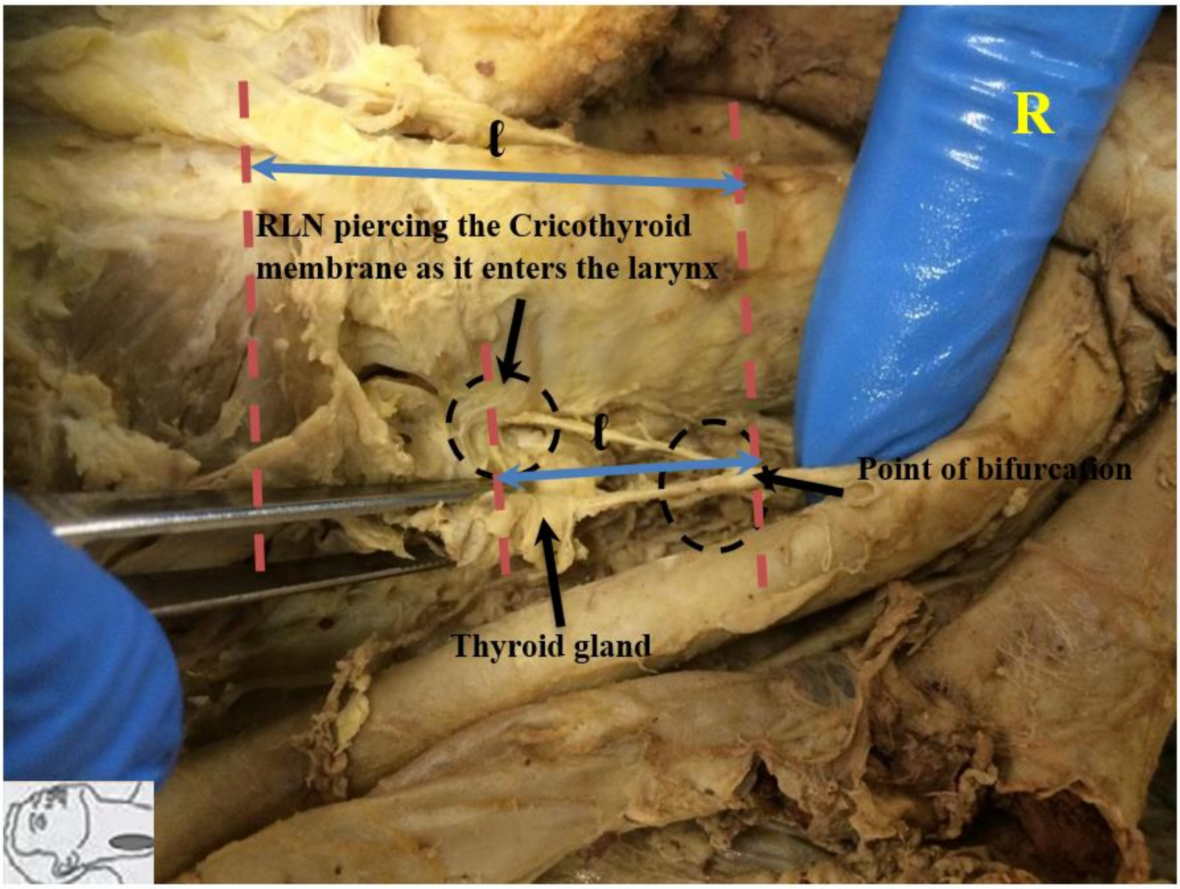


Figure 9. Photograph showing the point of bifurcation and entry into the larynx of the right RLN.

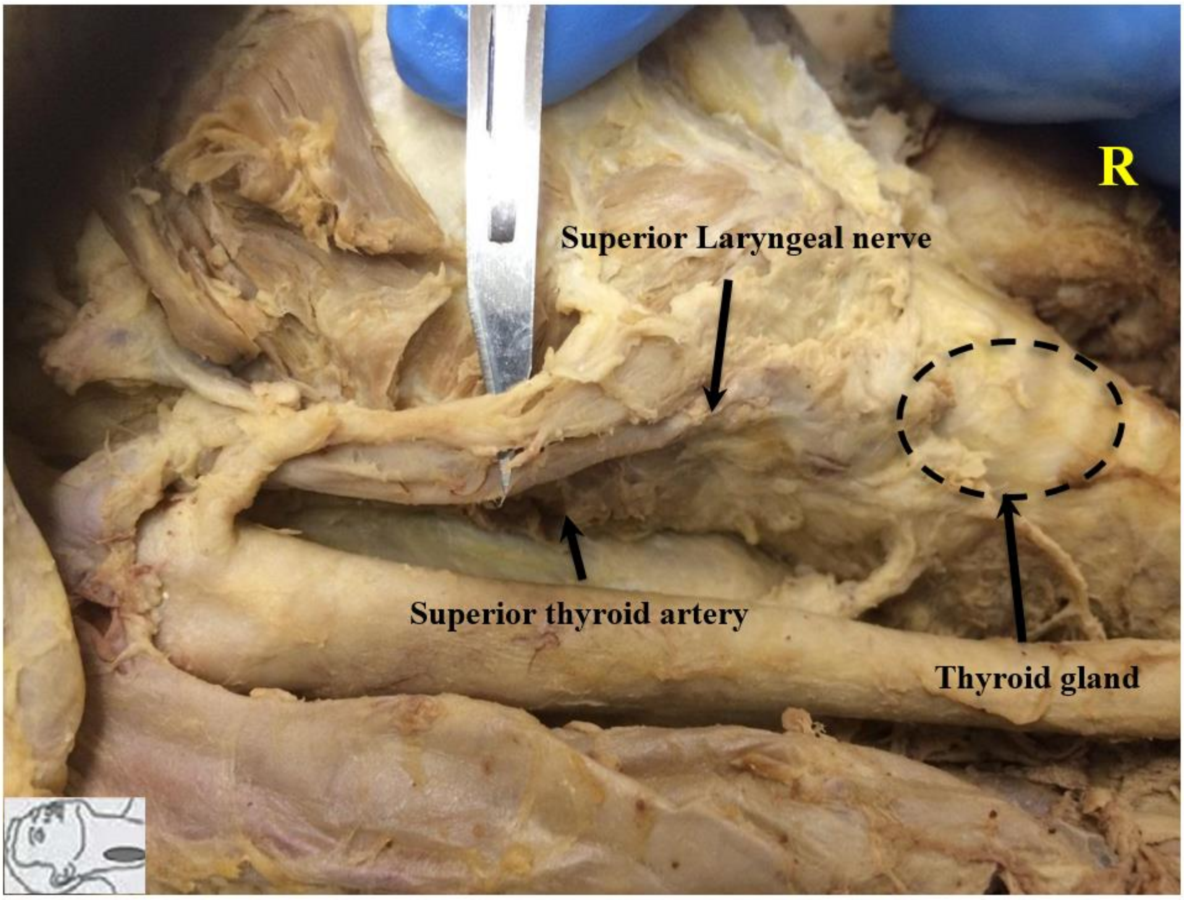


Figure 10. Photograph showing the relation between the superior laryngeal nerve, superior thyroid artery and the thyroid gland.

CHAPTER 4: RESULTS

A total of 50 adult cadavers, 31 paediatric cadavers and 30 thyroidectomy patients were recruited in this study. In total, 100 recurrent laryngeal nerves (RLN) from adult cadaveric specimens and 61 recurrent laryngeal nerves from paediatric cadaveric specimens were identified bilaterally. In the paediatric cadaver group, one of the specimens had an irreversibly damaged left RLN and the nerve was excluded from the study. In the adult patients undergoing thyroidectomies only 38 recurrent laryngeal nerves out of the possible 60 nerves were visualized bilaterally. The remaining 22 RLNs could not be visualized in thyroidectomy patients due to the invasiveness of the thyroid lesion and the risk to the patient of operative exploration to identify the nerve. Descriptive statistics and ancestry information of the entire study sample is shown in **Tables 3 and 4** below.

Table 3. Ancestry profile of the participants of the study.

Study sample	**Population**			
	White South African	Black South African	South African Indian	Total (N)
Adult cadavers	50 (100%)	0 (0%)	0 (0%)	50
Paediatric cadavers	8 (25.8%)	23 (74.2%)	0 (0%)	31
Surgical cohort	1 (3.3%)	28 (93.3%)	1 (3.3%)	30

Table 4. Descriptive statistics of the entire study sample.

	Cadaveric sample		Intraoperative (surgical cohort) sample
	Adult	Paediatric	-
N (Right RLN:Left RLN)	50 (50:50)	31 (31:30)	30 (19:19)
Male	50	16	0
Female	50	15	30
Age + SD	77.67 ±12.3 years	39.70±51.73 weeks	49.76±14.27 years
Range	42-95 years	0-144 weeks	21-80 years

SD: Standard deviation; RLN: recurrent laryngeal nerve

Table 5. Diameter of the recurrent laryngeal nerve

	Paediatric		Adult	
	Left	Right	Left	Right
Number	27	27	49	49
Mean diameter (mm)	0.74	0.72	1.75	1.63
Standard deviation	0.28	0.37	0.59	0.47
Median diameter (mm)	0.66	0.62	1.65	1.64
Shapiro-wilk test	0.39	0.03	0.03	0.98
Whitney Mann U p-value	0.62		0.37	

4.1. Adult cadaveric population

4.1.1. Diameter of the RLN in adult cohort

In the adult cadaveric population, the mean age was 77.67 ± 12.3 years (range 42 to 95 years). The male to female ratio was 1:1 with a total of 50 right sided and 50 left sided recurrent laryngeal nerves. The specimens belonged to the white ancestry as shown in **Table 3**. The demographic information and overall diameters and medians for the adult and paediatric cohort are shown in **Table 4 – 5**. The diameters of the RLN in both male and female adult cadavers are shown in **Table 6** below. The mean diameters of the RLN were 1.75 ± 0.59mm (median: 1.65mm) and 1.63 ± 0.47mm (median: 1.64mm) on the left and right sides respectively. The mean diameters of the RLN for the male specimens on both the left and the right sides were 1.69 ± 0.66mm and 1.71 ± 0.53mm, respectively. The mean diameters of the RLN for the female specimens on both the left and the right sides were 1.80 ± 0.51mm and 1.55 ± 0.40mm, respectively. Comparison of the left median diameters to the right median diameters was not statistically significant ($p \geq 0.05$). Comparison irrespective of side between the mean diameters of the RLN of males to that of females was not statistically significant ($p \geq 0.05$).

Table 6. Mean diameter of the left and right recurrent laryngeal nerve of adult male and female participants.

Sex	Side	Diameter (mm) ±SD
Male	Right	1.71±0.53
	Left	1.69±0.66
	Combined	1.70±0.59
Female	Right	1.55±0.40
	Left	1.80±0.51
	Combined	1.68±0.47

4.1.2. The course of the recurrent laryngeal nerve in the tracheosophageal groove in adult specimens

On the left side, the majority (71%) of the RLN followed the course of the tracheaesophageal groove (TEG), but 29% deviated from the TEG creating an angle of up to 45° in size. On the right side 10% of the RLN followed the TEG while 90% deviated from the TEG creating a maximum angle of greater than 45° in size (**Figures 11, 12 and 13**). 93.9% of the recurrent laryngeal nerves on the left side coursed within 0 - 15° of the TEG. While on the right side the RLNs coursed from 0 - 15° (46.9%) and 15 - 30° (40.8%) of the TEG in almost equal proportions as shown in **Figure 11**. Symmetry (the proportion of participants that have an identical angular course in the TEG) was observed in 6% of the entire adult cohort. Comparison of the course of the nerve in the TEG on left and right sides was statistically significant ($p \leq 0.05$; value = 0.0001) as shown in **Table 14**. The percentage frequency of nerves coursing at given angular range in the entire adult sample is shown in **Figure 11** and **Table 7**. Annotated photos and line drawings of dissected specimens displaying the anatomy, relation and course of the RLN can be seen in **Figures 16-19**.

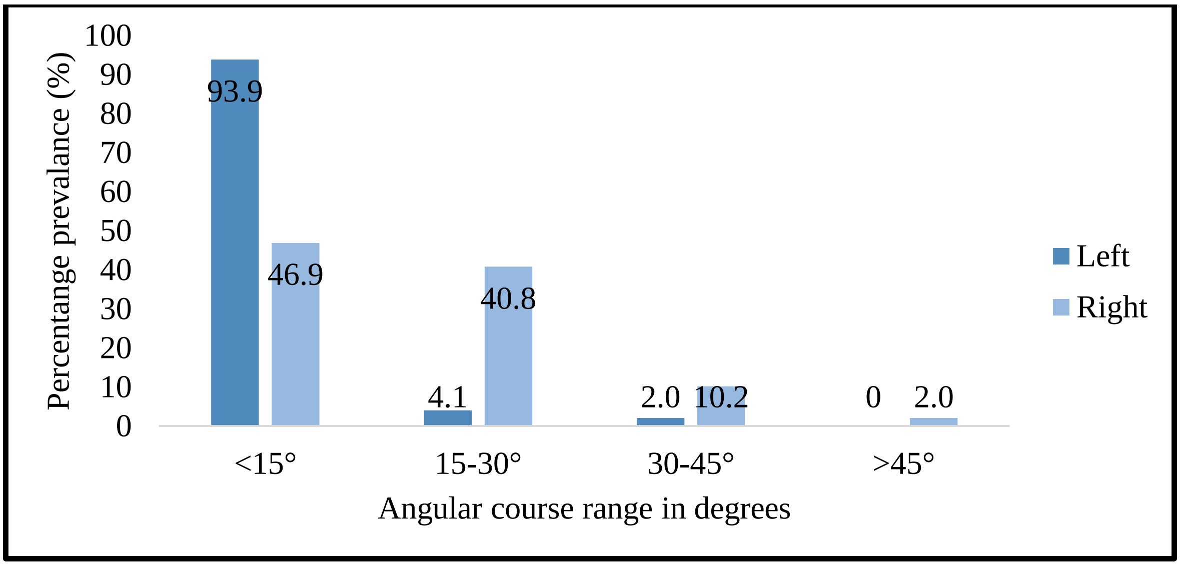


Figure 11. Bar chart showing the prevalence of the percentage angular course of the RLN in the TEG in adult cadavers.

Table 7. *The angular (degrees: mean ± SD: Standard Deviation) course of the RLN in relation to the TEG in adult cadavers*

		0 to 15°	**16 to 30°**	**31 to 45°**	**>45**
Male	Right	11.1±3.5	20.3±3.8	35.7±3.8	0
	Left	3.9±5.9	20.9±1.9	44.2*	0
Female	Right	8.3±3.0	20.3±4.2	37.2±7.2	0
	Left	6.3±6.0	22.7±4.9	0	57.5±0.2

*This is a single observation in that range (hence not an average).

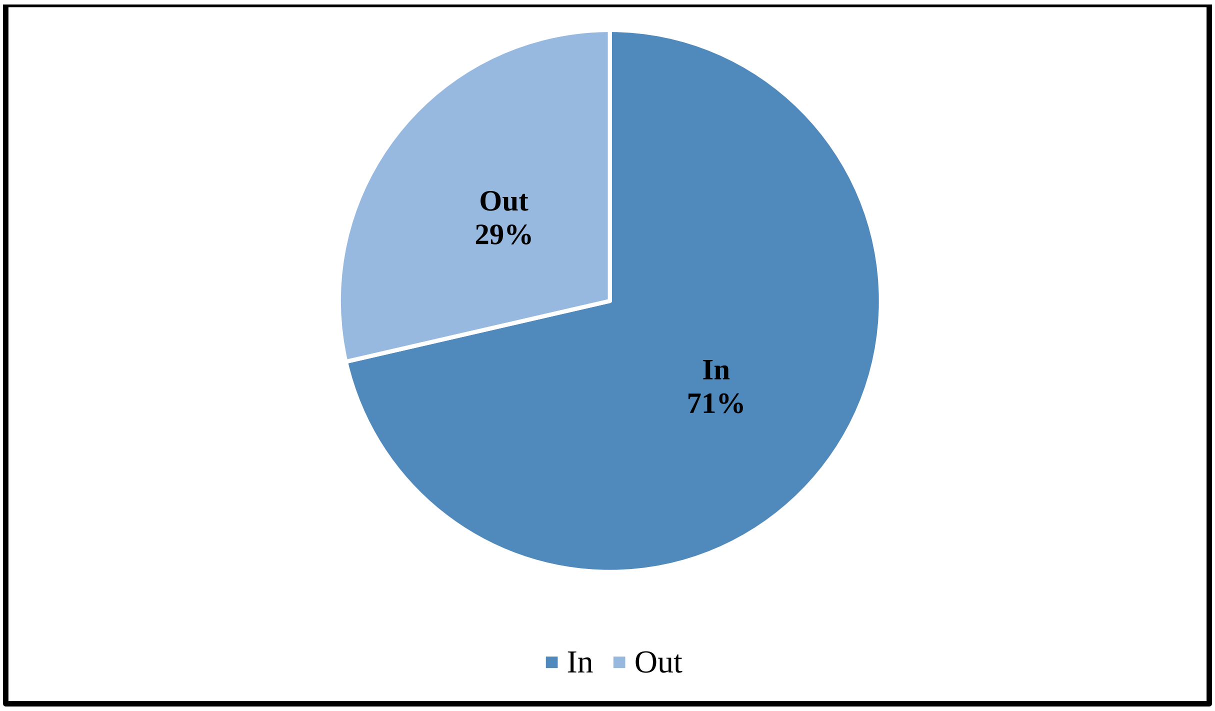


Figure 12. Pie chart of the course of the Left RLN in the TEG in adult cadavers

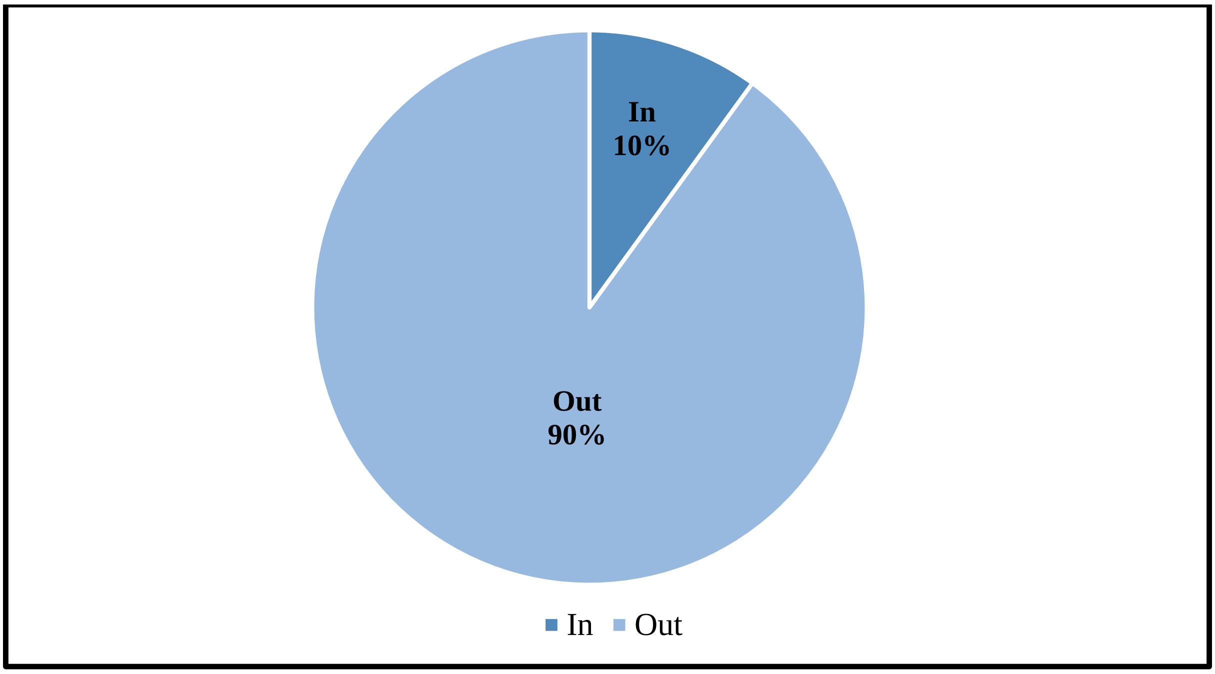


Figure 13. Pie chart of the course of the RLN in the TEG in adult cadavers

4.1.3. The relationship between the recurrent laryngeal nerve and the inferior thyroid artery in adult cadavers

All the recurrent laryngeal nerves in the studied adult population group followed a recurring course. On the right side, the RLN was related to the inferior thyroid artery (ITA) as follows: 24.4% anterior, 48.9% posterior and 26.5% in between the branches of the ITA (**Figure 14**). On the left side 39.5% of the RLNs coursed anterior, 14.5 % posterior and 45.8 % in between the branches of the ITA (**Figure 15**). There was no association between sex and the course of the RLN in relation to the ITA (anterior, posterior and in-between). The association between side and the relationship of the RLN to ITA was statistically significant ($p \leq 0.05$; value = 0.001355) as shown in **Table 14**.

An anomalous course of the vertebral artery was observed in two adult specimens (**Figure 20 and 21**). The anomalous vertebral arteries in both specimens originated directly from the arch of aorta as opposed to the conventional left Subclavian artery, they both assumed an anterior cranial course in the neck. In one of the two cases, a tortuosity of the blood vessel was observed at the level of the cricoid cartilage where it pierced the C4 vertebral body to assume a posterior course towards the circle of Willis (**Figure 21**). The other anomalous artery though non-tortuous followed the same course as the above-described vessel.

Table 8. Summary of the relationship between the RLN and the ITA stratified for sex and side in the adult cohort.

Sex	**Side**	**Relationship of RLN to ITA (%)**		
		Anterior	In between	Posterior
Male	Left	12.5	37.5	50.0
	Right	58.3	20.8	20.8
Female	Left	29.1	54.1	16.6
	Right	28.0	40.0	32.0

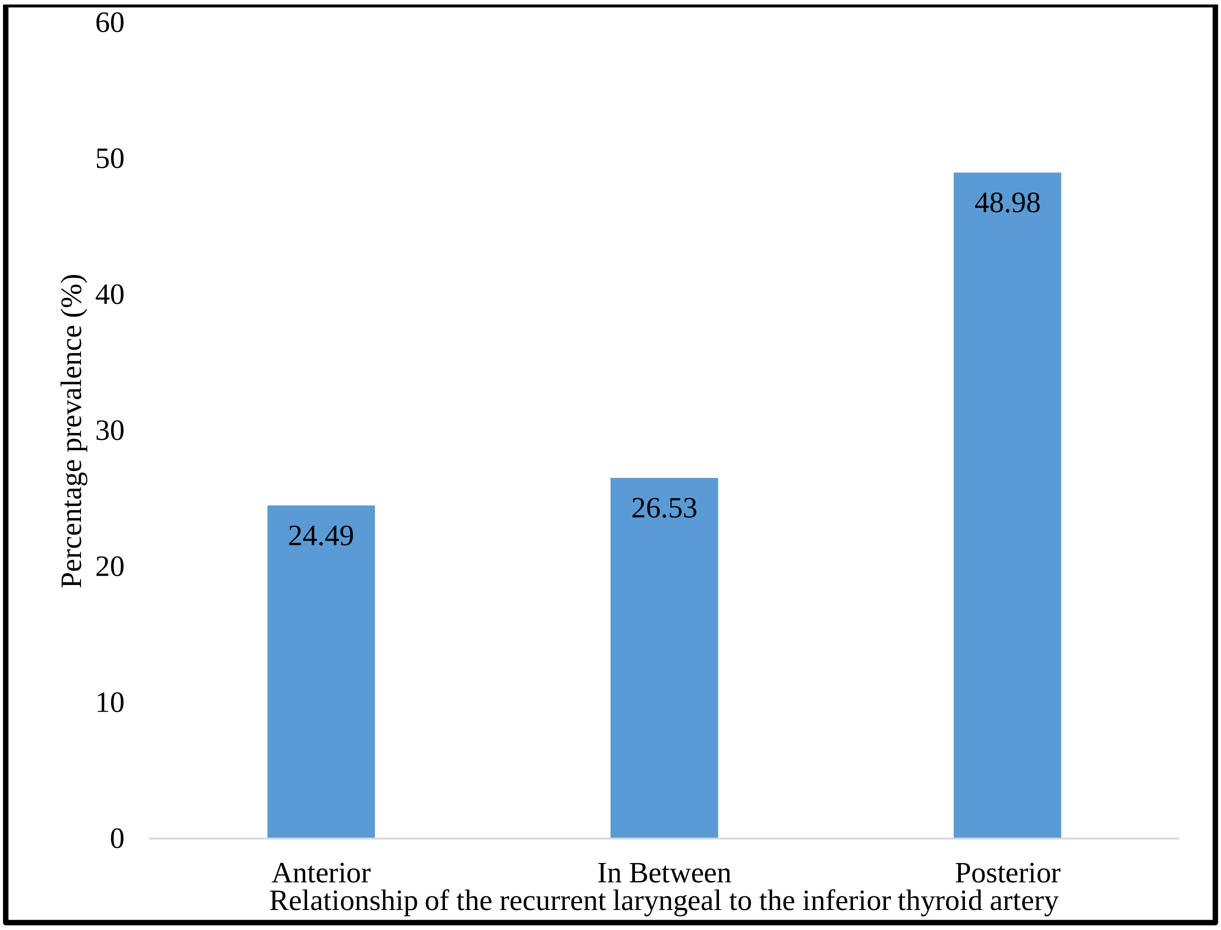


Figure 14. Bar graph of the relation of the RLN to ITA on the right side in the adult cadaveric population

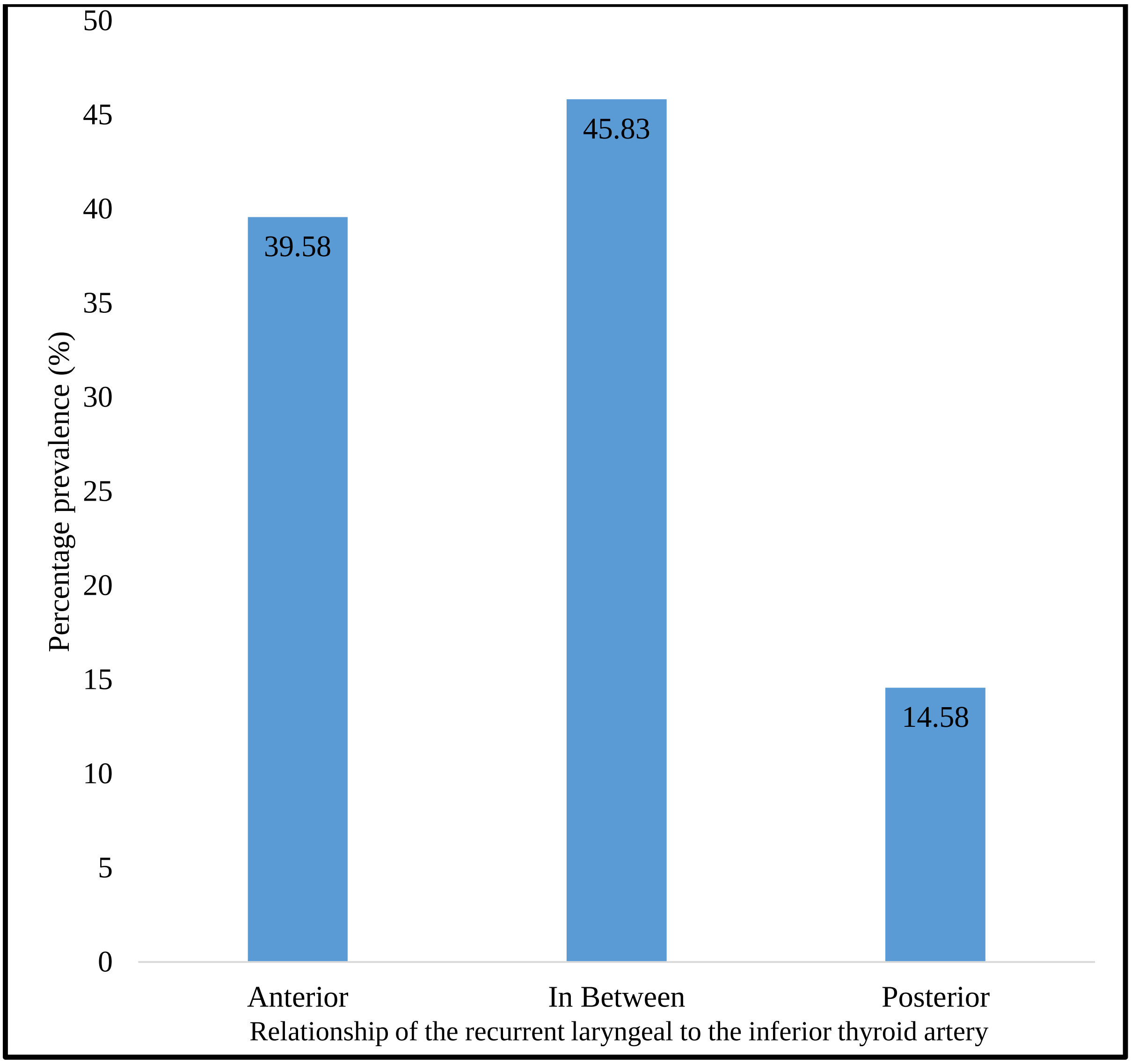


Figure 15. Bar graph of the relation of the RLN to ITA on the left side in the adult cadaveric population

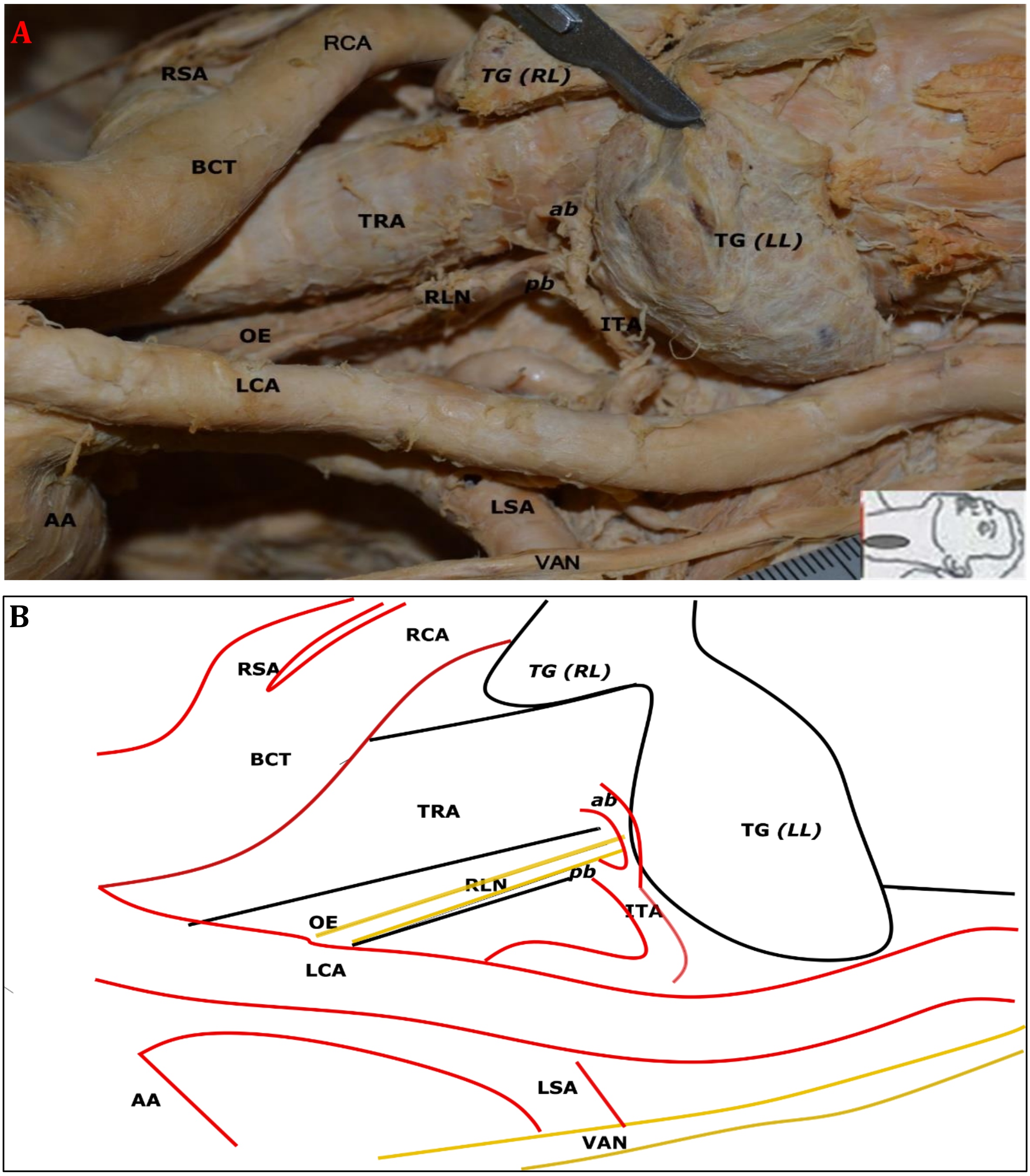
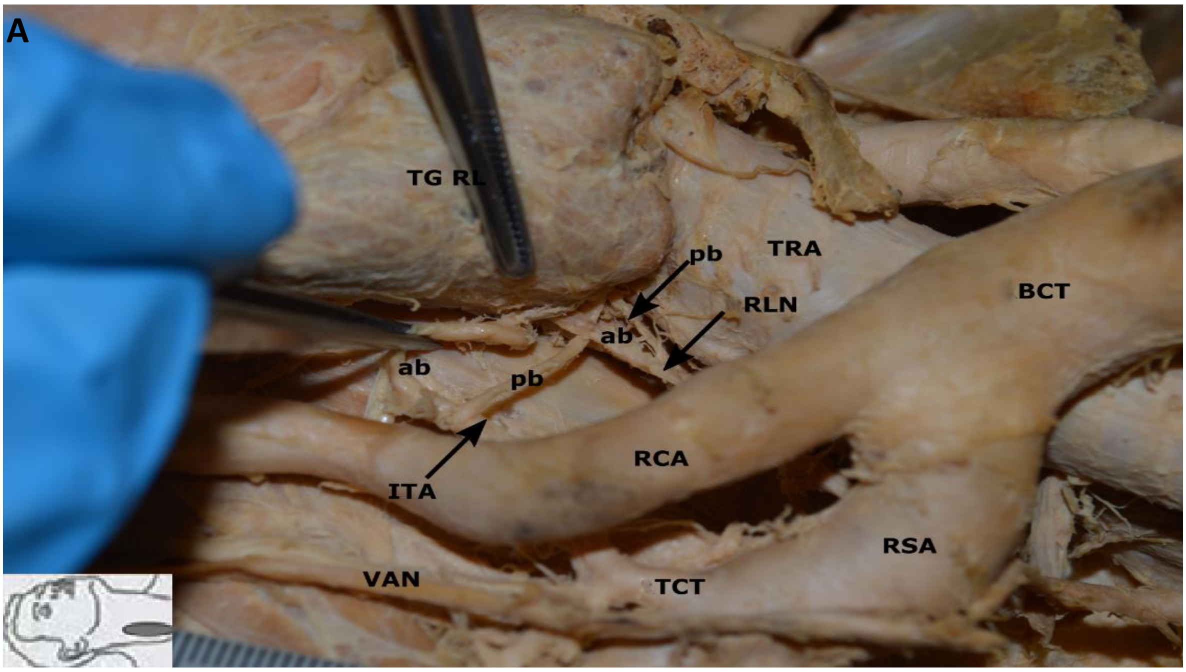


Figure 16. The diagrams (**A, B**) show the RLN coursing in between the branches of the ITA on the left side. **A**. Dissection photograph; **B.** Line drawing. Abbreviations RLN, left recurrent laryngeal nerve; ITA left inferior thyroid artery; ab, anterior branch of ITA ; pb, posterior branch of the ITA ; TG, thyroid gland; (RL), right lobe; (LL), left lobe ; TRA, trachea ; OE, oesophagus ; LCA, left common carotid artery ; BCT, brachiocephalic trunk; RSA, right Subclavian artery; LSA, left subclavian artery; AA, aortic arch; VAN, vagus nerve; RCA, right common carotid artery.



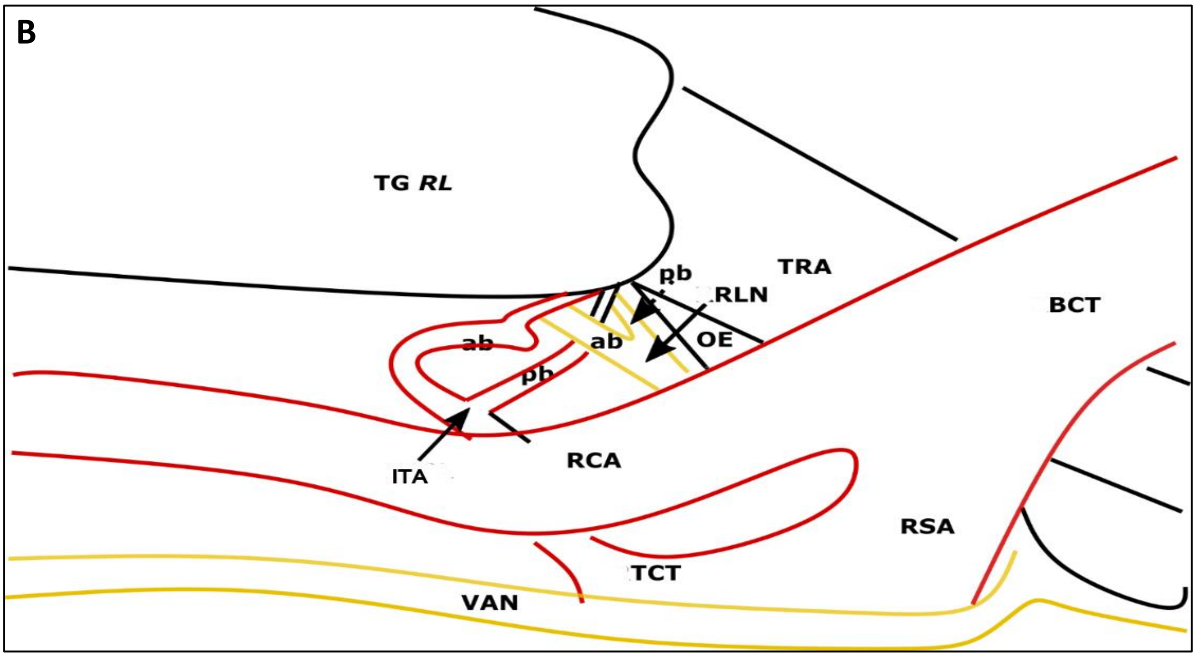


Figure 17. The diagrams (**A, B**) show the RLN coursing in between the branches of the ITA on the right side. **A.** Dissection photograph; **B**. Line drawing. Abbreviations RLN, right recurrent laryngeal nerve; ab, anterior branch of RLN; pb, posterior branch of the RLN; ITA inferior thyroid artery; ab, anterior branch of ITA; pb, posterior branch of the ITA; TG, thyroid gland; (RL), right lobe; TRA, trachea; OE, oesophagus; RCA, right common carotid artery; BCT, brachiocephalic trunk; RSA, right Subclavian artery; VAN, vagus nerve.

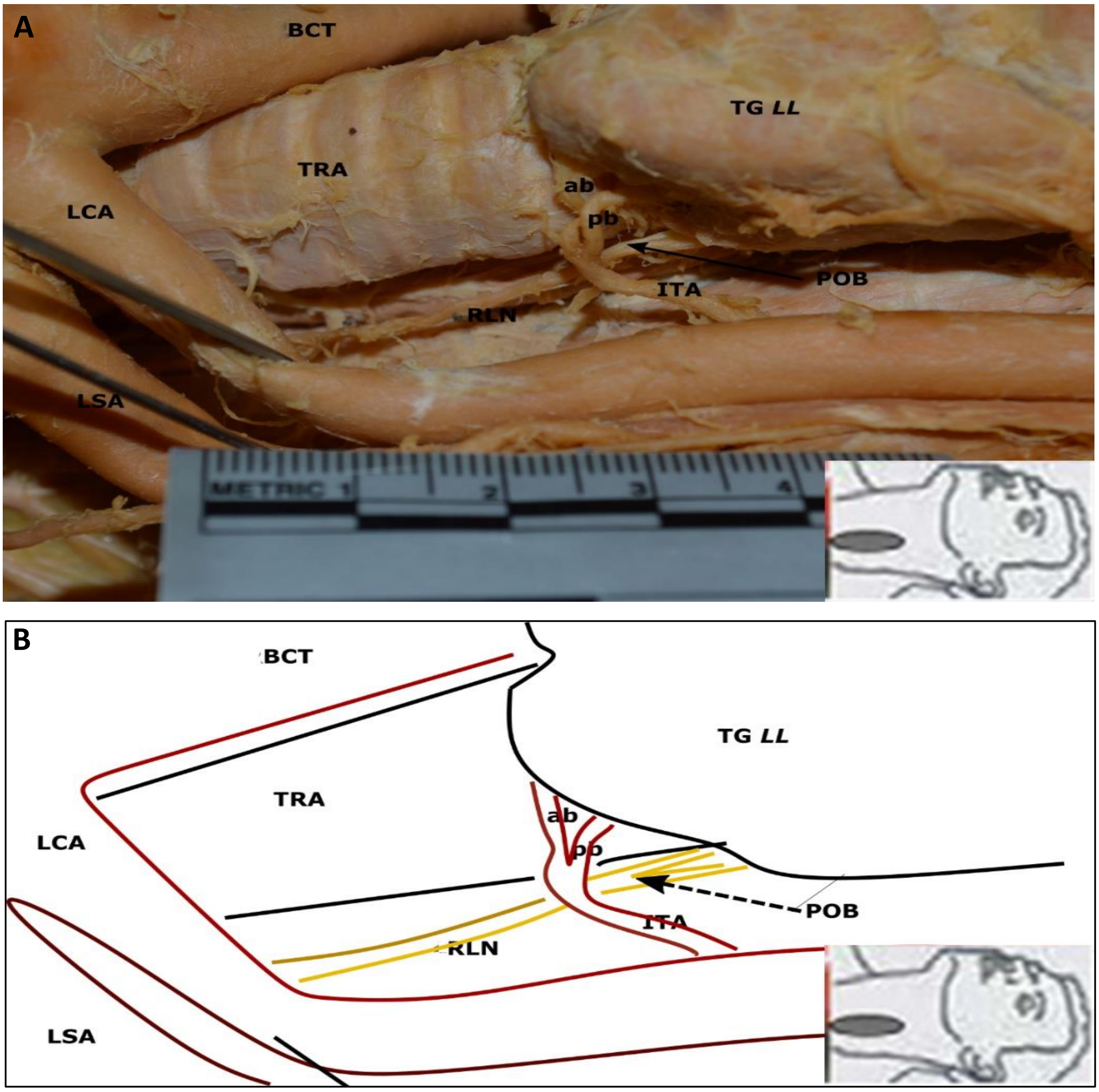


Figure 18. The diagrams (**A, B**) show the RLN coursing posterior to the ITA on the left side. **A**. Dissection; **B**. Line drawing. Abbreviations RLN, recurrent laryngeal nerve; ITA inferior thyroid artery; ab, anterior branch of ITA; pb, posterior branch of the ITA ; TG, thyroid gland; (LL), left lobe; TRA, trachea ; POB point of bifurcation; LCA, left common carotid artery ; LSA, left subclavian artery.

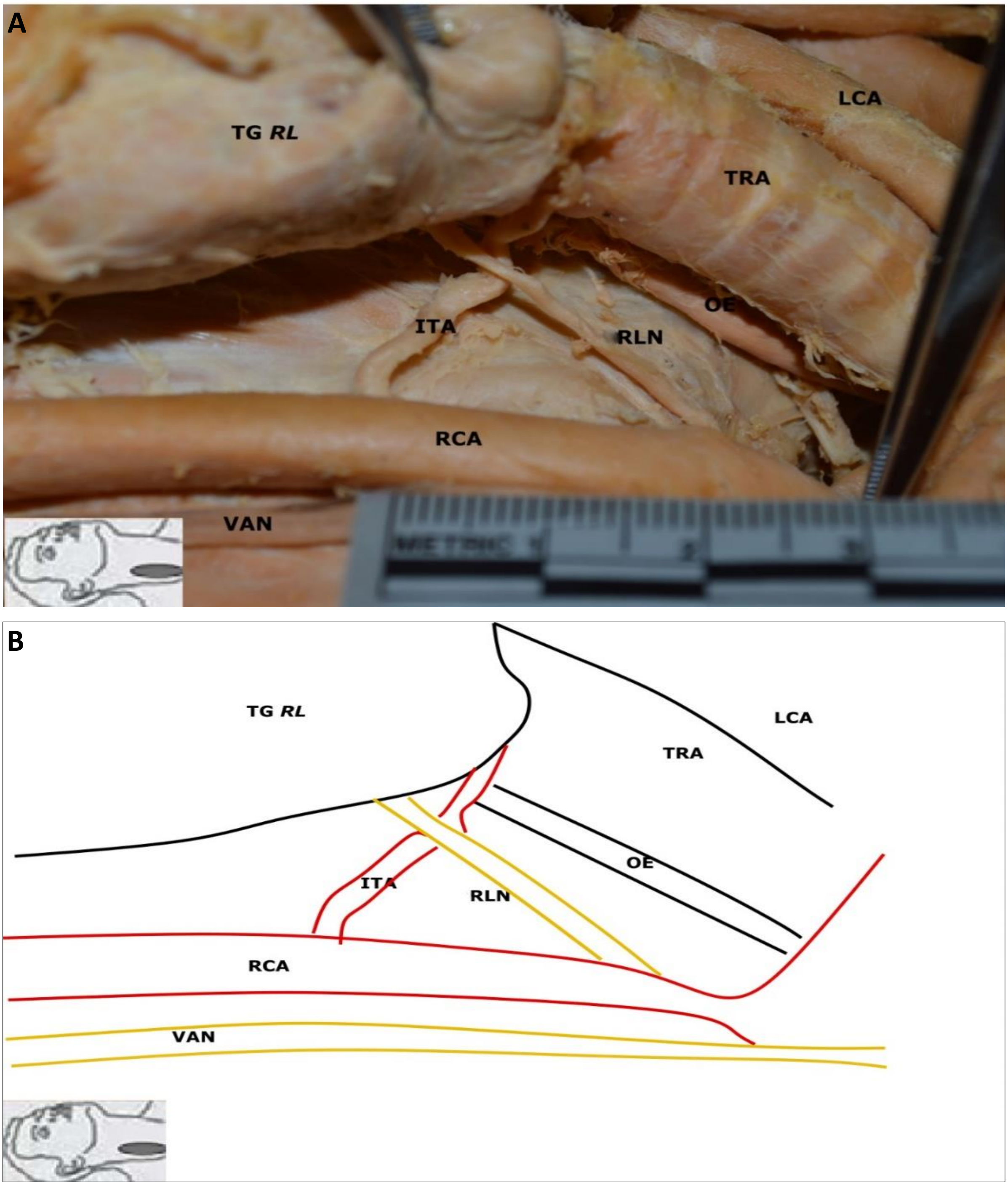


Figure 19. The diagrams (**A, B**) show the RLN coursing anterior to the ITA on the right side. **A**. Dissection photograph; **B**. Line drawing. Abbreviations ITA, inferior thyroid artery; TRA, trachea; OE, oesophagus; RCA, right common carotid artery; VAN, vagus nerve; TG, thyroid gland.

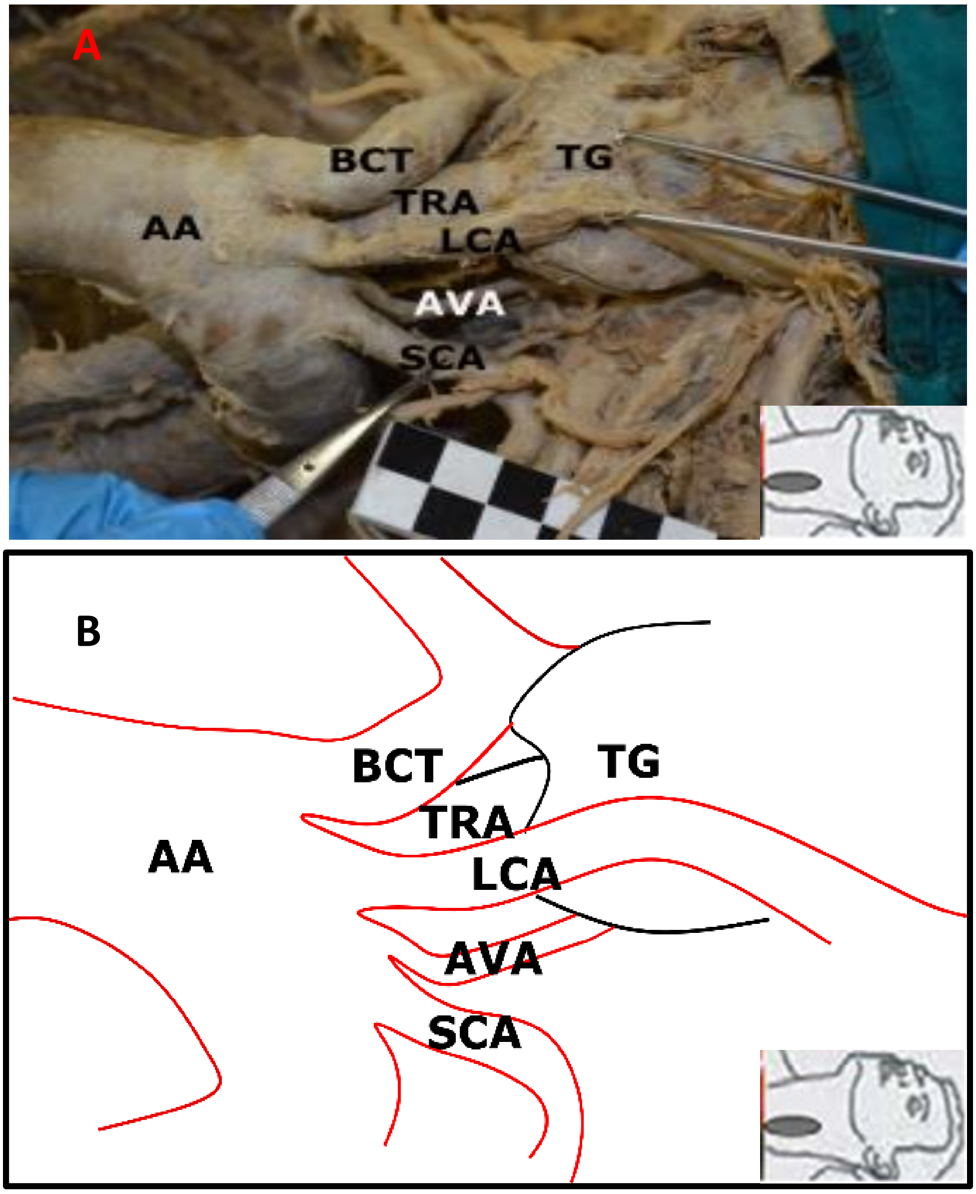


Figure 20. The diagrams (**A, B**) show the arch of the aorta branching to give four branches including an anomalous vertebral artery (**AVA**) on the left side. **A**. Dissection photograph; **B**. Line drawing. Abbreviations AA, aortic arch; BCT, brachiocephalic trunk; SCA, left Subclavian artery; TG, thyroid gland; TRA, trachea; LCA, left common carotid artery.

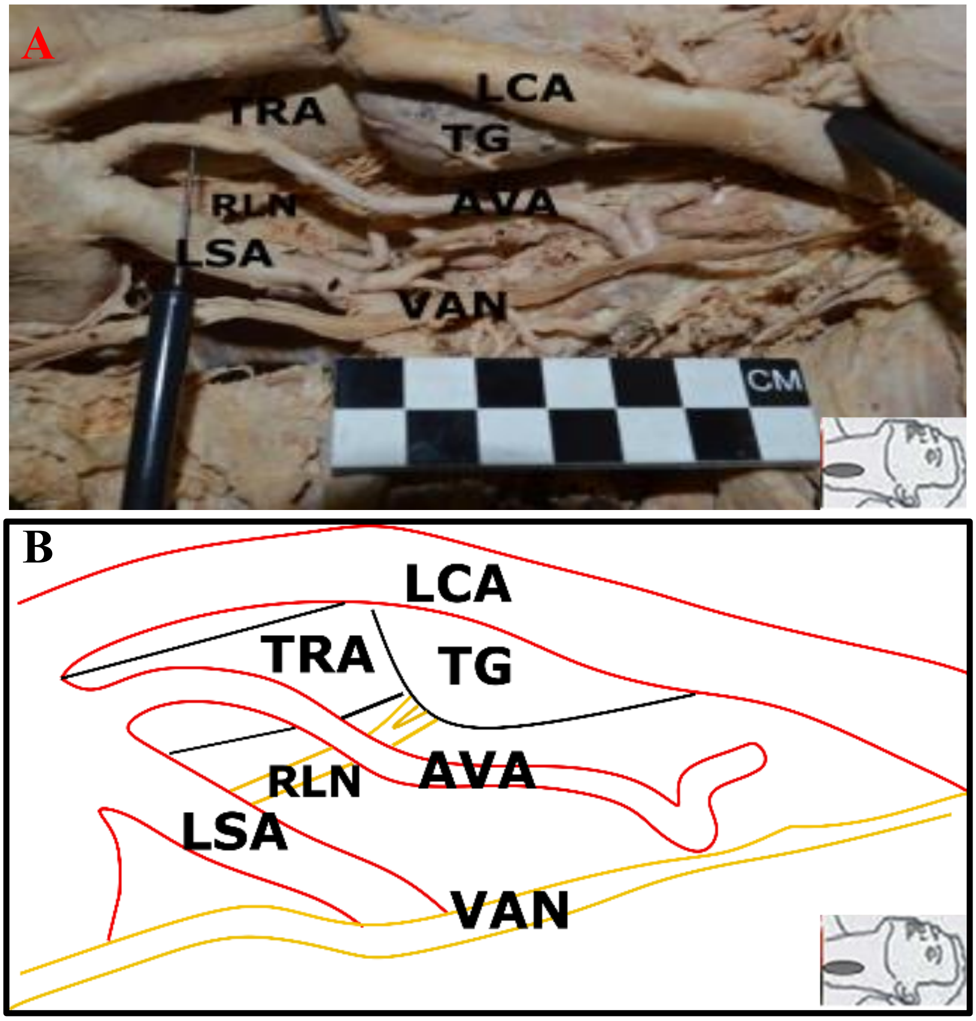


Figure 21. The diagrams (**A, B**) show the arch of the aorta branching to give four branches including an anomalous vertebral artery (**AVA**) on the left side. **A**. Dissection photograph; **B**. Line drawing. Abbreviations LSA, left Subclavian artery; LCA, left common carotid artery; TG, thyroid gland; TRA, trachea; RLN, recurrent laryngeal nerve; VAN, vagus nerve

4.1.4. Terminal branching of the RLN in the adult cadaveric population

Extra-laryngeal branching of the RLN was observed in 61% of the cases. Of these, 36.7% (18/49) revealed a symmetrical bilateral bifurcation (that is on both the left and right side equally). There was no association between the extra-laryngeal branching and side ($p \geq 0.05$) (**Table 14**). The highest number of branches observed in this study was 4 (quadrification) (**Figure 22**)

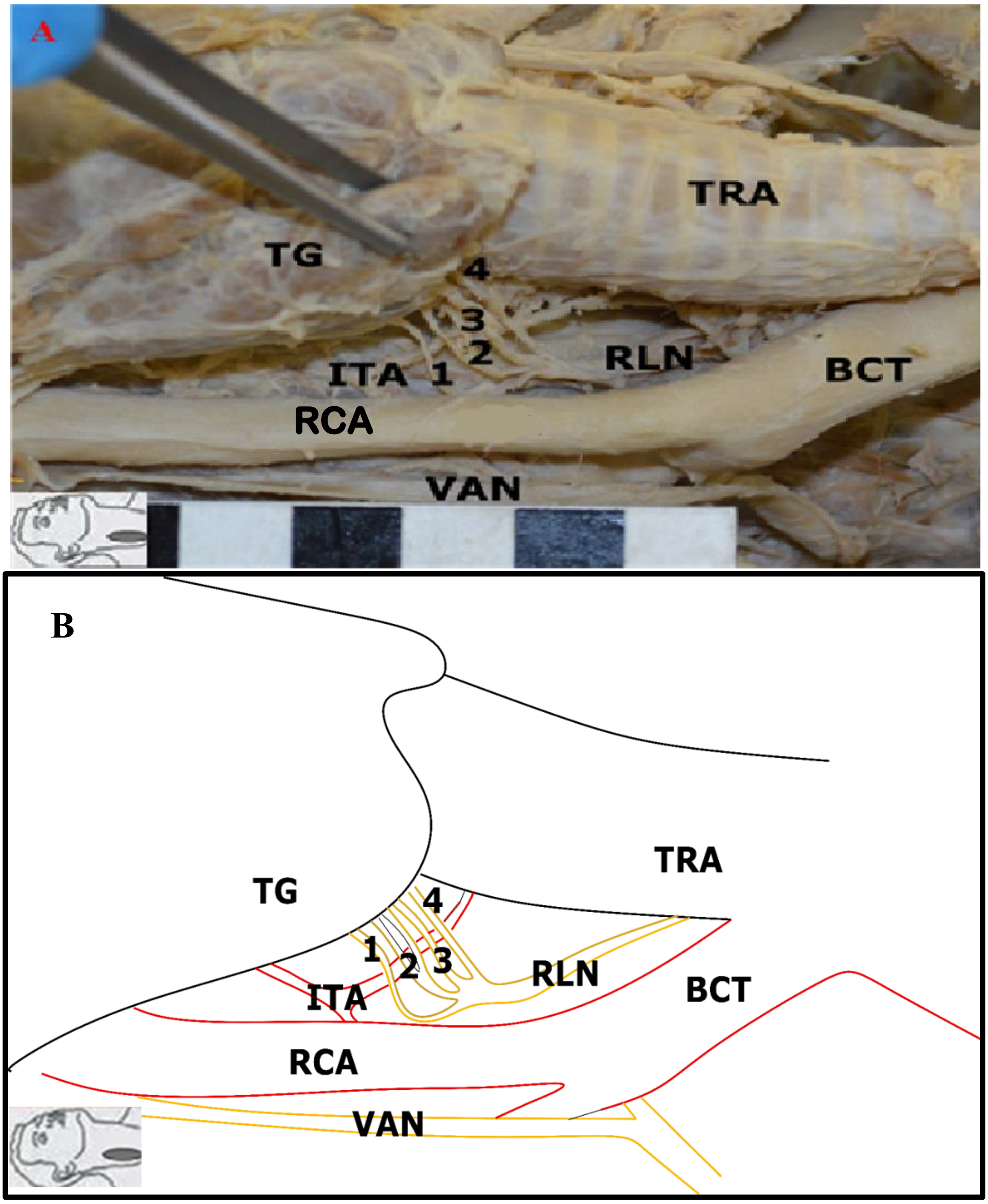


Figure 22. The figures (**A, B**) show a case of the recurrent laryngeal nerve branching into four (4) branches before entering the larynx. The branch labelled number 2 further bifurcates outside the larynx. Abbreviations; TRA, trachea; RLN, recurrent laryngeal nerve; VAN, vagus nerve; TG, thyroid gland; BCT, brachiocephalic trunk; RCA, right common carotid artery; ITA inferior thyroid artery.

4.2. Paediatric population

4.2.1. Demographic statistics and the diameter of the RLN in the paediatric cadaver population

In the paediatric cadaveric population, the average age was 39.7 ± 51.7 weeks (range 0 - 144 weeks). A total of 61 recurrent laryngeal nerves comprising of 31 on the right side and 30 on the left side were observed and analysed in the current study (**Table 4**). The study sample consisted of 23 (74.2%) South African blacks and 8 (25.8%) South African whites (**Table 3**). The diameters of the RLN in the paediatric cohort are presented in **Table 9.** The mean diameter of the RLN was 0.74 ± 0.28mm and 0.72 ± 0.37 on the left and right sides respectively. The median diameter of the RLN was 0.66mm and 0.62mm on the left and right side respectively. Comparison between the left and right median diameters was not statistically significant ($p \geq 0.05$; value = 0.6157) (**Table 14**). Comparison irrespective of side between the mean diameters of males versus female was not statistically significant ($p \geq 0.05$; value = 0.6674). The combined mean diameters of the left versus the right was also not statistically significant ($p \geq 0.05$; value = 0.9063). See **Table 14**.

Table 9. Mean diameter of the left and right recurrent laryngeal nerves of paediatric male and female participants.

Sex	Side	Diameter (mm) ±SD
	Right	0.73±0.44
Male	Left	0.72±0.29
	Combined	0.73±0.36
	Right	0.77±0.27
Female	Left	0.71±0.31
	Combined	0.74±0.28

4.2.2. The course of the recurrent laryngeal nerve in the TEG in the paediatric cadaver population

On the right side, 42% of the RLN followed the course of the TEG, but 58% deviated from the TEG creating an angle ranging from 6.7 - 44°. On the left side, the majority of RLN (89%) followed the TEG while 11 % deviated from the TEG creating an angle ranging from 0.9 - 37° (**Figures 23 - 24**) (**Table 10**). In total, 96.0% of the nerves on the left side were found coursing within 0 - 15° range of the TEG. While on the right side, 90.0% of the RLNs were found coursing along the TEG as follows: from 0 - 15° (65.0%) and 15 - 30° (25%) (**Figure 25**). Symmetrical angular course was observed in 40.74%. The course of the RLN showed a significant association between the side and the angle along the TEG ($p \leq 0.05$; value = 0.0170) (**Table 14**).

Table 10. The angular (degrees: mean ± SD) course of the RLN in relation to the TEG in paediatric cadavers.

		0 to 15°	**16 to 30°**	**31 to 45°**	**>45**
Male	Right	3.4±5.4	9.6 ±11.4	44*	0
	Left	0.9±3.5	0	0	0
Female	Right	6.3±7.2	20.5±0.7	31*	0
	Left	0	0	37*	0

*These are single observations in that particular range (hence not an average)

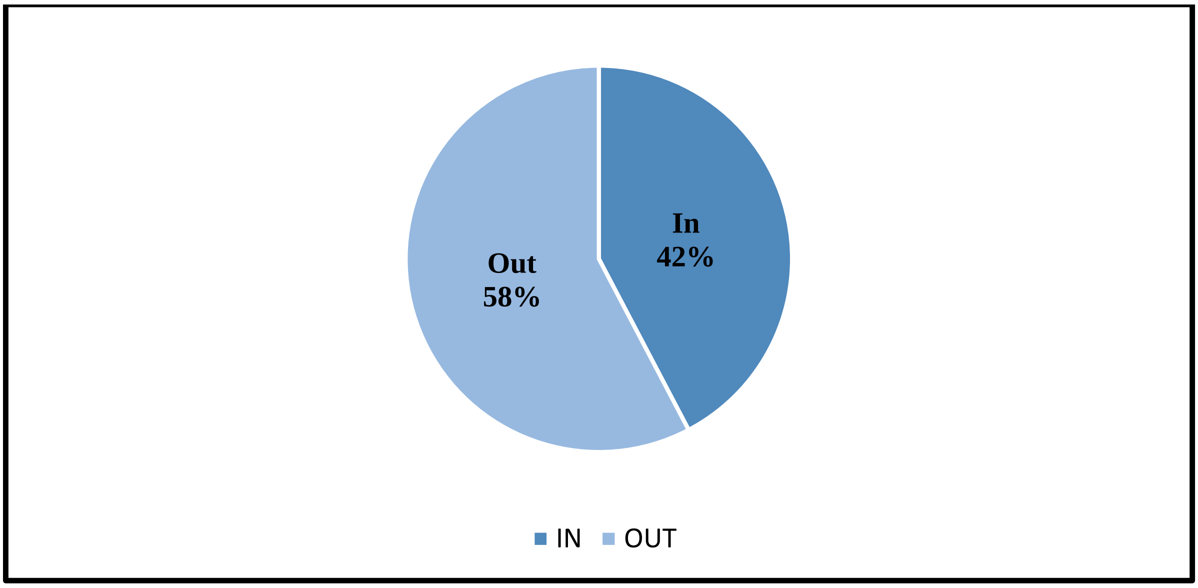


Figure 23. Pie Chart showing the proportion of nerves coursing in and out of the TEG on the right side.

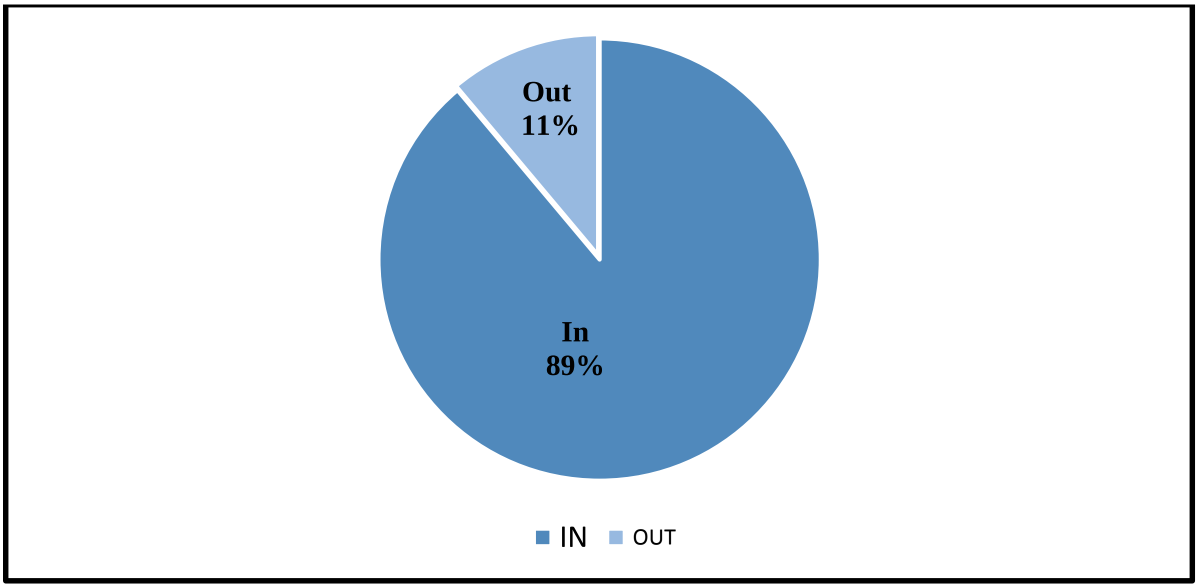


Figure 24. Pie Chart showing the proportion of nerves coursing in and out of the TEG on the left side.

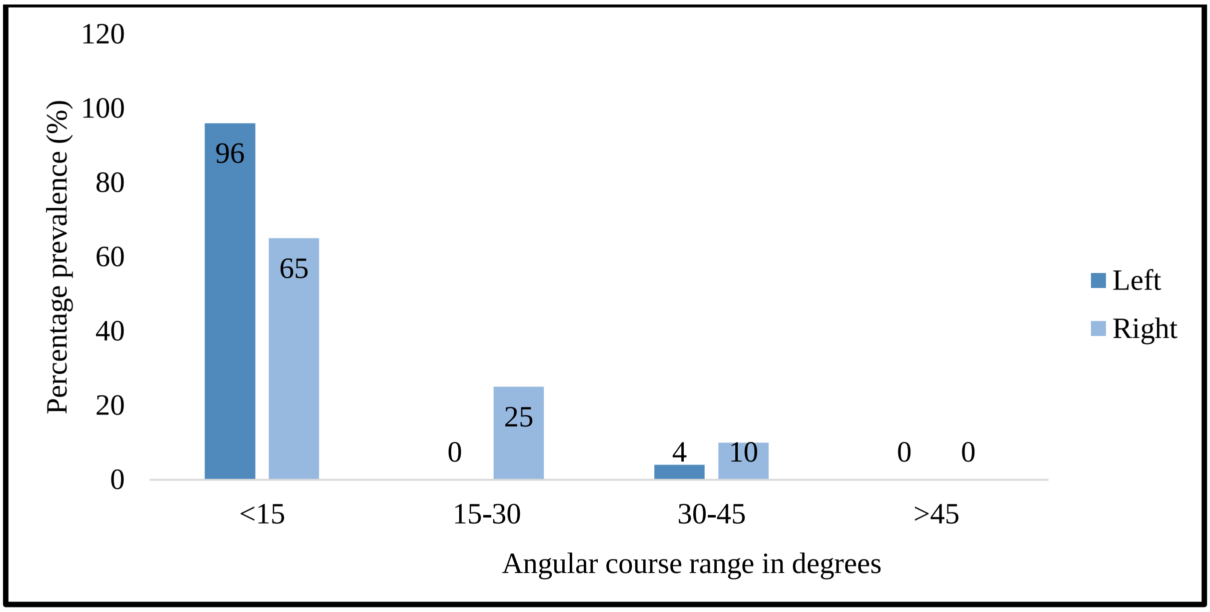


Figure 25. Bar chart showing the prevalence of the angular course of the RLN in the TEG.

4.2.3. The relationship between the recurrent laryngeal nerve to the inferior thyroid artery

All the recurrent laryngeal nerves in the paediatric cadaver population followed a recurring course. On the right side, the RLN was related to the ITA as follows: 67.7% anterior, 19.3% posterior and 12.9% in-between the branches of the ITA. On the left side, 56.6% of nerves coursed anterior, 40.0% posterior and 3.3% in-between the branches of the ITA (**Figures 26 and 27**) (**Table 11**). In one specimen, the left RLN was observed to recur on a distinctively large patent ductus arteriosus. A communicating vessel between the main portion of the pulmonary artery and the proximal portion of the descending aorta was also observed (**Figure 30**).

Table 11. Summary of the relationship between RLN and the ITA stratified by sex and side in the paediatric cadaver cohort.

Sex	**Side**	**Relationship or RLN to ITA (%)**		
		Anterior	In-between	Posterior
Male	Left	68.7	0.0	31.2
	Right	75.0	6.2	18.7
Female	Left	42.8	7.1	50.0
	Right	53.3	26.6	20.0

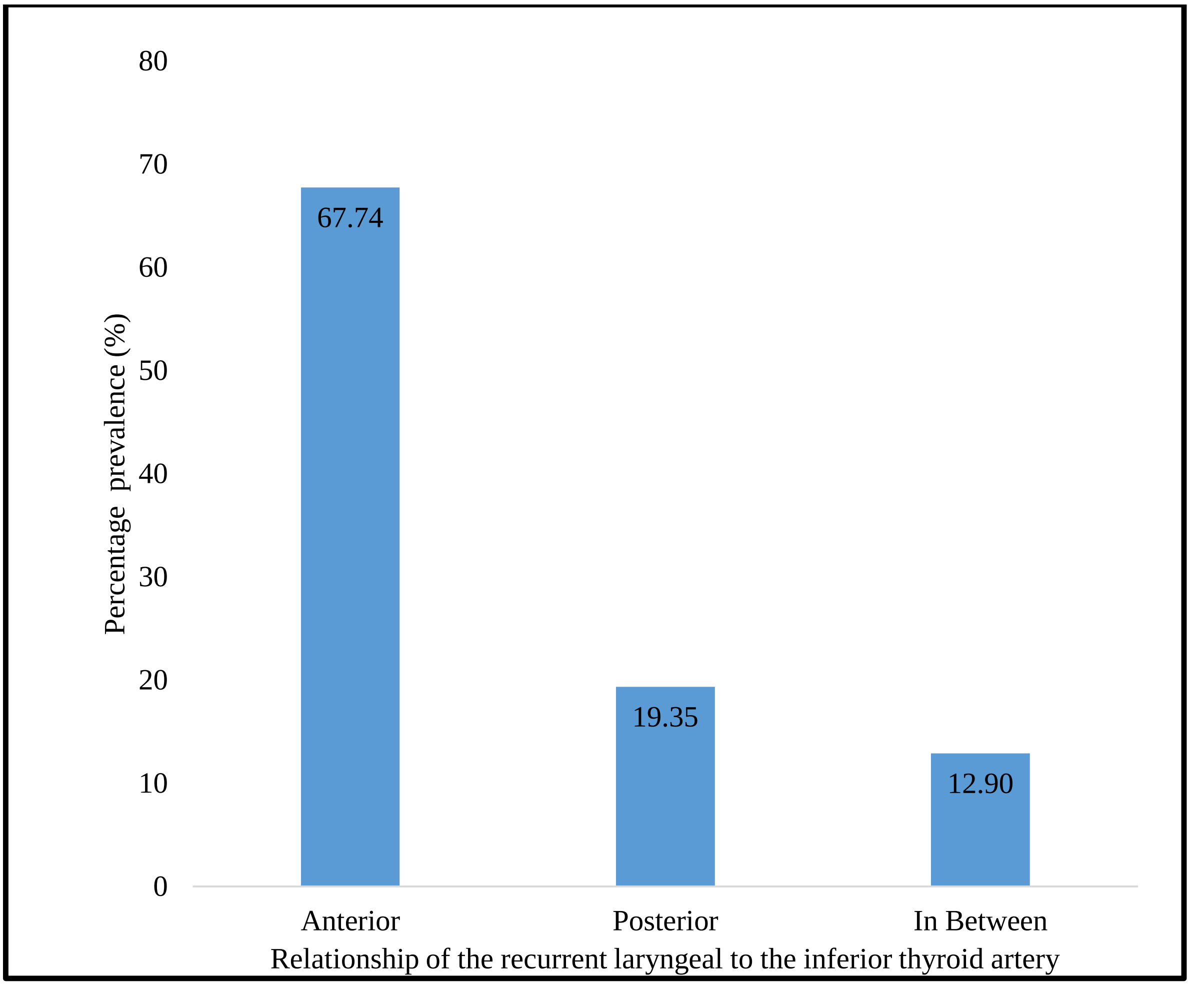


Figure 26. Bar chart showing the relation of the RLN to the ITA in the right side.

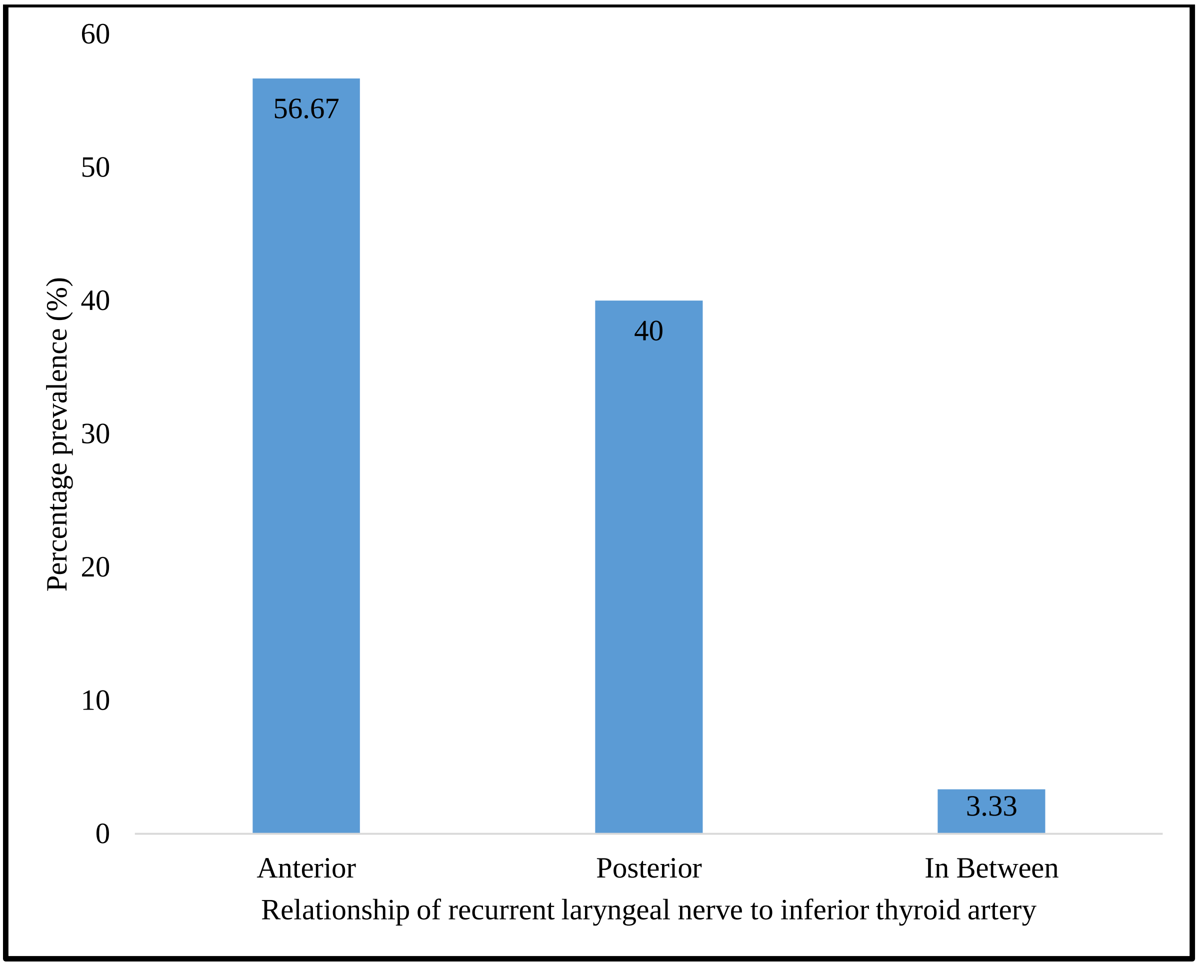


Figure 27. Bar graph showing the relationship of the RLN and ITA on the left side.

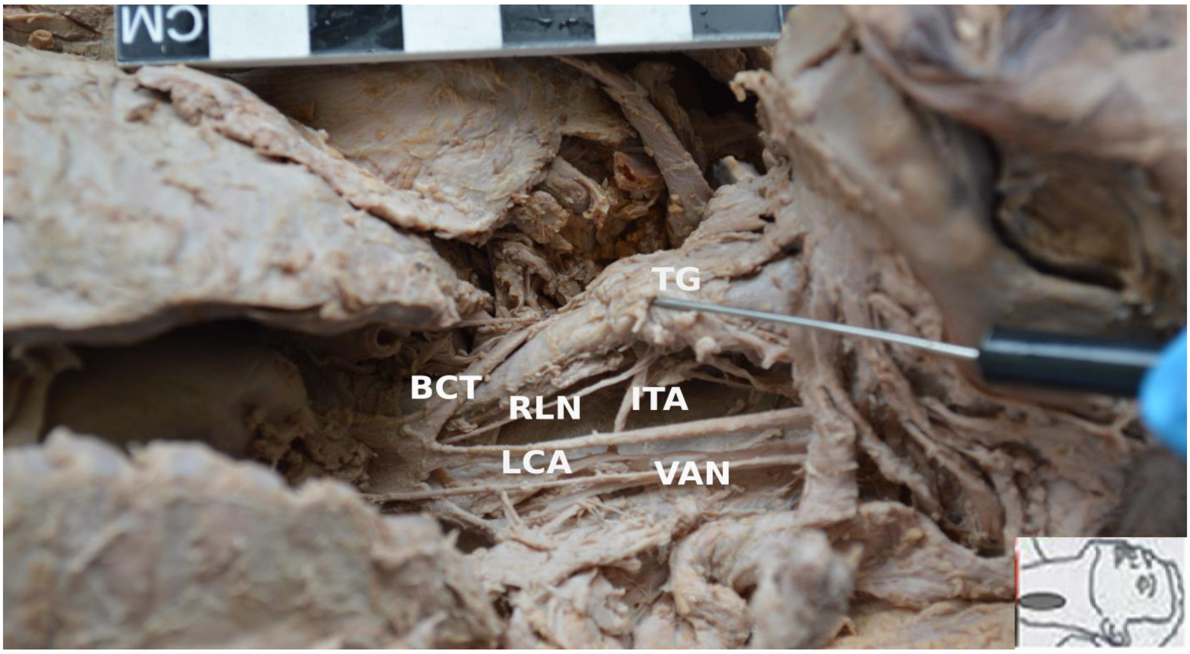


Figure 28. The photograph shows the RLN coursing anterior to the ITA on the left side. Abbreviations RLN, recurrent laryngeal nerve; ITA inferior thyroid artery ; TG, thyroid gland; LCA, left common carotid artery; VAN, Vagus nerve; BCT, brachiocephalic trunk.

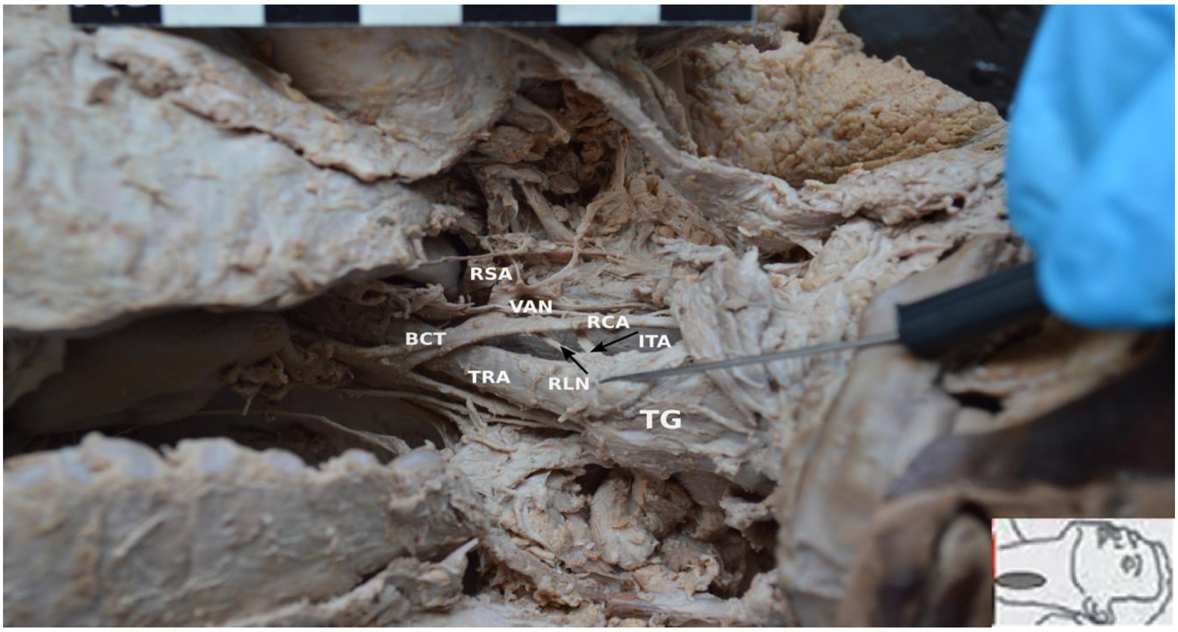


Figure 29. The photograph shows the RLN coursing anterior to the ITA on the right side. Abbreviations RLN, right recurrent laryngeal nerve; ITA, inferior thyroid artery; TG, thyroid

gland; TRA, trachea; RCA, right common carotid artery; BCT, brachiocephalic trunk; RSA, right subclavian artery; VAN, vagus nerve.

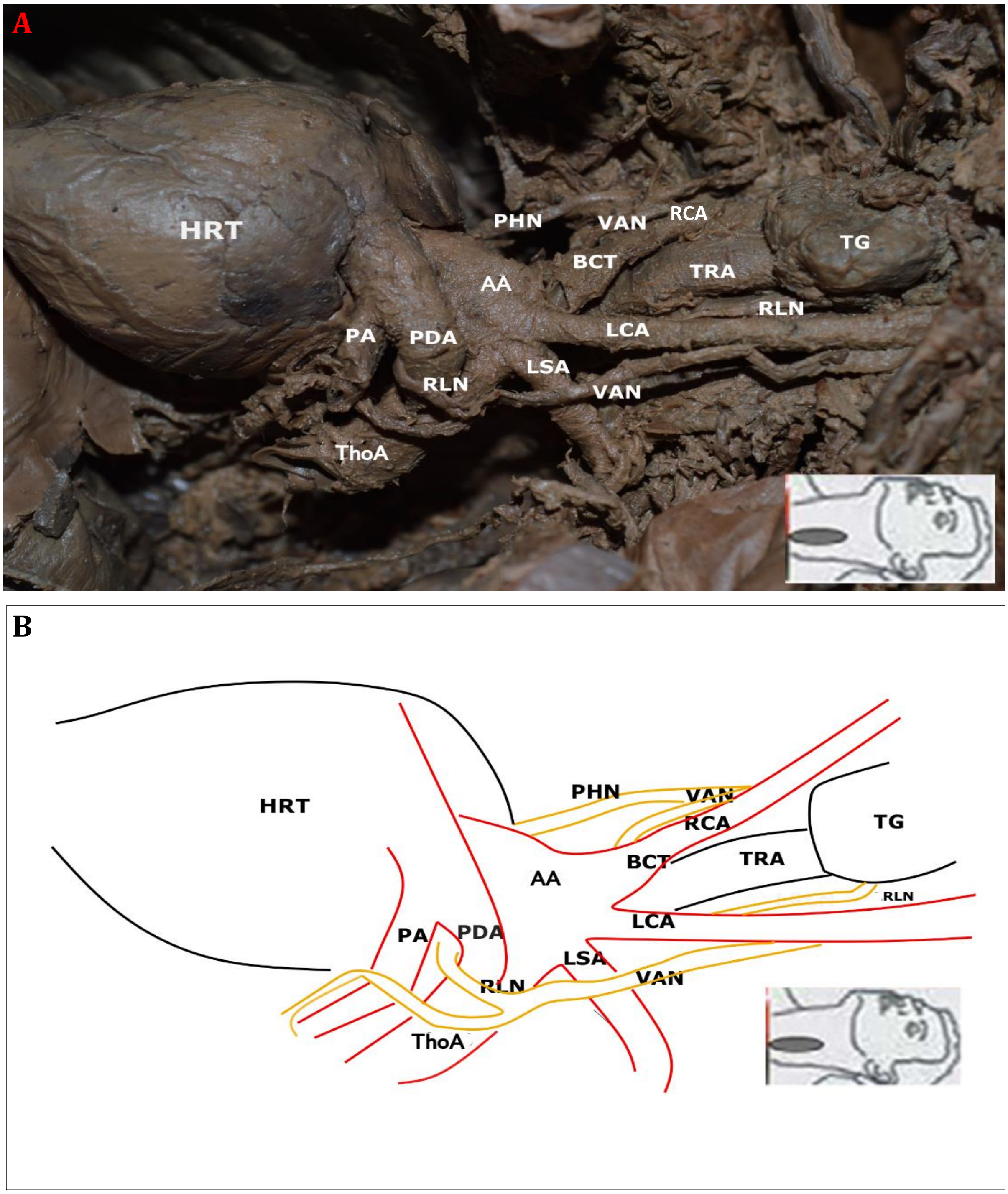


Figure 30. The diagrams (**A, B**) show the variant pattern of the RLN recurring below a patent ductus arteriosus on the left side. **A**. Dissection photograph **B**. Line drawing; Abbreviations RLN, left recurrent laryngeal nerve; TG, thyroid gland; TRA, trachea ; HRT, heart; PA, pulmonary artery; PDA, patent ductus arteriosus; ThoA, Thoracic Aorta; LCA, left common

carotid artery ; LSA, left subclavian artery; BCT, brachiocephalic trunk; RCA, right common carotid artery; PHN, phrenic nerve; VAN, vagus nerve

4.2.4. The terminal branching of the RLN in the paediatric cadaver cohort

Extra-laryngeal branching of the RLN was observed in 35.5% (11/31) of the cases. In 6.45% (2/31) of these cases, the RLN showed bilateral bifurcation (that is on both the left and right side). Term babies were more likely to demonstrate terminal branching than pre-term infants ($p < 0.05$; value= 0.008266) as shown in **Table 14**.

4.3. Surgical candidates

4.3.1. Descriptive statistics

In the thyroidectomy adult cohort, 19 patients (38 RLNs on both left and right sides) were analysed from a total sample of 30 female patients with a mean age 49.76 ± 14.27 years (age range: 21 - 80 years) as shown in **Table 5**. The ancestry profile in the sample was (28/30) (93.3%) South African Blacks, (1/30) (3.3%) South African Whites and (1/30) (3.3%) Indians (**Table 4**). The reduction in the observable RLNs in this cohort was due to the unanticipated high rate of intraoperative difficulty visualisation rate of the RLN (**Figure 32 and 33**). Measurements of the diameter could not be done due to the absence of scale bar in the majority of sterilized operative sets. A sample of annotated pictures of one of the patients recruited in the study is seen in **Figure 34**.

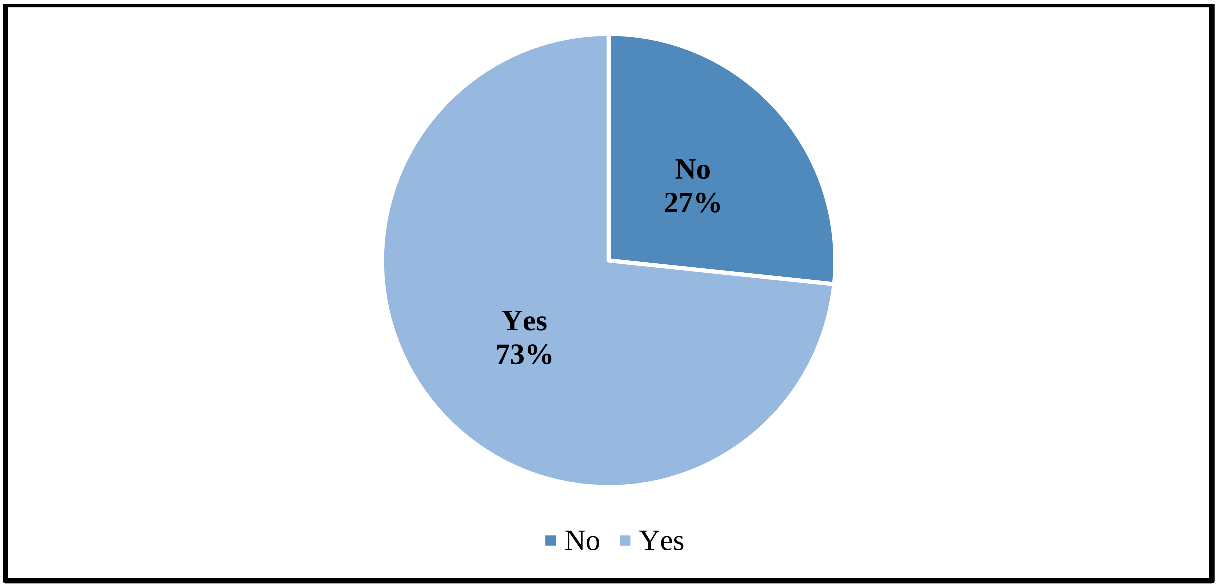


Figure 31. Pie chart showing the intraoperative visualization rate of the RLN in the adult surgical cohort.

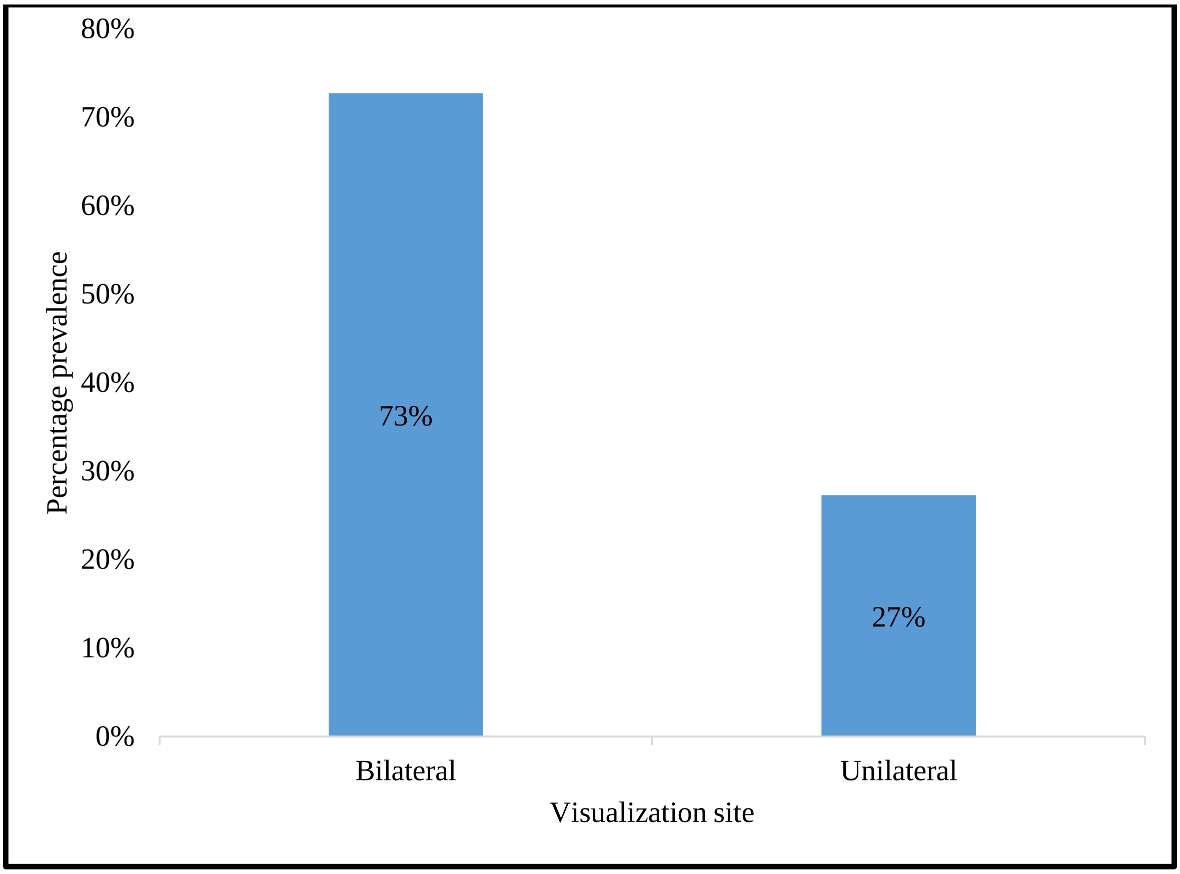


Figure 32. Bar chart showing the site of visualization of the recurrent laryngeal nerve

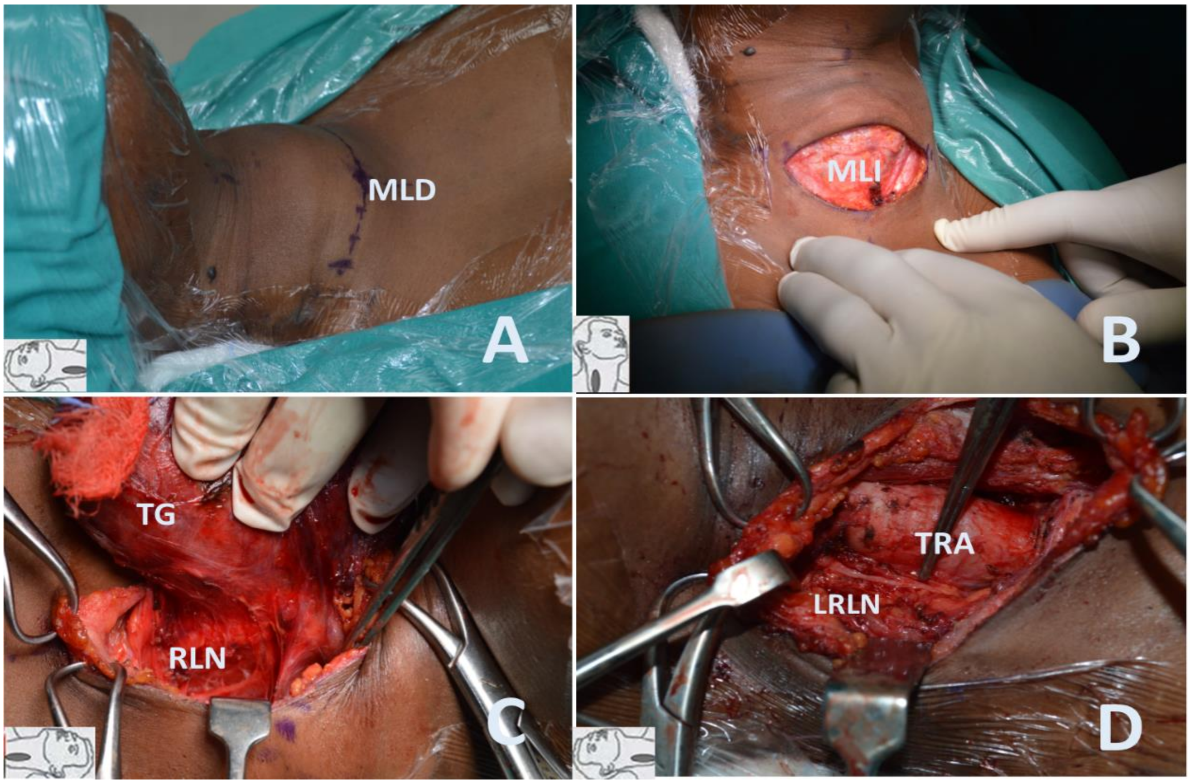


Figure 33. The photographs (**A,B,C,D**) depicts a summary of the steps intraoperatively to visualize and preserve the RLN. Abbreviations; **A.** MLD, midline demarcation; **B.** MLI, midline incision; **C**. TG, thyroid gland; RLN, recurrent laryngeal nerve; **D.** TRA, trachea; LRLN, left recurrent laryngeal nerve.

4.3.2. The relationship of the recurrent laryngeal nerve to the inferior thyroid artery in the surgical cohort

All the recurrent laryngeal nerves analysed in the surgical cohort followed a recurring course and no anomalously coursed nerves were observed. On the right side, the RLN was related to the ITA as follows: 21.0 % anterior, 78.9 % posterior and 0 % in-between the branches (**Figure 35**). On the left side, the RLN presented as follows: 21.0 % coursed anterior, 73.6 % posterior and 5.2 % in-between branches of the ITA (**Figure 36**). **Table 12** below shows proportions of the relationships of the RLN to the ITA stratified by sex and side. The relationship of the RLN to ITA on both left and right side did not show statistically significant side associations ($p \geq 0.05$; value = 0.5961).

Table 12. Summary of the relationship between the RLN and the ITA stratified by sex and side in adult surgical cohort.

Sex	**Side**	**Relationship or RLN to ITA (%)**		
		Anterior	In-between	Posterior
Male	Left	0.0	0.0	0.0
	Right	0.0	0.0	0.0
Female	Left	21.0	5.2	73.6
	Right	21.0	78.9	0.0

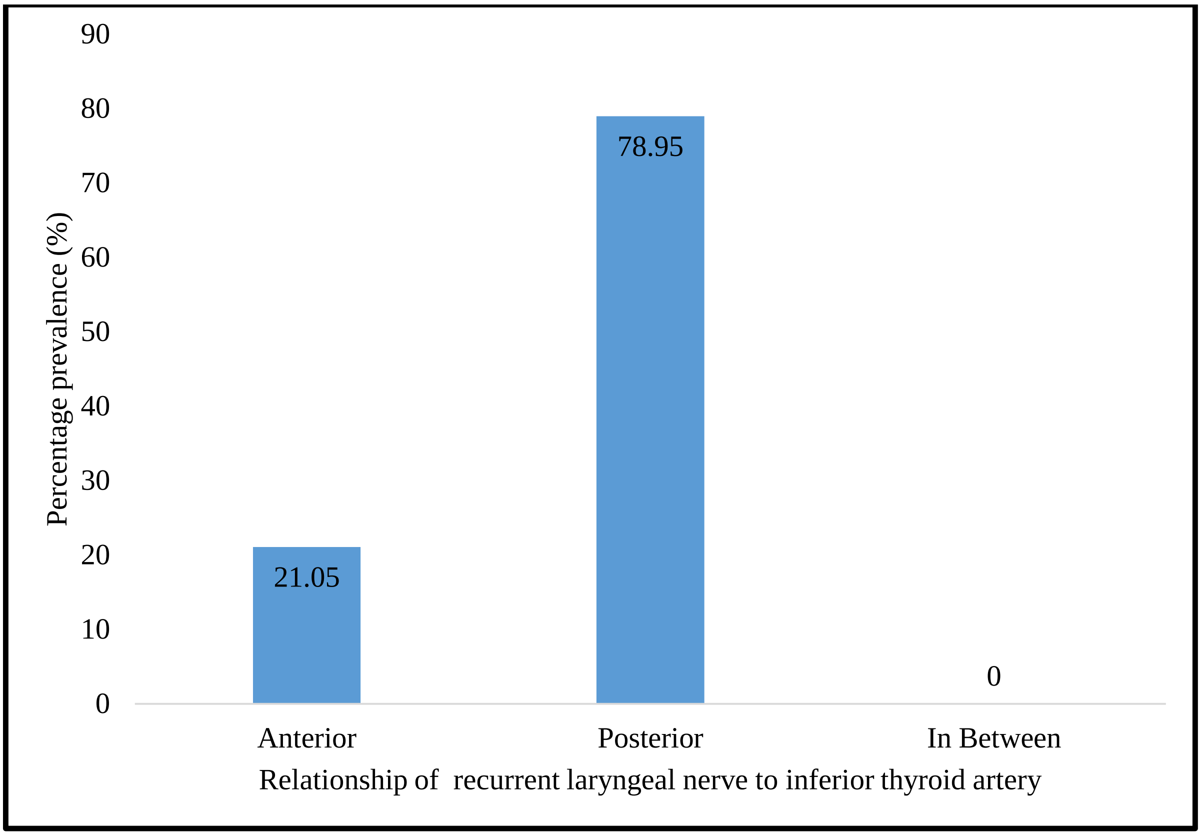


Figure 34. Bar Chart showing the relationship the right RLN and ITA in the adult surgical cohort

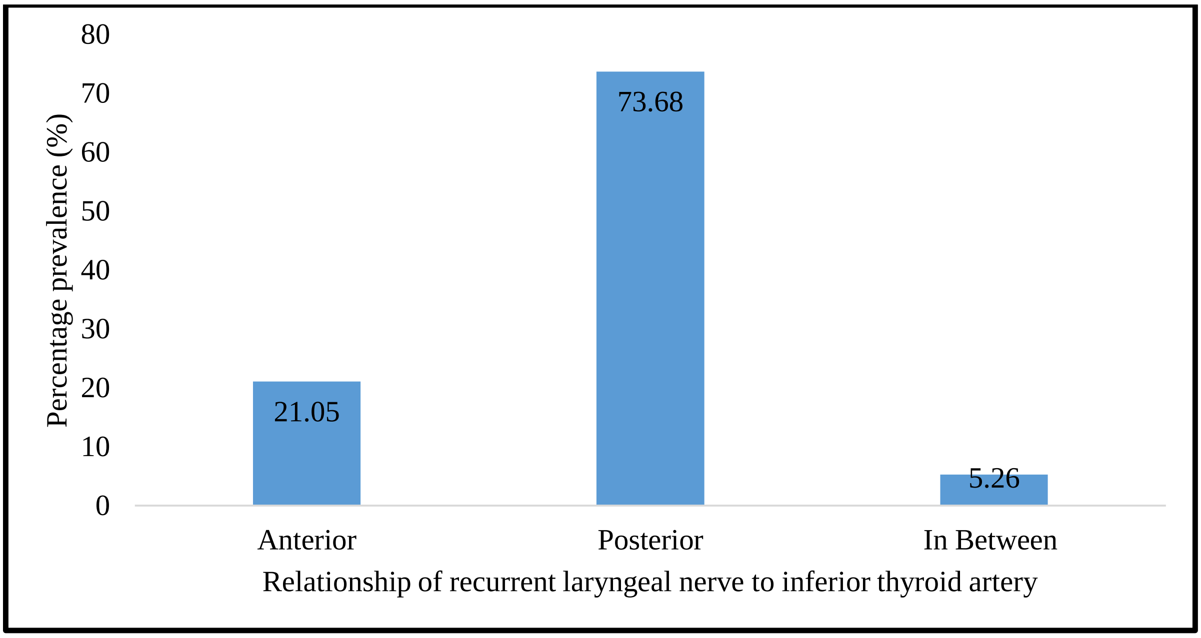


Figure 35. Bar Chart showing the relationship of the left RLN to ITA in the adult surgical cohort

4.3.3. The terminal branching of the nerve

The RLN gave extra-laryngeal branches in 9.52% of the cases on the left side and in 15% of the cases on the right side. No symmetrical branching was observed. The association between branching of the nerve and the side was not statistically significant ($p \geq 0.05$; value = 0.5922)

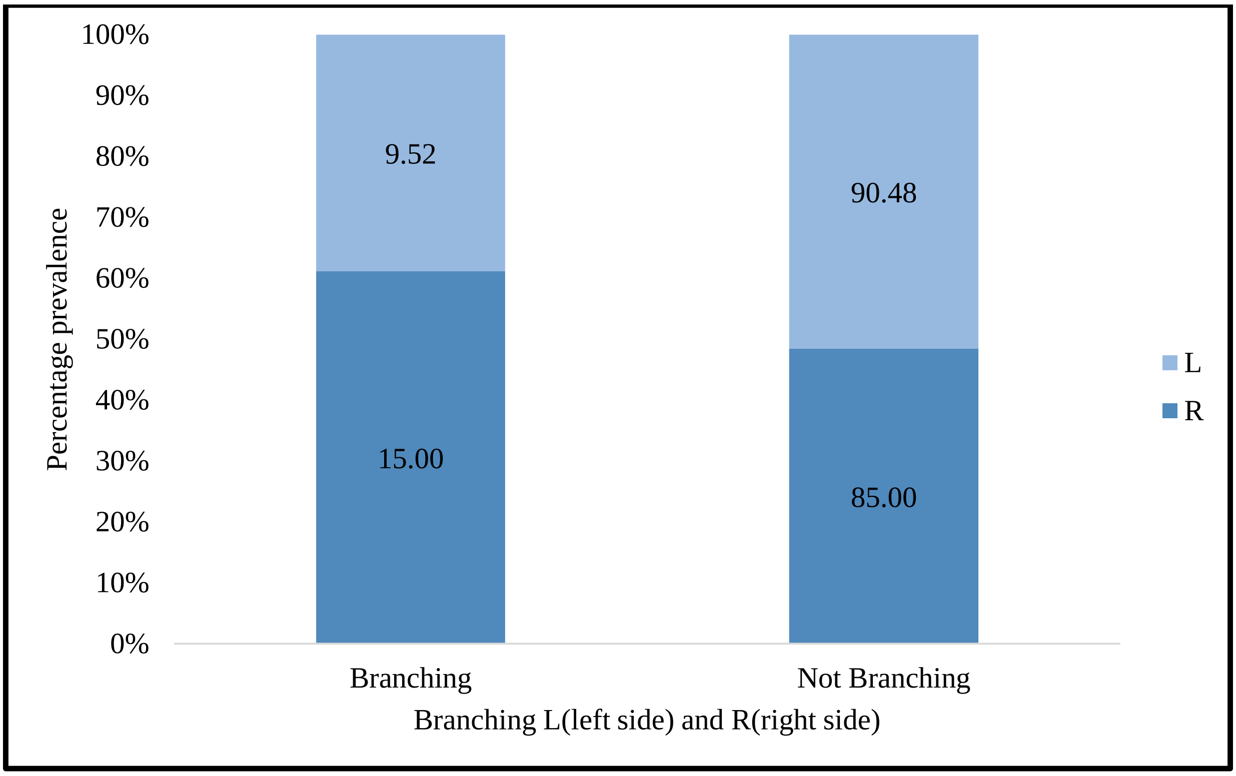


Figure 36. Bar chart showing the rate of branching of the recurrent laryngeal nerve on the left and the right side.

4.4. Comparative and inferential statistics across three studied cohorts

In the paediatric cadaver population, the combined mean diameters of both the left and right sides was 0.73 ± 0.32mm (range 0 - 1.73mm) while for the adult cadaver population it was 1.69 ± 0.53mm (range: 0 - 3.36mm). The Mann Whitney U test comparing diameters in paediatric and adult cadaveric sample groups was statistically significantly ($p \leq 0.05$; value = 0.0000). On the overall, most of the RLN irrespective of side, gender, age and population affinity, 40.3% coursed posteriorly, 38.7% anteriorly and 20.9% in-between the branches of the ITA. Similarities in the patterns of the RLN in relation to the ITA on both sides in all the three cohorts are presented below (**Table 13**).

Table 13. The presentation showing the relationship of the RLN and ITA in all three cohorts showing similarities in pattern on both sides.

	Anterior	In-between	Posterior	%	p-value
Adult (n=50)	2	6	3	22.0%	0.8075
Paediatric (n=31)	12	0	2	44.1%	
Surgical (n=19)	3	0	10	68.4%	

Table 14. Consolidated table of inferential statistics in adults, paediatrics and surgical study population

Description	p-value		
	Adults	Paeds	Surgery
Comparison of the relationship of the RLN to the ITA on the right and left sides	0.0013	0.1221	0.5961
Comparison the relationship of the RLN to the ITA in males and females	0.3733	0.1149	nil
Comparison of the diameter of the RLN on the right and left sides	0.3725	0.6157	nil
Comparison of course of the RLN in tracheoesgophageal groove on the right and left sides	0.0001	0.0170	nil
The association between RLN branching patterns and side	0.8388	nil	nil
T-test comparing mean diameter male versus female	0.8333	0.6674	Nil
Association between branching of RLN and gestational age	Nil	Nil	0.0083

Abbreviations: paeds: paediatric cadaver cohort

CHAPTER 5: DISCUSSION

5.1. Diameter of the RLN

In the current study, the mean diameter of the recurrent laryngeal nerve (RLN) in the paediatric cadaveric population was 0.74 ± 0.28mm and 0.72 ± 0.37mm on both the left and left respectively. On the other hand, in the adult cadaveric population, it was 1.75 ± 0.59mm (left) and 1.63 ± 0.47mm (right). Bargy et al. (1986) reported a mean diameter of 0.64 ± 0.116mm on a population of 12 term babies with average weight and standard deviation of 2775 ± 475 grams. In addition, the diameter of the RLN was found to positively correlate with birth weight and this would increase with increase in weight (Bargy *et al.,* 1986). In this study, the weight of the specimens was not obtained but the observed difference between mean diameters of the RLN in the paediatric cadaver cohort and adult cadaver cohort, we posit, may be associated with both age, and weight related changes. However, sex and sidedness did not influence the diameter of the RLN.

5.2. The relationship between the RLN and the tracheoesophageal groove (TEG)

In the adult cadaveric cohort, 40.8% of all the RLNs coursed in the TEG compared to 66% in the paediatric cadaveric cohort. These values are within the ranges (24.9 - 100%) reported by Ardito et al. (2004), Henry et al. (2016) and Henry et al. (2017b), Uen et al. (2006), Wiseman et al. (2004). The majority of RLNs that followed the course of the TEG were found on the left side (71.4% and 88.9% in the adult and paediatric cadaveric populations respectively) which was not consistent with the findings of Henry et al. (2017b) in a meta-analysis. The meta-analysis examined 5970 nerves and showed that there is a similarity on the proportions of RLN found in the TEG irrespective of side or ancestry (62.1% - right; 68.0% - left and on the overall 63.7%) (Henry *et al.,* 2017b). Symmetry, which refers to the proportion that will have an identical, bilateral course in the TEG in our study (adults 6% and paediatric 40.7%), is less compared to the pooled prevalence (61.1%) reported by (Henry *et al.,* 2017b) in the abovementioned meta-analysis. The TEG is therefore considered to be a less reliable landmark for the identification of the RLN due to its variable anatomy compared to other structures such as Berry's ligament (Henry *et al.,* 2017b).

In addition, most of the RLN on the left side in both adult (93.9%) and paediatric (96.3%) cadaveric populations coursed at angles less than 15° relative to the TEG. This is consistent with (Lee *et al.,* 2009) who demonstrated that 80% of RLNs on the left are likely to course at an angle relative to the TEG that is less than 30°. On the right side, the majority of the RLNs coursed at angles between 15 - 30° in both adult (87%) and peadiatric (76%) cadaveric populations. It has been shown that 50% of RLNs irrespective of side or gender will course between 15 - 30° and this proportion will increase to 78.6% if the nerve is sought in its course between 15 - 45° angle relative to the TEG (Lee *et al.,* 2009).

Table 15. The comparative analysis pooled data of the course the recurrent laryngeal nerve in or out of the TEG in various studied adult populations and this study.

	Meta-analysis[a] (adults)		Current study: Adults			Current study: Paediatric[b]		
Side	Inside (%)	Outside (%)	Side	Inside (%)	Outside (%)	Side	Inside (%)	Outside (%)
Right (1597 nerves)	62.1 (48.1-75.2)	37.9 (24.8-51.9)	Right	10.0	90.0	Right	42.0	58.0
Left (1554 nerves)	68.0 (56.4-78.6)	32.0 (21.4-43.6)	Left	71.0	29.0	Left	89.0	11.0
Overall (5970 nerves)	63.7 (55.3-77.7)	36.3 (28.3-44.7)	Overall	-		Overall	-	
Symmetry	61.1		Symmetry	6.0		Symmetry	40.7	

a. (Henry *et al.,* 2017b)

b. The paediatric data has been added here to provide a global view of results

Table 16. The comparative analysis pooled data of the course the recurrent laryngeal nerve in relation to the inferior thyroid artery.

	Meta-analysis[a] (adults)			**Current study: Adults**			**Current study: Paediatric[b]**			**Current study: Intraoperative**		
Side	Anterior	Posterior	In between	Anterior	Posterior	In between	Anterior	Posterior	In between	Anterior	Posterior	In between
Right	37.1	37.0	29.0	24.4	48.9	26.5	67.7	19.3	12.9	21.0	78.9	0
Left	17.2	62.6	20.2	39.5	14.5	45.83	56.6	40.0	3.3	21.0	73.7	5.3
Overall	27.6	50.7	21.7	32.0	32.0	36	62.3	29.5	0.082	21.1	76.3	2.6

a. (Henry *et al.,* 2017d): Overall (79 studies, 14269 nerves); right side (59 studies with 5617 nerves); left side (58 studies with 5455 nerves)

b. The paediatric data has been added here to provide a global view of results

5.3. The relationship between RLN and ITA

The relationship between ITA and RLN was initially classified by Reed (as cited by Henry *et al.,* 2017d) with 28 subtypes. Currently a more practical classification, which denotes the position of the RLN as either coursing anterior, posterior or in between the branches of the ITA is widely, used (Henry *et al.,* 2017a).

In the adult cadaveric cohort, the relationship of the RLN to ITA on the right side was as follows: anterior (24.4%), posterior (48.9%) and in-between (26.5%). On the left side, the RLN coursed anterior (56.6 %), posterior (40.0%) and least in-between (3.3%) the branches of the ITA. The current findings are inconsistent to the convention of numerous authors where the majority of RLNs were found to course either anterior to the ITA on the right side (37.1%) or posteriorly on the left side (62.6%) as shown in the **Table 16** adapted from results of a meta-analysis study (Henry *et al.,* 2017c).

In the paediatric cohort, on the right side the RLN coursed in relation to the ITA as follows: anterior (67.7%), posterior (19.3%) and in-between (12.9%). On the left side, the RLN course anterior (39.5 %), posterior (14.5%) and in-between (45.83%) in relation to the ITA. The right side's predominantly anterior course was consistent with the finding of Henry et al. (2017c) after analyzing 5617 nerves. However on the left side the RLN coursed most frequently in-between the branches of the ITA in the current study but in contrast to the majority of the previous studies (Ardito *et al.,* 2004; Ling and Smoll, 2016; Henry *et al.,* 2017c).

Nonetheless, in the surgical cohort 78.95% of RLNs unexpectedly coursed posterior to the ITA on the right and 73.68% on the left sides, which is in agreement with previous reports (Deveze *et al.,* 2003; Dolezel *et al.,* 2015; Henry *et al.,* 2017c; Lee *et al.,* 2009; Prades *et al.,* 2012; Sakamoto, 2013; Yalcin *et al.,* 2008). There was not any recognizable pattern and this is consistent with the fact that the anatomy of the RLN is highly variable (Henry *et al.,* 2017d; Sun *et al.,* 2001). Variations of the pattern of the RLN in cadaver and surgical populations reported herein may be due to ancestry differences since the majority of the cadavers were of European descent while the surgical cohort was mainly from the Africans. In the current study, the rate at which the relationship of the RLN and ITA was replicated bilaterally (i.e. symmetry) was 22%, 44.16% and 68.42% in the adult, paediatric and surgical cohort respectively. In a meta-analysis of 79 studies, symmetrical RLN / ITA relationships was observed in 36.6% of the cases (Henry *et al.,* 2017d).

Overall, in the current study most of the RLNs irrespective of side, gender, age and demography coursed in relation to the ITA as follows: posteriorly (40.3%); anteriorly (38.7%) and in-between (20.9%). This is consistent with most of the findings by various researchers if the demographics of subjects are not taken into consideration. Henry et al. (2017d) concluded that 50.7% of all RLNs assume a posterior course relative to the ITA. In addition, Ling and Smoll (2016) analyzing 32 studies with a total of 8655 nerves found that 50.95% of all RLN will course posterior to the ITA.

5.4. Prevalence of RLN postoperative palsy

In our study, postoperative RLN palsy was 0%. Temporary RLN injury varies from 0 to 8% whereas permanent injury ranges from 0 to 0.5% (Wiseman *et al.,* 2004). Ardito et al. (2004) reported similar permanent injury rates of the RLN of 0.7%. Chiang et al. (2010a) and Serpell (2010) in their studies report much higher ranges of permanent RLN injury rates (1 - 2%) and (0.3 - 3%) respectively. In the paediatric population undergoing corrective surgery for a patent ductus arteriosus, iatrogenic injury of the RLN can reach up to 11.5% (Pereira *et al.,* 2006). Generally, the prevalence of the postoperative injury to the RLN increases with time indicated by follow up studies on patients compared to immediate evaluations (Serpell, 2010). Follow up of patients in this study was not done to assess late onset complications associated with thyroidectomies. Other complications, which include hypocalcaemia and life-threatening bleeding, may contribute to possible causes of postoperative damage to the RLN (Dolezel *et al.,* 2015; Dralle *et al.,* 2008; Sun *et al.,* 2001).

The injury rate of the RLN in patients with no anatomical variation of the RLN is 2 - 5% (Casal *et al.,* 2010; Chiang *et al.,* 2010a; Chiang *et al.,* 2010b). This rate increases when the patients present with variations such as non-recurrent inferior laryngeal nerve NRILN or the RLN cannot be easily visualized and identified during surgery (Casal *et al.,* 2010; Deveze *et al.,* 2003; Dolezel *et al.,* 2015). In our study, we did not observe any NRILN. Previous reports show that prevalence of a NRILN on the right side is 0.32 - 1.6% and even rarer on the left side (0.004%) (Casal *et al.,* 2010; Chiang *et al.,* 2010a; Deveze *et al.,* 2003; Dolezel *et al.,* 2015; Toniato *et al.,* 2010). There were two cases of patients with an anomalous vertebral artery on the left, originating directly from the aortic arch.

The vertebral artery commonly originates from the posterior superior aspect of the first part of the subclavian artery on both sides (Yuan, 2016). Variation may occur at the origin of the vertebral artery and is seen to arise from the aortic arch, carotid arteries and rarely the descending thoracic aorta (Yuan, 2016).

If a persistent 8th cervical intersegmental artery fuses longitudinally with the first 7 cervical intersegmental arteries, the origin of the right vertebral artery no longer arises from the subclavian artery but from the proximal part of the descending thoracic aorta (Yuan, 2016). Furthermore, a duplication of the vertebral artery is a product of failure of fusion of two segmental metameric arteries and degeneration of the dorsal aorta in foetal life (Mukkannavar *et al.,* 2013; Meila *et al.,* 2012).

The RLN and Inferior thyroid artery (ITA) have a variable anatomy that are of clinical importance both general and endocrine surgeons (Casal *et al.,* 2010; Chiang *et al.,* 2010a). In the presence of poor haemostasis, neck pathology and poor surgical skills, the RLN can become very difficult to identify and thereby, compounding the rate of injury (Chiang *et al.,* 2010a; Dralle *et al.,* 2008).

5.5. Extra-laryngeal branching of the RLN

Sixty one percent of RLN in the adult cadaveric population compared to 29.03% in the paediatric cadaveric population demonstrated extra-laryngeal branching of the RLN. While, in the surgical cohort, the extra-laryngeal branching rate was 9.52%. This is within range (43 - 78%) of the adult prevalence of extra-laryngeal branching reported by Holt and Thompson (as cited by Sun et al. (2002). Ardito et al. (2004) after analyzing 2626 RLNs reported a similar bifurcation rate of 70.6% happening at 0.6 – 4.0cm distance prior to entering the larynx. Furthermore, Henry et al. (2016) in a meta-analysis of 79 studies looking at 14269 RLNs showed a 60% extra-laryngeal branching rate. It is generally accepted that the anterior branch of the RLN provide8s motor innervation whereas the posterior branch provides sensory innervation to the larynx (Chiang *et al.,* 2010a).

The point of branching is a vulnerable area for injury of the RLN (Chiang *et al.,* 2010a). In the current study, the maximum number of extra-laryngeal branches observed was four. Henry et al. (2016) reported that the terminal branching pattern of the RLN can be expected at about 2cm from the cricothyroid joint and number of terminal branches ranges from 1 - 6 branches. Similarly, Sun et al. (2002) after analyzing 100 cadaveric specimens demonstrated that most of the RLNs branch 10.13 ± 7.19mm (1cm) above the inferior pole of the thyroid gland. In addition, Uen et al. (2006) showed that the extra-laryngeal branching of the RLN can occur anywhere between 0.5 - 1.2cm (average 0.8cm) from the inferior horn of the thyroid cartilage Serpell et al. (2009) reported that there is more extra-laryngeal branching of the RLN on the right side than left side. In the same study, symmetrical bilateral bifurcation of the RLN was observed in 8.9% of 838 nerves examined (Serpell *et al.,* 2009). Henry et al. (2016) in a review, looking at 28387 nerves describes a wide range of extra-laryngeal branching of 5 - 100% with a symmetrical (bilateral) branching of 36.5%. Bifurcation, trifurcation and more than three branches were observed in 51.1%, 4.7% and 2.2% respectively (in a meta-analysis; Henry *et al.,* 2016). Prades et al. (2012) reported higher rates of extra-laryngeal branching of the RLN including bifurcation (88%), trifurcation (12%) and greater than 3 branches (0.2%).

In the current study, more extra-laryngeal branching of the RLN was observed in the adult specimens compared to the paediatric specimens. We hypothesize that disparity between the prevalence of extra-laryngeal branching the paediatric and adult cadaveric cohorts may associated with size of structures and the dissection technique that may make it difficult to observe the branching. The use of voice in a developing infant may also contribute to neuro-muscular development and may account for the size and the absence of extra-laryngeal branching. This notable difference is important information for paediatric endocrine or general surgery.

5.6. Visualization of the RLN during surgery and intraoperative nerve monitoring

In the surgical cohort the visualization rate of RLN was only 73% owing to the complete distortion of the regional anatomy of the neck due to neck masses. Visualization and identification of the RLN is advocated for by many authors and is considered as a standard of treatment to prevent RLN injury (Ardito *et al.,* 2004; Chiang *et al.,* 2010a; Ling and Smoll, 2016; Serpell *et al.,* 2009; Sun *et al.,* 2001; Uen *et al.,* 2006; Wiseman *et al.,* 2004). Although, it has been proven that visualization of the nerve is associated with a reduction in RLN injury, it is not always possible to see or dissect the nerve when resecting a locally invasive lesion in the thyroid gland (Casal *et al.,* 2010; Chiang *et al.,* 2010a). The remaining 27% of cases in the current study where RLN was not visualised did not show any clinical signs of RLN palsy postoperatively. Filaire et al. 2001 (as cited by Chiang *et al.,* 2010a) concedes that visualization of the RLN is not always possible even though it may be desired to prevent intraoperative injury.

Intraoperative neuro-monitoring (IONM) has been described as an important assistive intervention in cases of a difficult dissection of the RLN (Chiang *et al.,* 2010a; Sun *et al.,* 2001). In the current study, IONM was not consistently implemented due to the choice of approach by operating surgeons. Although, the use of IONM in reduction of RLN injury is debatable, its use can be valuable in identifying damaged nerves intraoperatively (Julien *et al.,* 2017; Périé *et al.,* 2013). Once intraoperative injury of the RLN on one side is demonstrated by IONM, the consensus is to abandon the surgical intervention to avoid bilateral iatrogenic injury (Périé *et al.,* 2013; Julien *et al.,* 2017). On the other hand, Ardito et al. (2004) argues that a thyroidectomy without routine visualization and identification of the RLN should be considered substandard care (Casal *et al.,* 2010; Dolezel *et al.,* 2015; Dralle *et al.,* 2008). Chiang et al., (2010) demonstrated that early intraoperative identification and visualization of the RLN can be achieved with the aid of IONM. Non-visualization and identification of the nerve in patients with anatomical variations of the RLN is more likely to compound the risk of nerve injury (Ardito *et al.,* 2004; Casal *et al.,* 2010; Dolezel *et al.,* 2015).

6.0. LIMITATIONS OF THE STUDY

Random sampling and stratification could not be achieved due to convenient sampling. Prior dissection on the specimens distorted the anatomy of some specimens. The number of paediatric specimens available for analysis were limited. Recruitment of patients undergoing thyroidectomy was limited by theatre time and the capacity of the intensive care unit. Adequate dissection of the recurrent laryngeal nerve intraoperative was not always feasible. Follow up was not done to assess late onset complications of the operative procedure. The intended stratification for age in the paediatric was not feasible due to the limited number of specimens in the paediatric collection used in this study.

7.0. CONCLUSION

The current study demonstrated the anatomy of the RLN varies in the same population and in different populations located in the same geographic area. Overall, irrespective of age, gender and side, the RLN courses posterior to the ITA in most cases. In addition, the majority of RLNs on left side coursed in the TEG while on the right side the nerve deviated from the TEG at an angle ranging between 0 - 30^{o}. Most of the RLNs demonstrated 2 or more extra-laryngeal branches bilaterally.

8.0. RECOMMENDATIONS

This study reiterates the importance of the anatomy of the RLN in the surgical management of the neck pathologies to avoid iatrogenic nerve injuries. It is therefore important to combine all the diagnostic modalities in order to visualize the RLN such as IONM and physical identification of the nerve during dissection. In addition, it is also recommended to identify the frequency of the extra-laryngeal branching of the RLN to reduce intraoperative injury. Above all, there is need to a consensus study to standardize and operationalize the terminology and branching patterns of the RLN to ensure consistence in the reporting of research findings on the various patterns of the RLN. For future studies, it is important to follow the extra-laryngeal branches to their final destinations to ascertain their importance in case of intra-operative injury.

REFERENCES

AKKARI, M., MAKEIEFF, M., JEANDEL, C., RAINGEARD, I., CARTIER, C., GARREL, R., GUERRIER, B., BLANCHET, C. & MONDAIN, M. 2014. Thyroid surgery in children and adolescents: a series of 65 cases. *Eur Ann Otorhinolaryngol Head Neck Dis,* 131**,** 293-7.

ARDITO, G., REVELLI, L., D'ALATRI, L., LERRO, V., GUIDI, M. L. & ARDITO, F. 2004. Revisited anatomy of the recurrent laryngeal nerves. *Am J Surg,* 187**,** 249-53.

BARGY, F., HOUETTE, A. & BARBET, P. 1986. The left recurrent laryngeal nerve at birth: anatomy and surgical applicationsLe nerf récurrent gauche du nouveau-né. Anatomie et applications chirurgicales. *Surgical and Radiologic Anatomy,* 8**,** 245-250.

BOJUNGA, J. 2018. Ultrasound of thyroid nodules. *Ultraschall Med*.

BOMBIL, I., BENTLEY, A., KRUGER, D. & LUVHENGO, T. 2014. Incidental cancer in multinodular goitre post thyroidectomy. *South African Journal of Surgery,* 52**,** 5-9.

CALO, P. G., MEDAS, F., ERDAS, E., PITTAU, M. R., DEMONTIS, R., PISANO, G. & NICOLOSI, A. 2014. Role of intraoperative neuromonitoring of recurrent laryngeal nerves in the outcomes of surgery for thyroid cancer. *International Journal of Surgery,* 12**,** S213-S217.

CALO, P. G., MEDAS, F., GORDINI, L., PODDA, F., ERDAS, E., PISANO, G. & NICOLOSI, A. 2016. Interpretation of intraoperative recurrent laryngeal nerve monitoring signals: The importance of a correct standardization. *International Journal of Surgery,* 28**,** S54-S58.

CASAL, D., PECAS, A., SOUSA, D. & ROSA-SANTOS, J. 2010. A non-recurrent inferior laryngeal nerve in a man undergoing thyroidectomy: a case report. *J Med Case Rep,* 4**,** 386.

CERNEA, C. R., HOJAIJ, F. C., DE CARLUCCI, D., JR., GOTODA, R., PLOPPER, C., VANDERLEI, F. & BRANDAO, L. G. 2009. Recurrent laryngeal nerve: a plexus rather than a nerve? *Arch Otolaryngol Head Neck Surg,* 135**,** 1098-102.

CHANG, S., ZHOU, L., LI, J., HUANG, Y., ZENG, Q., HE, F. & WANG, Z. 2010. [Identification techniques of the recurrent laryngeal nerve in endoscopic thyroidectomy]. *Zhong Nan Da Xue Xue Bao Yi Xue Ban,* 35**,** 377-80.

CHIANG, F. Y., LU, I. C., CHEN, H. C., CHEN, H. Y., TSAI, C. J., HSIAO, P. J., LEE, K. W. & WU, C. W. 2010a. Anatomical variations of recurrent laryngeal nerve during thyroid surgery: how to identify and handle the variations with intraoperative neuromonitoring. *Kaohsiung J Med Sci,* 26**,** 575-83.

CHIANG, F. Y., LU, I. C., CHEN, H. C., CHEN, H. Y., TSAI, C. J., LEE, K. W., HSIAO, P. J. & WU, C. W. 2010b. Intraoperative neuromonitoring for early localization and identification of recurrent laryngeal nerve during thyroid surgery. *Kaohsiung J Med Sci,* 26**,** 633-9.

CHIANG, F. Y., LU, I. C., KUO, W. R., LEE, K. W., CHANG, N. C. & WU, C. W. 2008. The mechanism of recurrent laryngeal nerve injury during thyroid surgery--the application of intraoperative neuromonitoring. *Surgery,* 143**,** 743-9.

CHIANG, F. Y., WANG, L. F., HUANG, Y. F., LEE, K. W. & KUO, W. R. 2005. Recurrent laryngeal nerve palsy after thyroidectomy with routine identification of the recurrent laryngeal nerve. *Surgery,* 137**,** 342-7.

CITTON, M., VIEL, G. & IACOBONE, M. 2016. Neck ultrasonography for detection of non-recurrent laryngeal nerve. *Gland surgery,* 5**,** 583.

DEVEZE, A., SEBAG, F., HUBBARD, J., JAUNAY, M., MAWEJA, S. & HENRY, J. F. 2003. Identification of patients with a non-recurrent inferior laryngeal nerve by duplex ultrasound of the brachiocephalic artery. *Surg Radiol Anat,* 25**,** 263-9.

DIONIGI, G., DONATINI, G., BONI, L., RAUSEI, S., ROVERA, F., TANDA, M. L., KIM, H. Y., CHIANG, F.-Y., WU, C.-W. & MANGANO, A. 2013. Continuous monitoring of the recurrent laryngeal nerve in thyroid surgery: a critical appraisal. *International Journal of Surgery,* 11**,** S44-S46.

DOLEZEL, R., JAROSEK, J., HANA, L. & RYSKA, M. 2015. Clinical relevance and surgical anatomy of non-recurrent laryngeal nerve: 7 year experience. *Surg Radiol Anat,* 37**,** 321-5.

DRALLE, H., SEKULLA, C., HAERTING, J., TIMMERMANN, W., NEUMANN, H. J., KRUSE, E., GROND, S., MUHLIG, H. P., RICHTER, C., VOSS, J., THOMUSCH, O., LIPPERT, H., GASTINGER, I., BRAUCKHOFF, M. & GIMM, O. 2004. Risk factors of paralysis and functional outcome after recurrent laryngeal nerve monitoring in thyroid surgery. *Surgery,* 136**,** 1310-22.

DRALLE, H., SEKULLA, C., LORENZ, K., BRAUCKHOFF, M., MACHENS, A. & GERMAN, I. S. G. 2008. Intraoperative monitoring of the recurrent laryngeal nerve in thyroid surgery. *World J Surg,* 32**,** 1358-66.

FEROCI, F., RETTORI, M., BORRELLI, A., COPPOLA, A., CASTAGNOLI, A., PERIGLI, G., CIANCHI, F. & SCATIZZI, M. 2014. A systematic review and meta-analysis of total thyroidectomy versus bilateral subtotal thyroidectomy for Graves' disease. *Surgery,* 155**,** 529-540.

FRESCHI, G., MASI, C., PICHI, M. G., PACIFICO, G., PANCONESI, R., CORSI, A. & SEGHI, P. 1994. Anatomic and surgical considerations regarding the recurrent laryngeal nerve in thyroidectomy. *Minerva chirurgica,* 49**,** 943-947.

GAO, E.-L., ZOU, X., ZHOU, Y.-H., XIE, D.-H., LAN, J. & GUAN, H.-G. 2014. Increased prediction of right nonrecurrent laryngeal nerve in thyroid surgery using preoperative computed tomography with intraoperative neuromonitoring identification. *World journal of surgical oncology,* 12**,** 262.

HENRY, B. M., PEKALA, P. A., SANNA, B., VIKSE, J., SANNA, S., SAGANIAK, K., TOMASZEWSKA, I. M., TUBBS, R. S. & TOMASZEWSKI, K. A. 2017a. The Anastomoses of the Recurrent Laryngeal Nerve in the Larynx: A Meta-Analysis and Systematic Review. *J Voice,* 31**,** 495-503.

HENRY, B. M., SANNA, B., GRAVES, M. J., SANNA, S., VIKSE, J., TOMASZEWSKA, I. M., TUBBS, R. S. & TOMASZEWSKI, K. A. 2017b. The Reliability of the Tracheoesophageal Groove and the Ligament of Berry as Landmarks for Identifying the Recurrent Laryngeal Nerve: A Cadaveric Study and Meta-Analysis. *Biomed Res Int,* 2017**,** 4357591.

HENRY, B. M., VIKSE, J., GRAVES, M. J., SANNA, S., SANNA, B., TOMASZEWSKA, I. M., HSIEH, W. C., TUBBS, R. S. & TOMASZEWSKI, K. A. 2017c. Variable relationship of the recurrent laryngeal nerve to the inferior thyroid artery: A meta-analysis and surgical implications. *Head Neck,* 39**,** 177-186.

HENRY, B. M., VIKSE, J., GRAVES, M. J., SANNA, S., SANNA, B., TOMASZEWSKA, I. M., HSIEH, W. C., TUBBS, R. S. & TOMASZEWSKI, K. A. 2017d. Variable relationship of the recurrent laryngeal nerve to the inferior thyroid artery: A meta-analysis and surgical implications. *Head & neck,* 39**,** 177-186.

HENRY, B. M., VIKSE, J., GRAVES, M. J., SANNA, S., SANNA, B., TOMASZEWSKA, I. M., TUBBS, R. S. & TOMASZEWSKI, K. A. 2016. Extralaryngeal branching of the recurrent laryngeal nerve: a meta-analysis of 28,387 nerves. *Langenbecks Arch Surg,* 401**,** 913-923.

JAN, I.-S., LEE, Y.-T., WANG, C.-M., CHENG, T.-Y., WANG, C.-Y., CHANG, T.-C. & SHIH, S.-R. 2019. The surgery and repeat aspiration outcomes of the atypia of undetermined significance/follicular lesion of undetermined significance category in

The Bethesda System for Reporting Thyroid Cytopathology. *Asian journal of surgery,* 42**,** 144-147.

JULIEN, N., FERRARY, E., SOKOLOFF, A., LAMAS, G., STERKERS, O. & BERNARDESCHI, D. 2017. Vagal and recurrent laryngeal nerves neuromonitoring during thyroidectomy and parathyroidectomy: a prospective study. *European annals of otorhinolaryngology, head and neck diseases,* 134**,** 77-82.

KIESER, J. & ALLAN, J. 2001. Practical Anatomy. Johannesburg: Witwatersrand University Press.

KONSCHAKE, M., ZWIERZINA, M. E., PECHRIGGL, E. J., MORIGGL, B., BRENNER, E., HÖRMANN, R. & PROMMEGGER, R. 2016. The nonrecurrent laryngeal nerve: a clinical anatomic mapping with regard to intraoperative neuromonitoring. Surgery, 160, 161-168.

LEDBETTER, D. J. 2014. Thyroid surgery in children. *Seminars in Pediatric Surgery,* 23**,** 60-65.

LEE, M. S., LEE, U. Y., LEE, J. H. & HAN, S. H. 2009. Relative direction and position of recurrent laryngeal nerve for anatomical configuration. *Surg Radiol Anat,* 31**,** 649-55.

LING, X. Y. & SMOLL, N. R. 2016. A systematic review of variations of the recurrent laryngeal nerve. *Clin Anat,* 29**,** 104-10.

MEILA, D., TYSIAC, M., PETERSEN, M., THEISEN, O., WETTER, A., MANGOLD, A., SCHLUNZ-HENDANN, M., PAPKE, K., BRASSEL, F. & BERENSTEIN, A. 2012. Origin and course of the extracranial vertebral artery: CTA findings and embryologic considerations. *Clinical neuroradiology,* 22**,** 327-333.

MORAIS, M., CAPELA-COSTA, J., MATOS-LIMA, L. & COSTA-MAIA, J. 2015. Nonrecurrent laryngeal nerve and associated anatomical variations: the art of prediction. *European thyroid journal,* 4**,** 234-238.

MUKKANNAVAR, S. B., KUTHE, S. A., MISHRA, A. K. & ROHIT, M. K. 2013. Aberrant right vertebral artery from descending thoracic aorta. *The Annals of thoracic surgery,* 96**,** 1074-1076.

NEMIROFF, P. M. & KATZ, A. D. 1982. Extralaryngeal divisions of the recurrent laryngeal nerve. Surgical and clinical significance. *Am J Surg,* 144**,** 466-9.

NGUYEN, M., JUNIEN-LAVILLAUROY, C. & FAURE, C. 1989. Anatomical intra-laryngeal anterior branch study of the recurrent (inferior) laryngeal nerve. *Surg Radiol Anat,* 11**,** 123-7.

NYEKI, A.-R. N., NJOCK, L.-R., MILOUNDJA, J., VOKWELY, J.-E. E. & BENGONO, G. 2015. Recurrent laryngeal nerve landmarks during thyroidectomy. *European annals of otorhinolaryngology, head and neck diseases,* 132**,** 265-269.

PEREIRA, K. D., WEBB, B. D., BLAKELY, M. L., COX JR, C. S. & LALLY, K. P. 2006. Sequelae of recurrent laryngeal nerve injury after patent ductus arteriosus ligation. *International journal of pediatric otorhinolaryngology,* 70**,** 1609-1612.

PÉRIÉ, S., AÏT-MANSOUR, A., DEVOS, M., SONJI, G., BAUJAT, B. & ST GUILY, J. 2013. Value of recurrent laryngeal nerve monitoring in the operative strategy during total thyroidectomy and parathyroidectomy. *European annals of otorhinolaryngology, head and neck diseases,* 130**,** 131-136.

PRADES, J. M., DUBOIS, M. D., DUMOLLARD, J. M., TORDELLA, L., RIGAIL, J., TIMOSHENKO, A. P. & PEOC'H, M. 2012. Morphological and functional asymmetry of the human recurrent laryngeal nerve. *Surg Radiol Anat,* 34**,** 903-8.

PRADES, J. M., GAVID, M., DUBOIS, M. D., DUMOLLARD, J. M., TIMOSHENKO, A. T. & PEOC'H, M. 2015. Surgical anatomy of the ansa cervicalis nerve: which branch to use for laryngeal reinnervation in humans? *Surg Radiol Anat,* 37**,** 139-45.

REED, A. F. 1943. The relations of the inferior laryngeal nerve to the inferior thyroid artery. *The Anatomical Record,* 85**,** 17-23.

SAKAMOTO, Y. 2013. Interrelationships between the innervations from the laryngeal nerves and the pharyngeal plexus to the inferior pharyngeal constrictor. *Surg Radiol Anat,* 35**,** 721-8.

SCHNEIDER, R., RANDOLPH, G. W., BARCZYNSKI, M., DIONIGI, G., WU, C. W., CHIANG, F. Y., MACHENS, A., KAMANI, D. & DRALLE, H. 2016. Continuous intraoperative neural monitoring of the recurrent nerves in thyroid surgery: a quantum leap in technology. *Gland Surg,* 5**,** 607-616.

SERPELL, J. W. 2010. New operative surgical concept of two fascial layers enveloping the recurrent laryngeal nerve. *Ann Surg Oncol,* 17**,** 1628-36.

SERPELL, J. W., YEUNG, M. J. & GRODSKI, S. 2009. The motor fibers of the recurrent laryngeal nerve are located in the anterior extralaryngeal branch. *Annals of surgery,* 249**,** 648-652.

SIERRA, M. S., SOERJOMATARAM, I. & FORMAN, D. 2016. Thyroid cancer burden in Central and South America. *Cancer epidemiology,* 44**,** S150-S157.

SUN, S.-Q., ZHAO, J., LU, H., HE, G.-Q., RAN, J.-H. & PENG, X.-H. 2002. An anatomical study of the recurrent laryngeal nerve: its branching patterns and relationship to the inferior thyroid artery. *Surgical and Radiologic Anatomy,* 23**,** 363-369.

SUN, S. Q., ZHAO, J., LU, H., HE, G. Q., RAN, J. H. & PENG, X. H. 2001. An anatomical study of the recurrent laryngeal nerve: its branching patterns and relationship to the inferior thyroid artery. *Surg Radiol Anat,* 23**,** 363-9.

TONIATO, A., MERANTE BOSCHIN, I., PAGETTA, C., CASALIDE, E. & PELIZZO, M. 2010. A "pilot light" of the right non-recurrent laryngeal nerve. *Acta Otorhinolaryngol Ital,* 30**,** 107-9.

UEN, Y.-H., CHEN, T.-H., SHYU, J.-F., SHYR, Y.-M., SU, C.-H., CHEN, J.-Y., LEE, C.-S. & LIU, J.-C. 2006. Surgical anatomy of the recurrent laryngeal nerves and its clinical applications in Chinese adults. *Surgery today,* 36**,** 312-315.

WAGNER, H. E. & SEILER, C. 1994. Recurrent laryngeal nerve palsy after thyroid gland surgery. *Br J Surg,* 81**,** 226-8.

WANG, Z., ZHANG, H., ZHANG, P., HE, L. & DONG, W. 2014. Preoperative diagnosis and intraoperative protection of nonrecurrent laryngeal nerve: a review of 5 cases. *Medical science monitor: international medical journal of experimental and clinical research,* 20**,** 233.

WHITE, W. M., RANDOLPH, G. W., HARTNICK, C. J. & CUNNINGHAM, M. J. 2009. Recurrent laryngeal nerve monitoring during thyroidectomy and related cervical procedures in the pediatric population. *Arch Otolaryngol Head Neck Surg,* 135**,** 88-94.

WISEMAN, S. M., TOMLJANOVICH, P. I. & RIGUAL, N. R. 2004. Thyroid lobectomy: operative anatomy, technique, and morbidity. *Operative Techniques in Otolaryngology-Head and Neck Surgery,* 15**,** 210-219.

YALCIN, B., TUNALI, S. & OZAN, H. 2008. Extralaryngeal division of the recurrent laryngeal nerve: a new description for the inferior laryngeal nerve. *Surg Radiol Anat,* 30**,** 215-20.

YUAN, S.-M. 2016. Aberrant origin of vertebral artery and its clinical implications. *Brazilian journal of cardiovascular surgery,* 31**,** 52-59.

APPENDIX A

R14/49 Dr JBL Kiluba

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M160664

NAME: **(Principal Investigator)**	Dr JBL Kiluba
DEPARTMENT:	Anatomical Sciences Medical School
PROJECT TITLE:	A topical and surgical anatomical description of the recurrent laryngeal nerve in adult and paediatric populations of South Africa
DATE CONSIDERED:	24/06/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Professor TE Luvhengo
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	22/11/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially reviewed in **June** and will therefore be due in the month of **June** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX B

School of Anatomical Sciences

University of the Witwatersrand, Johannesburg
7 York Rd, Parktown, 2193, South Africa • Tel: +27 11 717 2713 • Fax: +27 86 589 7863 • www.wits.ac.za

SCHOOL OF ANATOMICAL SCIENCES ETHICS WAIVER CLEARANCE LETTER

Faculty of Health Sciences
School of Anatomical Sciences
University of the Witwatersrand
Johannesburg

Re: In terms of Chapter 8, sections 62-64 of the National Health Act No 61 of 2003 donated bodies and their tissues may be used for, among other purposes, health and research. Use of such Material is subject only to permission from the responsible person in the School of Anatomical Sciences – the Head or person designed by the Head.

Human Research Ethics Committee (Medical) Clearance Certificate:
W-CJ-140604-1

This letter serves to confirm that the **Head of School**, based in the School of Anatomical Sciences, Faculty of Health Sciences, has reviewed the research proposal entitled:

A topographical and surgical anatomical description of the recurrent laryngeal nerve in adult and paediatric populations of South Africa

and has granted clearance to access the blanket ethics waiver to conduct the abovementioned research study.

…………………………………………………………… **Dated** : **3rd June 2019**

Professor Maryna Steyn
Head of School
School of Anatomical Sciences
Health Sciences Faculty

APPENDIX C

Human Research Ethics Committee (Medical)

Research Office Secretariat: Senate House Room SH 10005, 10th floor. Tel +27 (0)11-717-1252
Medical School Secretariat: Medical School Room 10M07, 10th Floor. Tel +27 (0)11-717-2700
Private Bag 3, Wits 2050, www.wits.ac.za. Fax +27 (0)11-717-1265

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

Ref: W-CJ-140604-1 25/01/2016

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator: School of Anatomical Sciences (Head: Prof M Steyn - Previously Prof T J M Daly, initial approval 04/06/2014 – recertified 27/01/2016).

Project title: Research on Cadaveric Material.

Reason: In terms of Chapter 8, sections 62-64 of the National Health Act No 61 of 2003 donated bodies and their tissues may be used for, among other purposes, health, and research. Use of such Material is subject only to permission from the responsible person in the School of Anatomical Sciences – the Head or person designed by the Head.

Professor Peter Cleaton-Jones

Chair: Human Research Ethics Committee (Medical)

UNIVERSITY OF THE WITWATERSRAND OFFICE OF THE DEPUTY VICE CHANCELLOR RESEARCH 29 JAN 2016 RESEARCH OFFICE HREC (Medical) JOHANNESBURG

Copy - HREC (Medical) Secretariat: Rhulani Mkansi, Zanele Ndlovu.

APPENDIX D

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences
Department of Anatomy

30.08.2018

RE: Permission to use cadavers within the Department of Anatomy, University of Pretoria

To Whom It May Concern

Dr Joshua Kiluba is a colleague from the University of the Witwatersrand and is conducting collaborative research within the Department of Anatomy, University of Pretoria. This study forms part of his MSc in Medicine thesis titled; *A topographical and surgical anatomical description of the recurrent laryngeal nerve in adult and paediatric populations in South Africa*.

Dr Kiluba already has permission to conduct this study from the University of the Witwatersrand postgraduate research committees (see associated acceptance letters) and has presented his protocol to the Department of Anatomy for review. Please accept this letter as both permission for Dr Kiluba to access the paediatric cadavers stored within the Department of Anatomy, University of Pretoria (under the rules and regulations set in the South African National Health Act, 61 of 2003) as part of his research study, as well as approval from the departmental research committee.

Should you have any queries, you are welcome to contact me directly.

Kind regards

Prof. Albert-Neels van Schoor
ASSOCIATE PROFESSOR
Section Head: Clinical Anatomy
Department of Anatomy

Room 4-9, Level 4, Basic Medical Science Building
University of Pretoria, Private Bag X323
Arcadia 0007, South Africa
Tel +27 (0)12 319 2315
Fax +27 (0)12 319 2240
Email albert.vanschoor@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Departement Anatomie
Lefapha la Disaense tša Maphelo
Kgoro ya Anatomi