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Clinical Implications of Vocal Cord-Carina Distance and Tracheal Length in the Indian Population

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Abstract

Objective: Securing the tracheal tube (TT) at a fixed recommended depth of 21/23 cm in female and male patients, respectively, may result in inappropriate placement of the TT in some patients. The aim of the present study was to determine the vocal cord-carina distance (VCD) and tracheal length (TL) to ascertain the optimal depth of TT placement during orotracheal intubation in the adult Indian population.

Methods: A total of 92 adults undergoing elective surgery under general anaesthesia with orotracheal intubation were studied. Surface anatomy airway measurements were noted. A cuffed TT (female size 7 mm ID and male size 8 mm ID) was inserted with the intubation guide mark at level with the vocal cords (VCs). Fiberoptic bronchoscopy-guided measurements were obtained for VCD, TL, TT tip-carina distance, VC-cricoid distance and lip-carina (L-C) distance.

Results: The mean±SD VCD was 12.82±2.05 and 12.02±1.44 cm, and TL was 10.14±2.04 and 9.37±1.28 cm in male and female patients, respectively. Statistically significant differences were observed between male and female patients in VCD ($p=0.033$), TL ($p=0.032$), L-C distance ($p<0.001$) and lip-TT tip distance ($p<0.001$); lip-TT tip distance was 19.50±1.39 cm in male patients and 18.17±1.28 cm in female patients. The L-C distance correlated with patient height, weight and neck length. L-C distance=7.214+0.049×Height+0.320×Neck length+0.033×Weight.

Conclusion: We recommend placing the TT with its proximal guide mark at the level of VCs in the Indian population. The 21/23 cm rule for tube placement depth in female and male patients, respectively, cannot be routinely followed in the Indian population.

Keywords: Fibreoptic bronchoscope, tracheal intubation, tracheal length, tracheal tube, vocal cord-carina distance

Introduction

Incorrect placement or inappropriate depth of tracheal tube (TT) placement during tracheal intubation is associated with major complications. Carinal impingement or endobronchial intubation may occur consequent to too deep a placement of the TT (1); conversely, inadequate depth of TT placement may result in vocal cord (VC) injury or inadvertent extubation (2). Physical parameters and airway distances could help in predicting tracheal length (TL). This could be useful in identifying patients with a short trachea and to ascertain the optimal depth of TT placement (maintaining safe distances between the TT tip and carina and also between the TT cuff and VCs).

Few studies have investigated the correct depth of placement of TT in the Indian population, but these have assessed limited parameters (3, 4). We hypothesised that physical parameters and airway distances affect the vocal cord-carina distance (VCD) and TL that, in turn, could affect the depth of TT placement. The aim of the present study was to determine the VCD, TL and optimal depth of TT placement during orotracheal intubation in the adult Indian population. The correlation of VCD and TL with various physical parameters and airway distances was also determined.

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Methods

This cross-sectional observational study was approved by the hospital ethics committee. A total of 92 adults aged 18-65 years with American Society of Anesthesiologists physical status I or II undergoing elective surgery under general anaesthesia with orotracheal intubation were included in the study. Patients with anticipated difficult intubation, modified Mallampati oropharyngeal view class 3/4, neck swelling or contracture, distorted tracheal anatomy, need for intubation with flexometallic or preformed tubes, bleeding diathesis and patients on anticoagulants or at risk of aspiration were excluded from the study. Written informed consent was obtained from each patient.

The following airway characteristics and distances (cm) were assessed preoperatively by one investigator (using a rigid ruler) to reduce inter-observer variability: thyromental distance measured as the straight distance from the thyroid notch to the inner bony mentum (head extended, mouth closed), sternomental distance measured as the straight distance from the upper border of the manubrium sterni to the inner bony mentum (head extended, mouth closed), upper incisor to the manubriosternal joint (UI-MSJ) distance (head extended), sternothyroid length (STL) measured as the straight distance from the sternal notch to the thyroid notch (neutral head position), sternal length (SL) measured as the straight distance between the sternal notch and the xiphisternum (neutral head position), thyrosternal length obtained by adding STL and SL, neck length (NL) measured as the straight distance from the mastoid process to the sternal head of the clavicle (neutral head position), horizontal mandibular length measured as the straight distance between the angle of the mandible and symphysis menti and range of head and neck movement < or >80° as described by Wilson et al. (5).

All patients fasted overnight and received oral alprazolam 0.25 mg or 0.5 mg (< or >50 kg body weight, respectively) the night before and the morning of surgery. In the operating room, standard monitoring was established (electrocardiogram, pulse oximetry, capnography and non-invasive blood pressure). Intravenous access was secured. The patient's head was placed in the sniffing position. The operating table height was adjusted such that the plane of the patient's face was at level with the anaesthesiologist's xiphisternum.

The anaesthetic protocol was standard. Anaesthesia was induced with fentanyl (2 μg kg^{-1}) and propofol (2-2.5 mg kg^{-1}) until loss of verbal contact. Tracheal intubation was facilitated by vecuronium 0.1 mg kg^{-1}. Lungs were ventilated with oxygen, nitrous oxide (50:50) and isoflurane 0.6% for 3 min. Laryngoscopy was performed using a Macintosh size 3 blade. The laryngoscopic view was graded by the Cormack-Lehane grading scale (6). External laryngeal manipulation, if required, was done. Patients with Cormack-Lehane grades 3 and 4 were excluded.

A cuffed TT (size 7 mm ID for female patients and size 8 mm ID for male patients) was inserted until the intubation guide mark was at level with the VCs. An assistant stabilised the patients' head and neck to avoid TT movement during laryngoscope removal. After confirming TT placement by five-point auscultation and capnography, the TT was secured at the right angle of the mouth using an adhesive tape. Lignocaine 2% (2 mL) was instilled into the TT. With the head in neutral position, a fiberoptic bronchoscope (FOB) was inserted through the TT via a port for FOB insertion in the swivel connector.

The FOB tip was inserted to the following depths: carina, TT tip, cricotracheal junction, intubation guide mark and lip. At each depth, an adherent marker was placed on the bronchoscope where it enters the swivel connector. Measurement readings were noted at end expiration by brief cessation of ventilation; in between measurements, the lungs were ventilated normally. Measurements from the FOB tip to each of the markers were noted. For determining the cricotracheal junction, the FOB was withdrawn until the TT intubation guide mark was just visible through the FOB. The FOB light was switched off, and a sharp light was placed externally at the cricotracheal membrane (the beginning of the proximal end of the trachea). The FOB depth (with its light "off") was adjusted until the extra-tracheal glow was best visible intratracheally (3).

The following airway distances/lengths were measured (cm): lip to carina (L-C), lip to TT tip (L-TT tip, depth of TT insertion), lip to cricotracheal membrane, lip to VC (lip to intubation guide mark placed at the level of VCs), VCD, TL, TT tip-carina distance and VC-cricoid distance.

Fifteen samples of TT sizes 7.0 and 8.0 mm ID were reviewed. The TTs were measured with the tube straightened for measurement along the line of the air channel for cuff inflation. The cuff was inflated to allow the clear identification of the proximal cuff edge. The distances between the proximal end of the tracheal cuff and intubation guide mark and TT tip were noted.

Surgery commenced after obtaining the measurements. Anaesthesia was maintained with oxygen in 66% nitrous oxide and 0.6% isoflurane with controlled ventilation. At the end of surgery, residual neuromuscular blockade was antagonised with glycopyrrolate (10 μg kg^{-1}) and neostigmine (50 μg kg^{-1}). All patients were observed for at least 24 h postoperatively for sore throat, hoarseness or other upper airway symptoms.

Statistical analysis

With reference to a previous study (3), it was found that patient factors correlated with various airway distances in male and female patients. For tests of association using bivariate correlations, a moderate correlation between VCD and various physical parameters and airway distances was considered to be significant. To detect a moderate correlation (r=0.30), a sample of 92 analysable subjects will provide 90% power to discover that the correlation is significantly different from there being no correlation (i.e. that the correlation would be zero) at the 0.05 level.

Statistical analysis was performed by the Statistical Package for the Social Sciences programme for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA). Continuous variables are presented as mean±SD, and categorical variables are presented as absolute number (n) and percentage (%). Unpaired t test was used to compare normally distributed continuous variables. Chi-square test or Fisher's exact test was used to analyse categorical variables. A univariate analysis was performed to identify the potential factors associated with L-C distance. Multivariate linear regression model has been used to identify the independent risk factors using a stepwise approach to enter new terms into the model, with a limit of $p<0.05$ to enter the terms. Linear regression analysis of data was used to show the best model for the calculation of the L-C distance from among the patient factors studied.

Table 1. Patient characteristics, Mallampati class and Cormack-Lehane laryngoscopic view

Parameters	Total cases (n=92)	Male (n=46)	Female (n=46)	p
Age (year)	34.92±10.37	34.15±11.08	35.70±9.67	0.478
Height (cm)	161.98±9.259	169.41±5.59	154.54±5.49	<0.001
Weight (kg)	59.02±11.16	60.80±11.48	57.24±10.66	0.126
Body mass index (kg m^{-2})	22.85±4.49	21.53±4.01	24.18±4.59	0.004
Mallampati class I:II	73:19	38:8	35:11	0.440
Cormack-Lehane grade 1:2	68:24	33:13	35:11	0.635

Values are expressed as mean±SD or numbers, as appropriate

Table 2. Surface anatomy airway measurements

Parameters	Total cases (n=92)	Male (n=46)	Female (n=46)	
Thyromental distance	6.8±0.859	7.13±0.81	6.46±0.86	<0.001
Sternomental distance	15.84±1.84	16.57±1.56	15.10±1.81	<0.001
Upper incisor-manubriosternal joint	22.49±2.25	23.68±2.01	21.30±1.81	<0.001
Sternothyroid length	7.00±1.52	7.87±1.35	6.13±1.13	<0.001
Sternal length	18.88±1.86	19.49±1.68	18.26±1.84	<0.001
Thyrosternal length	25.88±2.56	27.37±1.77	24.40±2.35	<0.001
Mandible length	9.28±0.73	9.49±0.47	9.07±0.88	0.005

Values are expressed as mean±SD

Table 3. Airway distances measured through the fiberoptic bronchoscope

Distances (cm)	Total cases (n=92)	Male (n=46)	Female (n=46)	
Lip to carina	22.15±1.82	22.97±1.54	21.32±1.72	<0.001
Lip to tube tip	18.83±1.49	19.50±1.39	18.17±1.28	<0.001
Lip to cricotracheal membrane	12.63±1.6	13.18±1.70	12.07±1.28	0.001
Lip to vocal cord	9.39±1.29	9.74±1.35	9.04±1.12	0.008
Vocal cord-carina	12.42±1.81	12.82±2.05	12.02±1.44	0.033
Tracheal length	9.76±1.73	10.14±2.04	9.37±1.28	0.032
Tube tip to carina	3.41±1.37	3.58±1.43	3.23±1.33	0.228
Vocal cord-cricoid	3.30±1.13	3.43±1.31	3.17±0.91	0.274

Results

A total of 92 patients were studied. Patient characteristics, Mallampati class and Cormack-Lehane grade are presented in Table 1. The surface anatomy airway measurements showed a statistically significant difference between male and female patients (all p<0.05) (Table 2). Airway distances measured through FOB are shown in Table 3. Statistically significant differences were observed between male and female patients in VCD (p=0.033), TL (p=0.032), L-C distance (p<0.001) and L-TT tip distance (p<0.001). The TT tip-carina distance and VC-cricoid distance were comparable be-

Table 4. Tracheal tube tip to carina distance

Tube tip to carina distance (cm)	Total (n=92)	Male (n=46)	Female (n=46)
TT tip-carina (cm)	3.41±1.369	3.58±1.43	3.23±1.33
TT tip-carina <3 cm, n (%)	37 (40.21)	14 (30.4)	23 (50)
RTT tip-carina (cm)	0.61±1.45	0.72±1.47	0.5±1.45
RTT tip-carina <3 cm, n (%)	86 (93.47)	41 (89.1)	45 (97.8)
RTT tip-carina (0 or negative), n (%)	43 (46.73)	20 (44.4)	23 (48.9)

Values are expressed as mean±SD or frequency (%), as appropriate. TT tip-carina, tracheal tube tip to carina distance; RTT tip-carina, tracheal tube tip to carina distance when the tracheal tube is fixed at the recommended 21 cm in female patients and 23 cm in male patients; RTT tip-carina 0, tracheal tube tip is at carina; RTT tip-carina negative, tracheal tube tip is endobronchial.

Table 5. Correlation of vocal cord to carina distance, tracheal length and lip to carina distance with patient characteristics and surface anatomy airway measurements

Parameters		Vocal cord to carina distance	Tracheal length	Lip to carina distance
Age	R	0.003	−0.069	−0.074
	p value	0.975	0.511	0.484
Sex (male:female)	R	−0.324**	−0.263*	−0.480**
	p value	0.002	0.011	<0.001
Height	R	0.372**	0.344**	0.557**
	p value	<0.001	0.001	<0.001
Weight	R	0.175	0.207*	0.305**
	p value	0.095	0.047	0.003
Body mass index	R	−0.052	0.011	−0.056
	p value	0.620	0.915	0.597
UI-MSJ distance	R	0.224*	0.310**	0.392**
	p value	0.032	0.003	<0.001
Thyromental distance	R	0.260*	0.213*	0.376**
	p value	0.012	0.042	<0.001
Sternomental distance	R	0.249*	0.237*	0.278**
	p value	0.017	0.023	0.007
Sternothyroid length	R	0.319**	0.241*	0.444**
	p value	0.002	0.021	<0.001
Sternal length	R	0.212*	0.246*	0.228*
	p value	0.042	0.018	0.029
Thyrosternal length	R	0.318**	0.308**	0.410**
	p value	0.002	0.003	<0.001
Mandible length	R	0.16	0.173	0.312**
	p value	0.127	0.099	0.002
Neck length	R	0.425**	0.215*	0.467**
	p value	<0.001	0.039	<0.001

*Significant at p value <0.05, **Significant at p value <0.01. UI-MSJ: upper incisor-manubriosternal joint

Table 6. Linear regression analysis results

Model		Unstandardised coefficients B	Std. Error	Standardised coefficients Beta	t	Sig
1	(Constant)	6.587	2.924		2.252	0.027
	Height	0.096	0.018	0.49	5.329	<0.001
2	(Constant)	7.431	2.906		2.557	0.012
	Height	0.065	0.024	0.331	2.756	0.007
	Neck length	0.265	0.131	0.242	2.013	0.047
3	(Constant)	7.214	2.849		2.532	0.013
	Height	0.049	0.024	0.249	2.013	0.047
	Neck length	0.32	0.131	0.292	2.437	0.017
	Weight	0.033	0.015	0.203	2.179	0.032

a. Dependent variable: L-C distance

Table 7. Model summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	0.490[a]	0.240	0.231	1.5966
2	0.522[b]	0.273	0.257	1.5702
3	0.557[c]	0.310	0.287	1.5382

Results of linear regression analysis of data show that among all patient factors studied, Model 3 is the best for the calculation of the lip to carina distance. The lip to carina distance (L-C distance) correlated with patient height, weight and neck length. L-C distance=7.214+0.049×Height+0.320×Neck length+0.033×Weight.

tween male and female patients. No patient had endobronchial intubation.

With the TT fixed with the intubation guide mark at VCs, the L-TT tip distance was 19.50±1.39 cm in male patients and 18.17±1.28 cm in female patients. The TT tip-carina distance was 3.58±1.43 cm in male patients and 3.23±1.33 cm in female patients. The TT tip-carina distance was <3 cm in 14 (30.4%) male patients and 23 (50%) female patients (Table 4). Analysis of data from patients in whom the TT tip-carina distance was ≥3 cm showed that L-TT distance was 19.29±1.40 cm and 18.06±1.36 cm in male and female patients, respectively.

The correlation of VCD, TL and L-C distance with patient characteristics and surface anatomy airway measurements is presented in Table 5. Results of linear regression analysis of data show that among the patient factors studied, Model 3 is the best for the calculation of the L-C distance (Tables 6 and 7). The L-C distance correlated with patient height, weight and NL. L-C distance=7.214+0.049×Height+0.320×Neck length+0.033×Weight.

TT characteristics are presented in Table 8. The incidence of hoarseness and sore throat at 24 h was 2.2% and 4.3%, respectively.

Table 8. Tracheal tube parameter measurements

	Tracheal tube Size 7 ID	Tracheal tube Size 8 ID
Tracheal tube length (cm)	31.76±0.40	34.39±0.17
Intubation guide mark length (cm)	1.0±0.0	1.0±0.0
Cuff length (cm)	3.98±0.04	4.05±0.28
Intubation guide mark to proximal cuff (cm)	2.95±0.14	3.17±0.16
Intubation guide mark to tube tip (cm)	9.37±0.05	10.08±0.10
Distal cuff to tube tip (cm)	2.39±0.54	2.95±0.18

Values are expressed as mean±SD.

Discussion

The recommended depth of TT placement is 21 cm and 23 cm from incisors in female and male patients, respectively (7, 8). The VCD and TL may vary with patient size and body proportion. Therefore, securing the TT at a fixed recommended depth may result in inappropriate endobronchial intubation or endolaryngeal placement of the TT cuff in some patients. In our study, the mean±SD VCD was 12.82±2.05 cm and 12.02±1.44 cm, TL was 10.14±2.04 cm and 9.37±1.28 cm and optimal depth of TT placement was 19.29±1.40 cm and 18.06±1.36 cm in male and female patients, respectively.

The VCD measured in the overall Indian population (12.42±1.81 cm) in the current study is comparable with that reported in Chinese patients (12.6±1.4 cm) (9) and in Taiwanese patients (12.1±1.8) (10). The VCD best correlated with NL in our study. A poor correlation (9) and a moderate correlation (11) have been reported between the VCD and patient height. In Caucasians, the VCD was 13.6±1.4 and 11.8±1.3 cm in male and female patients, respectively (12). These values are remarkably similar to those in our study despite differences in height in the two population groups (179±8 and 163±8 cm vs 169.4±5.6 and 154.5±5.5 cm in Caucasian and Indian male and female patients, respectively). Interestingly, Caucasian VCDs are similar to those measured in the Chinese and Indian populations despite differences in heights of different populations (12).

TL in our study population was 10.14±2.04 cm and 9.37±1.28 cm in male and female patients, respectively. This is comparable to TL reported by Varshney et al. (3) (9.83±1.26 cm and 9.27±0.99 cm in male and female patients, respectively). Begum et al. (13), in a cadaveric study in Bangladesh, found lower values for TL compared to the Western population, with the TL increasing with increasing age. Our findings are similar and not surprising as the Bangladeshi study cohort is ethnically close to our study population. However, we did not find age to be a significant predictor of TL. A correlation between TL and SL has been reported (10).

With the TT placed with the intubation guide mark at VCs, the L-TT tip distance (depth of TT placement) was 19.50±1.39 cm in male patients and 18.17±1.28 cm in female patients. This is comparable to that reported by Varshney et al. (3) (20.63±1.14 cm and 19.34±0.92 cm in male and female patients, respectively). The TT tip-carina distance was 3.58±1.43 cm in male patients and 3.23±1.33 cm in female patients in this situation in our study. Literature states that the TT tip-carina distance should be at least 4 cm (7, 8). This will prevent complications associated with the movement of the head and neck, head down patient tilt or creation of pneumoperitoneum. Neck flexion from a neutral head position can advance the TT up to 3.1 cm towards the carina (14); neck extension can move the TT by 5.2 cm towards the VCs (14). Goodman et al. (15) recommended a mean TT tip-carina distance of 5±2 cm that will prevent carinal impingement and endobronchial intubation.

We considered a TT tip-carina distance of 3 cm to be safe. With the TT fixed at the above stated L-TT distances (19.50±1.39 cm in male patients and 18.17±1.28 cm in female patients), 14 (30.4%) of 46 male patients and 23 (50%) of 46 female patients had a TT tip-carina distance of <3 cm. Analysis of data from patients in whom the TT tip-carina distance was >3 cm showed that the L-TT tip distance was 19.29±1.4 cm and 18.06±1.36 cm in male and female patients, respectively. This depth of insertion may be regarded as the optimal depth of TT placement in our study population.

In the adult Western population, Roberts et al. (16) found that corner-of-the-mouth placement of the TT at 21 cm mark in male patients and 23 cm mark in female patients would have led to proper placement in 81 (97.6%; 95% confidence interval 89.6%-99.7%) of 83 patients. Owen et al. (17) found that using the 21/23 reference mark for securing the TT significantly reduces the likelihood of inadvertent endobronchial TT placement in the Western population. Had the recommended 21/23 cm tube placement depth rule been followed in our patients, the mean±SD tube tip to carina distance would have been 0.72±1.47 cm in male patients and 0.50±1.45 cm in female patients. A TT tip-carina distance of <3 cm would have been found in 41 (89.1%) of 46 male patients and 45 (97.8%) of 46 female patients; 20 (43.5%) of 46 male patients and 23 (50%) of 46 female patients would have had the TT tip either at the carina or endobronchial. This contrasts with the mean±SD TT tip-carina distance of 3.58±1.43 cm in male patients and 3.23±1.33 cm in female patients when the depth of TT placement was guided by the intubation guide mark at the VCs. In this situation, no patient of either gender had a TT tip placed at the carina or endobronchial. This suggests that the intubation guide mark reference is more suited to the Indian population than the recommended 21/23 cm TT placement depth.

Gómez et al. (18) suggested that the optimal TT insertion depth can be reliably estimated by prediction equations based on patient height; use of the 21/23 cm rule resulted in a higher incidence of endobronchial intubations. TT insertion depth determined topographically (by adding measured airway distances from the right mouth corner to the right mandibular angle and the right mandibular angle to the centre point of the transverse line through the middle of the sternal manubrium) resulted in fewer TTs positioned within 4 cm of the carina than 21/23 cm insertion depth (19). Ong et al. (20) also found that adopting the 21/23 reference marks do not result in the ideal positioning of TT. They postulated that this was because the Asian population is shorter than those in previous studies which had achieved the ideal TT positioning using these reference marks.

The distance between the proximal intubation guide mark to the proximal cuff end was ≈3 cm. The VC-cricoid distance was 3.17±0.91 cm in female patients and 3.43±1.31 cm in male patients. Therefore, no patient was at risk of VC damage or accidental extubation in our study.

The mean±SD L-C distance in our study and that reported by Varshney et al. (3) in the Indian population are comparable (22.97±1.54 cm, 21.32±1.72 cm and 24.32±1.81 cm, 21.62±1.34 cm in male and female patients, respectively). Mukherjee et al. (4) reported a longer L-C distance of 25.66±1.91 cm and 23.59±1.73 cm in male and female patients, respectively. Airway length measurements made by Mukherjee et al. (4) were from the upper incisor to the carina instead of the angle of the mouth to the carina. The L-C distance is shorter when the TT is fixed at the angle of the mouth than midline fixation at the upper lip (21). The correlation between external anatomical measurements and airway length has been reported. The UI-MSJ distance is a significant predictor of incisor-carina length (4, 22). The UI-MSJ distance showed a positive correlation with L-C distance in our study. Results of linear regression analysis of our data show that among the patient factors studied, patient height, NL and weight correlated best with L-C distance.

Varshney et al. (3) suggested that the L-C distance from the right angle of the mouth can be calculated by the formula: L-C distance=0.478+0.14×Height (cm). Other formulae suggested for the length of the orotracheal tube (cm) are Patient height (cm)/10+5 (23) and Patient height (cm)/10+4 (Chula formula) (11). Results of linear regression analysis in our study suggest the formula: L-C distance (cm)=7.214+0.049×Height+0.320×Neck length+0.033×Body weight. Subtracting 3 cm from the L-C distance will provide the L-TT tip distance or depth of TT placement that is a safe distance of 3 cm above the carina.

Our study has some limitations. The results of our study are valid only for the Indian population and may not be applicable to other ethnic populations. In addition, TTs by a single manufacturer were evaluated. Safe distances between the VC and proximal cuff and between the TT tip and carina may not apply to TTs by other manufacturers. A limitation of the technique of placing the guide mark at the level of VCs is that it can only be used when the VCs are visible at laryngoscopy.

Conclusion

We conclude that the 21/23 cm rule for TT fixation cannot be followed in the Indian population because it may lead to endobronchial intubation and carinal impingement. We recommend placing the TT with its proximal guide mark at the level of VCs in the Indian population. This ensures safe distances between VCs and proximal tracheal cuff and also between the TT tip and carina. The formula derived for the optimum depth of tube placement in our study can be used as a guide when the recommended method of tube placement cannot be used because of inability to visualise the VCs during intubation in patients with Cormack-Lehane grade 3 or 4 laryngoscopy view.

Ethics Committee Approval: Ethics committee approval was received for this study from Institute Ethics committee (S No. IEC/VMMC/SJH/October-2015 dated 2/11/2015).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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