Evaluation of the Cricothyroid Muscle Innervation Pattern Through Intraoperative Electromyography

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Abstract

Objectives: We observed significant contractions in the cricothyroid muscle (CTM) after recurrent laryngeal nerve (RLN) stimulation in some patients. We aimed to evaluate whether these contractions resulted from the laryngeal-muscle movement due to the contraction of other intrinsic muscles or actual CTM contraction, with objective real-time intraoperative electromyography (EMG) recordings.

Methods: This study was performed prospectively in 106 consecutive patients who underwent intraoperative neural monitoring-guided primary thyroid surgery due to various thyroid diseases between February-2015 and February-2016. After completion of the thyroidectomy procedure; the RLN, vagus nerve (VN), external branch of the superior laryngeal nerve (EBSLN), plexus pharyngeus (PP), and contralateral EBSLN (CEBSLN) were stimulated and the responses from the CTM and CPM were recorded and evaluated by EMG through needle electrodes.

Results: 182 CTMs of 106 patients, with the mean age of 45, were evaluated regarding their innervation patterns. Positive EMG waveforms were achieved from 181 CTMs with EBSLN stimulation. A total of 132 (74%) positive EMG responses were recorded after the stimulation of 179 RLNs. The mean amplitude obtained with CTM EMG with RLN stimulation was 5.5% of that with EBSLN stimulation. The CTM amplitude was 39% of the vocal cord amplitude with RLN stimulation. Positive EMG responses of 96 CTMs (55%) with VN stimulation were recorded. The mean amplitude through CTM EMG with VN stimulation was 6% of that with EBSLN stimulation. Positive EMG responses were achieved from 10 (0.6%) CTMs with the stimulation of 170 PPs. The mean amplitude obtained from CTMs with PP stimulation was 4.3% of that with EBSLN stimulation. Positive EMG amplitudes of 35 (67%) CTMs were obtained with stimulation of 52 CEBSLN. Temporary vocal cord paralysis was detected in six patients (5% of patients and 3.3% of the nerves) postoperatively.

Conclusion: The RLN contributes significantly to the innervation of the CTM. Despite the findings associated with the contribution of the PP and CEBSLN to the CTM innervation, further studies are needed. We are of the opinion that these are among the significant factors that contribute to the differences in clinical findings between patients with EBSLN injuries.

Keywords: Cricothyroid muscle, intraoperative electromyography, intraoperative nerve monitoring

Please cite this article as “Aygun N, Mihmanli M, Isgor A, Uludag M. Evaluation of the Cricothyroid Muscle Innervation Pattern Through Intraoperative Electromyography. Med Bull Sisli Etfal Hosp 2022;56(1):145–153”.

Thyroidectomy usually has a very low complication rate when performed by an experienced surgeon. The larynx with its complex neural supply is at the center of the thyroid and parathyroid operative field. Post-operative voice change is one of the most feared complications of thyroid and parathyroid surgery and recurrent laryngeal nerve (RLN) injury is one of the most common causes of post-operative voice change.1 In addition, the external branch
of the superior laryngeal nerve (EBSLN) is at risk of injury, which can be as high as 58%, during the dissection and ligation of the superior thyroid vessels. Such injury leads to impaired high-pitch, changes in frequency, and decreased sound projection, which may be significantly problematic for women and professional singers.\(^1,^2,^3\)

Conventional laryngeal neuroanatomical definitions suggest that all the intrinsic muscles of the larynx, except the cricothyroid muscle (CTM), are innervated by RLN, whereas CTM is innervated by the EBSLN.\(^4\) Although we have gained more information on the anatomy and physiology of the larynx in recent years, some questions still remain unanswered, one of which is why the vocal cords exhibit different positions following injury by the same laryngeal nerve. With the development of laryngeal electromyography (LEMG),\(^5\) it was suggested that the position of the vocal cords was correlated with the CTM activation,\(^6,^7\) which was in contradiction with the confirmation of different LEMG patterns in paralyzed vocal cords.\(^8,^9\) These contradictory findings demonstrate the fact that the connections between the laryngeal nerves may have a potential role in the varying positions of paralytic vocal cords, as well as the unknown aspects of laryngeal neuroanatomy.\(^10\)

In recent years, intraoperative nerve monitoring (IONM) has been widely used for the identification of both RLN and EBSLN as an adjunct to the gold standard visual identification of the nerve.\(^11\) EBSLN monitoring is based on two distinct outcome measures following the stimulation of the EBSLN: (1) Evaluation of cricothyroid twitch and (2) electromyographic glottis response of vocal cord depolarization identified via endotracheal tube surface electrodes. At present, available data suggest such glottis response through current endotracheal tube surface electrodes may be identified in 70–80% of patients with EBSLN stimulation.\(^3,^12\) Meticulous anatomical studies of cadaver larynges revealed terminal branches of the EBSLN reaching the thyroarytenoid muscle and communicating with the branches of the RLN in the larynx. These branches are called “human communicating nerves.”\(^13-15\) Some studies reported CTM activation with stimulation of RLN,\(^10,^12,^16\) which was confirmed by the real-time electromyography (EMG) recordings with the use of IONM and needle electrodes inserted into the CTM.\(^12,^16\) The present anatomical studies are mostly on cadavers. Various dissection studies noted that RLN was associated with the internal or EBSLN in 15–100% of all cases.\(^17\)

In the literature, there is a limited number of studies including intraoperative EMG monitoring of other muscles in the operative field, except for monitoring the function of the vocal cord during IONM-guided thyroidectomy, which has been widely used in recent years. It is difficult to assess the accurate prevalence of the superior laryngeal nerve injury based on the limited data and different methods applied in the studies. It has been shown that CTM EMG is the only definitive way to detect EBSLN injury because of the variability in vocal symptoms and findings at the post-operative laryngeal examination.\(^3\) Laryngeal EMG is the gold standard method used to evaluate the function of laryngeal muscles and nerves.

We similarly observed intrinsic motor activity in the relevant CTM after EBSLN stimulation in the patients whom were applied IONM. We also observed significant contractions in the CTM after RLN stimulation in some patients. We aimed to evaluate whether these contractions resulted from the laryngeal-muscle movement due to the contraction of other intrinsic muscles or actual CTM contraction, with objective real-time intraoperative EMG recordings.

**Methods**

This study was performed prospectively in 106 consecutive patients who underwent thyroidectomy between February 2015 and February 2016 in our clinic after obtaining approval from Şişli Hamidiye Etfal Training and Research Hospital Ethics Committee. All participating patients were informed and their written consent was obtained before the study. The study included patients aged between 18 and 75 years of age who underwent IONM-guided primary thyroid surgery due to various thyroid diseases.

Patients who refused to participate in the study, patients with secondary intervention, preoperative vocal cord paralysis (VCP), who required intraoperative resection of the EBSLN, vagus nerve (VN) or RLN, patients with non-RLN and those whom were unable to be applied IONM and/or EMG due to technical issues, were excluded from the study.

All patients underwent vocal cord examination with fiberoptic laryngoscopy by an independent otolaryngologist on the pre-operative and post-operative first 2 days. Patients with post-operative VCP underwent regular vocal cord examinations at the 15\(^{th}\) day and 1\(^{st}\), 2\(^{nd}\), 4\(^{th}\), and 6\(^{th}\) months until the post-operative vocal cord function returned to normal. VCP was defined as temporary if the vocal cord functions improved within the 6 months, whereas it was defined as permanent if the vocal cord function did not improve at the 6\(^{th}\) month.

All surgical operations were performed by the same surgeon (MU) experienced in endocrine surgery and use of IONM. Each side of the neck was considered as a separate entity. The demographic findings, pre-operative indications, intraoperative anatomical findings of the nerves, intraoperative loss of signal, intraoperative EMG findings, pre-operative,
and post-operative vocal cord examinations of all participating patients were recorded in a prospective database.

**Surgical Technique**

Thyroidectomy and/or central neck dissection was performed via a 4–6 cm collar transverse incision. All patients underwent lobectomies or total thyroidectomies. Subplatysmal flaps were raised, strap muscles were divided along the midline, the thyroid lobe was medialized, and middle thyroid vein was divided. Identification and monitoring of RLNs and EBSLNs were carried out systematically, as described previously.\(^{[18,19]}\)

**IONM Technique**

IONM of the RLN and EBSLN and intraoperative EMG of the CTM and CPM were carried out using a NIM 3.0 Nerve Monitoring System (Medtronic Xomed, Jacksonville, FL, USA) with four channels. All monitoring setup, applications, and EMG modality parameters were in accordance with the International Neural Monitoring Study Group (INMSG) Guidelines. Patients were examined under general anesthesia without the use of paralytic agents, except for an initial low-dose neuromuscular blockade (rocuronium 0.3 mg/kg) at induction, and patients were intubated using a Medtronic Xomed Nerve Integrity Monitor Standard Reinforced Electromyography Endotracheal Tube with surface electrodes (size 6.0, 7.0, or 8.0) (Medtronic Xomed, Jacksonville, FL, USA). Standard RLN (4-step procedure (V1, R1, R2, and V2)) and EBSLN IONM were performed in accordance with the INMSG guideline.\(^{[3,11]}\) The EBSLN, RLN, VN, plexus pharyngeus (PP), and contralateral EBSLN (CEBSLN) were stimulated with a monopolar stimulator probe (Medtronic Xomed, Jacksonville, FL, USA) at 1 mA with the impulse duration set at 100‐ms, 4‐Hz frequency and amplitude threshold of 100 µV. An EMG waveform of ≥100 μV and an audible alarm from the vocal cords after nerve stimulation were accepted as a positive response. Positive EMG waveform achieved from the vocal cord represented the vocal cord adduction. Continuous or intermittent IONM were applied. Standard RLN monitoring was performed in four steps as described below (V1, R1, R2, and V2). The function of IONM with EBSLN was assessed based on the presence of CTM contraction following the stimulation of a source nerve. Some cases may exhibit an audible alarm from the vocal cord and a positive EMG waveform after the stimulation of EBSLN. The waves obtained from any vocal cord signals after EBSLN stimulation were also recorded. EBSLN monitoring was performed in accordance with these standards. Intermittent or continuous RLN monitoring was performed according to the difficulty of the case and the surgeon’s preference.

**Neuromonitoring of CTM and CPM**

After completion of the thyroidectomy procedure; the RLN, VN, EBSLN, PP, and CEBSLN were stimulated and the responses from the CTM and CPM were recorded and evaluated by EMG through needle electrodes. The EMG waveforms from the TAM, which is the main adductor muscle of the vocal folds, were recorded by surface electrodes on the endotracheal tube. The main motor innervation of the CTM is provided by the EBSLN. EMG recordings from the CTMs were obtained using a pair of needle electrodes inserted into the CTMs. A pair of needle electrodes was inserted into the pars rectus of the CTM and in the midline of the CPM superior to the entry point of the RLN into the larynx (Fig. 1).

The needle electrodes inserted within the CTM were connected to channel 3 of the monitoring device, whereas the needle electrodes within the CPM were connected to channel 4. Following the needle insertion, the EBSLN (Fig. 1), RLN, VN, PP, and CEBSLN were stimulated with a monopolar stimulator probe at a current of 1 mA (Fig. 2). The vocal cord and the CPM EMGs were also recorded as the RLN is the main supplier for the vocal cord and the PP is for the CPM. The positive EMG findings of these muscles with the stimulation of RLN and PP were used to confirm that they are the main suppliers. The nerve stimulation points were measured to standardize latency time. EBSLN was stimulated 2 cm proximal to its insertion into the CTM. The PP was traced with a probe across the inferior pharyngeal muscle fibers posterior to the lamina of the thyroid cartilage and was detected with a muscle twitch. The PP was

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Figure 1. Placement of needle electrodes into the cricothyroid and cricopharyngeal muscles and probe stimulation of EBSLN.

CTM: Cricothyroid; CPM: Cricopharyngeal; RLN: Recurrent laryngeal nerve; EBSLN: External branch of the superior laryngeal nerve; APS: Automated Periodic Stimulation
stimulated with a probe posterolateral to the superior thyroid vessels at the same level as EBSLN and the EMG waves were recorded. The RLN was stimulated 2 cm proximal to its insertion into the larynx and the EMG waveforms were recorded. The VN was stimulated at the level of the 2–4th tracheal rings and the EMG waveforms were recorded. During the stimulation of these nerves, the EMG recordings of the left and right vocal cords, CTMs and CPMs were obtained through the first, second, third, and fourth channels of the NIM 3.0 Nerve Monitoring System, respectively. Visible contractions of the CTMs and CPMs with nerve stimulation were recorded. The EMG responses of the CPMs and CTMs were analyzed and evaluated considering the conditions in the studies of Faaborg-Andersen[5] and Martin-Oviedo et al.[10] A positive response had to be at least 4 times greater than that of the noninnervated muscle. In our study, the mean response in the contralateral CPM was 19.1 µV with stimulation of the EBSLN, and an evoked response was defined as positive when the value was ≥100 µV.

Statistical Analysis
Assessment of the variability of the investigated parameters was presented by number and percentages for categorical variables while mean, standard deviation, minimum, and maximum were used to summarize numerical variables. The student’s t-test was performed to compare numerical variables with normal distribution between the two independent groups while the Kruskal–Wallis test was used when a normal distribution was not achieved. The parametric Tukey’s test and the non-parametric Mann–Whitney U were used to compare the subgroups, while the Bonferroni correction was applied for the evaluation. The Chi-square test was used to test the difference in distribution of categorical variables between the independent groups. A value of p<0.05 was accepted statistically significant.

Results
A total of 106 patients with a mean of age 45 were evaluated in terms of innervation patterns of 182 CTMs on 182 neck sides. Demographic profile, preoperative diagnoses and surgical procedures of the patients are given in Table 1. 76 patients had bilateral, while 30 patients had unilateral operations. The amplitudes and latencies of the CTM with EBSLN, VN, RLN, PP, and CEBSLN stimulations, and additionally the amplitudes and latencies of the main suppliers of the vocal cords and the CPM are summarized in Table 2.

EMG was performed for a total of 182 CTMs using needle electrodes. Positive EMG amplitudes were achieved from 181 CTMs with EBSLN stimulation. CTM EMG could not be performed, since 1 EBSLN could not be detected with the probe. A total of 179 CTM EMG amplitudes were recorded with RLN stimulation, but no EMG waveforms were obtained from 3 CTMs due to the LOS. With VN stimulation, 177 CTM EMG amplitudes were achieved, but no EMG waveforms were obtained from the remaining 5 CTMs due to LOS. With PP stimulation, 170 CTM EMG amplitudes were obtained, while the EMG amplitudes of the remaining muscles could not be recorded due to the unidentified 12 PPs. A total of 52 CTMs were evaluated by EMG with CEBSLN stimulation. Positive amplitudes were achieved from all 181 CTMs with the EBSLN stimulation. The mean amplitude was 6812 µV with the mean latency time of 3.29 ms (Fig. 3). A total of 132 (74%) positive EMG responses were recorded after the stimulation of 179 RLNs. The mean amplitude obtained with CTM EMG with RLN stimulation was 5.5% of that with EBSLN stimulation. The mean positive EMG wave amplitude was 373 µV with the mean latency time of 4.22 ms. The CTM amplitude was 39% of the vocal cord amplitude with RLN stimulation. When the data of the same patient were evaluated, the CTM amplitudes with RLN stimulation were below 20% in 42 muscles (32%), between 20% and 50% in 45 muscles (34%), and between 50% and 100% in 24 muscles (18.1%), compared to the vocal cord amplitudes. A total of 21 CTMs (15.9%) demonstrated higher amplitudes than those of the vocal cord, doubling it around 1.1–8.4 times.

Positive CTM EMG responses were achieved in a total of 96 (55%) CTMs with VN stimulation. The mean positive EMG amplitude was 406 µV and the mean latency was 6.23 ms. The mean amplitude through CTM EMG with VN stimula-
tion was 6% of that with EBSLN stimulation. The mean amplitude through CTM EMG with VN stimulation was 56% of the vocal cord amplitude with vagus stimulation. Separate evaluation of each patient data revealed that the CTM amplitude with VN stimulation was below 20% in 23 muscles (24%), between 20% and 50% in 28 muscles (29.2%), and between 50% and 100% in 29 muscles (30.2%), compared to the vocal cord amplitude. A total of 16 CTMs (16.6%) showed higher amplitudes than those of the vocal cord, doubling it around 1.1–9.2 times.

Positive CTM EMG waveforms were obtained from 10 (0.6%) CTMs with the stimulation of 170 PPs. The mean positive CTM EMG amplitude was 296 µV and the mean latency was 4.78 ms. The mean amplitude obtained from CTMs with PP stimulation was 4.3% of that with EBSLN stimulation. The mean amplitude of the CTMs with PP stimulation was 12% of the CPM amplitude. Separate evaluation of each patient data revealed that the CTM amplitude with PP stimulation was below %20 in 7 (70%) muscles, and between 50% and 100% in 3 (30%) muscles, compared to the CPM amplitude.

Positive EMG responses were obtained in 35 (67%) CTMs with stimulation of the 52 CEBSLNs. The mean positive CTM EMG amplitude was 210 µV and the mean latency was 3.82 ms. The CTM amplitude with CEBSLN stimulation was 3.1% of the mean amplitude with ipsilateral EBSLN stimulation. The muscle amplitude was below 20% in 34 CTMs and between 20% and 50% in 1 CTM.

The comparison of amplitude and latency times revealed that the mean amplitude of CTM with RLN stimulation was significantly lower than that of the vocal cord (p<0.0001), whereas the latency time was significantly longer (b vs. e, p=0.001). The mean amplitude of CTM with VN stimulation was significantly lower than that of the vocal cord (p<0.0001), with no statistically significant difference in the latency times.

Table 1. Demographic and clinical profile, type of intervention

| Variables                      | n   |
|--------------------------------|-----|
| Gender (Female/Male)           | 80/26|
| Mean age (range) (years)       | 45 (18–75) |
| Preoperative diagnosis         |     |
| Benign thyroid disease         | 77   |
| Malignant or Suspicious malignancy | 29   |
| Intervention type              |     |
| Bilateral approach             |     |
| TT                             | 66   |
| TT+CND                         | 5    |
| TT+CND+LND                     | 5    |
| Unilateral approach            |     |
| Lobectomy                      | 30   |
| RLNs at risk                   | 182  |

TT: Total thyroidectomy, CND: Central neck dissection, LND: Lateral neck dissection, RLN: Recurrent laryngeal nerve

Table 2. Amplitudes and latencies obtained from the laryngeal muscles with nerve stimulations

| Nerve-muscle          | Amplitude (µV) Mean±SD (min-max) | Latency (ms) Mean±SD (min-max) |
|-----------------------|----------------------------------|--------------------------------|
| EBSLN-CTM             | 6812±5941 (599–29991)            | 3.29±1.06 (1.10–8.90)          |
| RLN-CTM†              | (373±554) (102–3631)             | 4.22±1.07 (2.20–7.10)          |
| VN-CTM†               | 406±551 (103–2950)               | 6.23±1.65 (3.30–11.13)         |
| PP-CTM‡               | 296±308 (100–1104)               | 4.78±1.83 (2.1–7.3)            |
| RLS-VC                 | 961±871 (105–5523)               | 3.86±1.22 (1.70–9.30)          |
| VN-VC†                | 729±720 (101–4140)               | 6.4±1.81 (2.90–11.70)          |
| PP-CPM                 | 2390±1638 (101–8041)             | 5.28±1.53 (1.30–10)            |
| CEBSLN-CTM‡            | 211±216 (100–1404)               | 3.82±1.15 (1.8–6.30)           |

Comparison of amplitudes: * vs †: p<0.0001, * vs ‡: p<0.0001, * vs §: p=0.005, * vs ¶: p<0.0001, * vs ‖: p=0.917, * vs ‧: p<0.0001, * vs ‧*: p<0.0001, * vs ‧*: p=0.008.
Comparison of latencies: * vs †: p<0.0001, * vs ‡: p<0.0001, * vs §: p=0.02, * vs ¶: p<0.0001, * vs ‖: p=0.043, * vs ¶: p<0.001, * vs ‥: p=0.392, * vs ‧: p=0.0001, * vs ‧*: p=0.764, * vs ‧*: p=0.944.
EBSLN: External branch of the superior laryngeal nerve, CTM: Cricothyroid muscle, RLN: Recurrent laryngeal nerve, VN: Vagus nerve, PP: Pharyngeal plexus, VC: Vocal cord, CPM: Cricopharyngeal muscle, CEBSLN: Contralateral external branch of the superior laryngeal nerve, SD: Standard deviation, min: Minimum, max: Maximum.

Figure 3. Positive electromyography waveforms of the cricothyroid muscle (channel 3) and cricopharyngeal muscle (channel 4) with external branch of the superior laryngeal nerve stimulation.
Near-field potentials generated by multiple sources, have small amplitudes, and are significantly higher than far-field potentials. Far-field potentials reflect the evoked activity in the specific muscle as those recorded at a considerable distance. In the studies discussing surface EMG, in which it was detected over a non-active muscle generating EMG activity and defined as “crosstalk” EMG signal. Near-field potentials are defined as those recorded in relative close proximity to the source muscle, whereas far-field potentials are defined as those recorded at a considerable distance. Near-field potentials reflect the evoked activity in the specific muscle and are significantly higher than far-field potentials. Far-field potentials generated by multiple sources, have smaller amplitudes, and are more difficult to interpret. Cross-talk contamination can be minimized by reducing the inter-electrode distance. A study conducted in leg muscles using surface electrodes with 10 mm spacing noted that the crosstalk contamination could reach up to 17% of the target muscle amplitude.

Liddy et al.[26] evaluated bilateral vocal cords, CTMs, strap muscles with EBSLN and RLN stimulations using EMG in patients undergoing thyroid surgery. The target muscles for RLN and EBSLN were vocal cords and CTM, respectively, and they found that the target muscle mean amplitudes were significantly higher than in non-target muscles. They noted visible contraction in the CTM and strap muscles, and assessed the glottis response of vocal cord depolarization with the palpation of the posterior cricoarytenoid muscle (PCAM), or through intraoperative laryngoscopy in some patients. The authors observed high EMG amplitudes and visible contraction in the CTM with EBSLN stimulation, PCAM twitching with RLN stimulation or vocal cord contraction through laryngoscopy. They

Discussion

The function of intrinsic laryngeal muscles that regulate vocal cord movements is highly important for sound production and respiration. The classical anatomical understanding suggested that the CTM was only innervated by the EBSLN, whereas the intrinsic laryngeal muscles, except for the CTM, were only innervated by the RLN.[17] However, later morphological studies revealed that the laryngeal nerves consist of different anastomoses with a rich and complex neural supply.[14,15,20] These morphological studies do not disclose any information about the function of nerve fibers. There is a limited number of electrophysiological studies on laryngeal nerves.[10,12]

In the present study, high EMG amplitudes were obtained from all CTMs with EBSLN stimulation in accordance with the anatomical findings. In addition, it was observed that the motor innervation of the CTM was affected at varying rates by the RLN, PP, and CEBSLN stimulations. Positive EMG responses were obtained from 74% of the CTMs with RLN stimulation, which proved that it contributed to the muscle’s motor innervation. This rate is 55% with VN stimulation which is lower than that with RLN stimulation and may be related to the fact that we stimulated both the RLN and the VN with 1 mA current. Since the position of the RLN fibers is variable in the VN, the probe may not directly contact these fibers during the vagus stimulation leading to obtain negative EMG response, but a positive EMG near the threshold with the RLN stimulation.

On the other hand, a study in which both the RLN and the VN were stimulated at 2 mA noted visible CTM contractions and similar EMG responses.[12] In a similar study, Masuoka et al.[12] observed that the stimulation of the RLN yielded a visible contraction with evoked amplitude of >300 μV in 27 of the 70 CTMs (39%) and an evoked amplitude of >300 μV with no visible contraction in 15, equaling up to a total of 42 CTMs (60%) with motor activity. The authors only evaluated the amplitudes above 300 μV as a positive response although they observed visual contraction in 9 muscles (13%) below 300 μV. Either visual contraction or EMG activity above 300 μV was detected in 24 muscles. Martin-Oviedo et al.[10] reported a positive EMG response of CTM with RLN stimulation in seven out of 13 patients scheduled for total laryngectomy. In addition, they found cricothyroid connection between the RLN and the EBSLN in a morphological study performed on the laryngectomy specimens they obtained from the patients.

The EMG amplitude of the CTM with RLN stimulation was 5.5% of the EMG amplitude with EBSLN stimulation, which supported that the CTM innervation was supplied by the anastomoses between the RLN and EBSLN in a small proportion of the muscle, rather than a direct RLN stimulation. Anatomical studies report cricothyroid anastomosis, which is called “human communicating nerve,” between the RLN and EBSLN at a rate ranging between 44% and 88%.[14,15,21] In addition, it was revealed that the anastomosis of the cricothyroid yielded thin branches[10,14] which were proposed to be an alternative innervation to the CTM from the RLN. The absence of CTM atrophy in EBSLN paralysis was also indicative of this alternative innervation.[10] Since the RLN is not the motor nerve of the CTM, it can be thought that the contraction of the main muscles of the nerve with stimulation may be an artifactual EMG response in the adjacent muscle. The neurological literature mentioned stimulus artifact in neighboring muscle groups in the studies discussing surface EMG, in which it was detected over a non-active muscle generating EMG activity and defined as “crosstalk” EMG signal.[22]
also obtained EMGs with significantly lower amplitudes in non-target muscles compared to those of the target muscles, with no sign of muscle contraction. These EMGs revealed similar latency in the target muscles and the others. These similar EMG responses, which we detected with the RLN, vagus, PP, and CEBSLN stimulation, were defined as far-field potentials. However, EMG is the gold standard for evaluating motor innervation of a muscle. Hydman et al. evaluated approximately 2200 RLNs and revealed the sensitivity and specificity of the laryngeal palpation test as 69.3% and 99.7%, respectively, with a positive predictive value of 92.1%. Laryngeal palpation fails to detect approximately 30% of VCP. Masuoka et al. stated that the absence of visible contraction in the CTM despite positive EMG response might be caused by innervation of only a small portion of the CTM and inappropriate location of the needle electrodes, or partial innervation that is sufficient to yield an electromyographic response but insufficient to cause macroscopic contraction of the muscle. Our findings suggest that EMG potentials generated by stimulation of nerves other than EBSLN in the CTM were not far-field potentials and they contributed to the muscle intervention. The needle electrodes were closely inserted into the stimulated muscle, and recorded activity in the confined space around them. Needle electrodes are inserted into the target muscle near each other and record activity in the confined space around them. In the present study, the mean CTM amplitude with RLN stimulation was 39% of the vocal cord amplitude with RLN stimulation. Separate evaluation of each patient parameter revealed that the CTM amplitude after RLN stimulation was below 20% in 32% of the muscles (32%), between 20% and 50% in 34% of the muscles, and between 50% and 100% in 18.1% of the muscles, compared to the vocal cord amplitude. A total of 21 CTMs (15.9%) showed higher amplitudes than those of the vocal cord, doubling it up to 8.4 times.

In the study, we observed a rare positive EMG waveform in CTM with PP stimulation at a rate of 0.6%, the amplitude of which was 12% of the CPM amplitude and between 50% and 100% in three of the 10 CTMs exhibiting positive innervation. There was no significant difference in the CPM and CTM latency times, which also suggests that PP may contribute to CTM innervation through anastomoses between the nerves, rather than direct innervation. This is the first study in which CTM innervation findings with electrophysiological PP stimulation are reported. The contribution of PP to the vocal cord innervation has been recently revealed in two experimental studies in rats. Similarly, it was reported in some cases that PP also contributes to human vocal cord innervation. The CTM innervation after PP stimulation may be associated with the anastomoses between EBSLN and PP. Some anatomical studies noted anastomoses between EBSLN and PP. In addition, it has been reported that the superior laryngeal nerve can consist of two roots, one of which from the vagus and the other from the glossopharyngeal nerve. It has also been shown that EBSLN may rarely contribute to innervation of PP, although PP mainly consists of branches of the glossopharyngeal and VN. Further studies are needed to evaluate PP contribution to CTM innervation more clearly despite the high amplitudes in some cases and complex anatomical data.

A positive EMG response was achieved in the CTM at a rate of 67% with CEBSLN stimulation, the amplitude of which accounted for 3.1% of the ipsilateral EBSLN stimulation. The EMG amplitude after the CEBSLN stimulation was significantly lower than that of the ipsilateral stimulation, whereas the latency was significantly longer. The high latency time indicate contraction by innervation, other than the contralateral CTM twitching although the amplitude of the CEBSLN stimulation was significantly low. Martin-Oviedo et al. have recently detected twitching in the contralateral CTM, TAM, and arytenoid muscles with EBSLN stimulation, which was attributed to the nerve connections from the deep arytenoid plexus by the authors. Anatomy studies report the incidence of arytenoid plexus as 28–100%. Although complex connections between internal laryngeal nerves have been reported in many anatomical studies, its clinical significance is still controversial. Since the 19th century, there has been a debate suggesting that such complex neural network may be related to the varying vocal cord positions occurring after laryngeal nerve injuries. Many studies have reported that the innervation status of the CTM does not influence the vocal fold position in laryngeal paralysis. The CTM dysfunction in unilateral vocal fold paralysis does not affect the vocal cord position, but may impair sound quality due to impaired mucosal vibration. Clinical findings may vary in CTM dysfunction. Contribution of RLN to CTM innervation may change findings related to voice quality in EBSLN injuries. Clinical findings may be milder in cases while the RLN, or in some circumstances, the PP, innervates the CTM.

**Conclusion**

It has been reported by many anatomical studies that the intralaryngeal innervation has a complex structure, which is also supported by the electrophysiological findings in our study. The RLN contributes significantly to the innervation of the CTM. Despite the findings associated with the contribution of the PP and CEBSLN to the CTM innervation, further studies are needed. We are of the opinion that these are among the significant factors that contribute to the differences in clinical findings between patients with EBSLN injuries.
Disclosures

Ethics Committee Approval: The study was approved by the Sisli Hamidiye Etfal Training and Research Hospital Ethics Committee (Date: 14/04/2015, No: 934).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – N.A.; Design – N.A., M.U.; Supervision – A.I., M.A.; Materials – N.A., M.U.; Data collection &/or processing – N.A., M.U.; Analysis and/or interpretation – M.M., A.I., M.U.; Literature search – N.A., M.U.; Writing – N.A.; Critical review – A.I., M.U.

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