Quantifying the shrinkage of laryngeal laser excisions: a case control study

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**Meetings**

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**Abstract**

*Introduction*

Heat from transoral laser microsurgery can cause tissue shrinkage, impacting the surgical margin. The aim of this study was to compare shrinkage between cold steel and carbon dioxide laser resections of laryngeal lesions.

*Materials*

A European Laryngological Society type II resection was performed on 10 mm ‘lesions’ marked on both the true and false cords of fresh frozen human larynxes: laser resection on the right side and cold steel on the left side.

*Results*

Twenty-eight larynxes were included. Tissue shrinkage was significantly higher in laser resection (35-45%) compared to cold steel resection (8-14%) (*p* <0.0001). In most cases, there was no significant difference in shrinkage between true and false cord sites.

*Discussion*

This study demonstrates specimen shrinkage is significantly higher in laser resections. This shrinkage will affect the size of the margin; surgeons and pathologists should be aware of this when considering positive and close margins.

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**Keywords**

Case-Control Studies

Laryngeal Neoplasms

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**Introduction**

Laryngeal cancer is diagnosed in approximately 2,400 people every year in the United Kingdom.1 Treatment options include transoral laser microsurgery (TLM), transoral robotic surgery (TORS) and open partial laryngeal surgery for early stage disease (stage I/II).

In the United Kingdom, TLM using the carbon dioxide (CO2) laser, when surgical access permits its use, is a cost-effective modality of treatment when compared to radiotherapy2 with comparable disease free and laryngeal preservation rates.3 The laser has a wavelength of 10,600 nm and a high coefficient of extinction in water which limits its penetration and collateral effects.4 It provides an ideal and precise cutting instrument for laryngeal lesions.5

The vocal cords of the human larynx are composed of five histological layers each with a specific function. Preservation of these layers whilst ensuring adequate cancer clearance presents a dilemma to the surgeon. Unnecessary damage to healthy tissues should be minimised to reduce the effects on functional outcomes such as voice.6 A clear margin, normally defined as >1.00 mm, indicates the absence of cancer cells at the resection margin at the microscopic level. A close margin can be considered to be ≤1.00 mm.7 Positive margins require further treatment; close margins may require further treatment depending on tumour characteristics.8

Heat causes tissue shrinkage due to collagen contracture at temperatures above 50 degrees Celsius.9 TLM is a thermally mediated treatment, hence shrinkage of the resected laryngeal specimen is expected.10 The thermal effects from the laser can have detrimental effects to the surgical mucosal margin which can shrink after excision.11 If the surgical margins shrink after excision, even by only 1-2 mm, it can be difficult for the histopathologist to accurately report the margins to determine whether or not they are involved. This can result in difficulties in deciding whether or not the patient undergoes revision surgery or adjuvant treatment, such as radiotherapy, that may be unnecessary.

Following resection, some surgeons use alcohol-dried cucumber to mount the specimen, providing the histopathologist with an orientation of the resected laryngeal specimen.12 It is possible that this process may further alter the size of the specimen, and therefore affect the interpretation of the surgical margins.

The aim of the current study was to quantify the amount of shrinkage of a type II laryngeal excision specimen,13 comparing shrinkage between cold steel (control) and CO2 laser resection and to determine the additional effects of mounting the specimen on cucumber.

**Materials and Methods**

*Cadaveric material*

Harvested fresh frozen human larynxes were removed using the same operating technique. The larynxes were stored in a freezer between -18 and -21 degrees Celsius.

*Cucumber preparation*

For mounting resected specimens onto cucumber, a 10-blade scalpel was used to cut slices of cucumber measuring 5 mm thick with digital callipers. A triangular shaped section was cut from the centre of each slice. The cucumber slices were submerged into an airtight container with 99% isopropyl alcohol which was replaced every 24 hours for three consecutive days. The final slices were stored in absolute alcohol until required, at which point they were dried with a paper towel to remove excess fluid.12

*Dissection protocol*

The frozen larynxes were defrosted by submerging into warm water for 15 minutes before use. They were dried with surgical gauze. A midline incision was made in the posterior aspect of each larynx to improve access. The larynx was placed on a flat board, covered in damp surgical gauze. A self-retaining retractor was used to keep the larynx open (Figure 1). A 10 mm ruler was held using non-toothed forceps on the true vocal cord and then false vocal cord. The CO2 laser was used to mark the top, middle and bottom of the resection, using the ruler as a marker for all specimens. A European Laryngological Society type II laryngeal resection was performed between these marks.13 Laser resection was performed on the right side of each specimen and, as a control, cold steel resection was performed on the left side. Resections were all performed by the senior author (AG) using the Zeiss OPMI 1FC S21 microscope. The CO2 laser used was the Jena Surgical SmartXide2 C60 (1.0W, dot shaped Ultra Pulse, continuous delivery). Cold steel resection was performed using a 10-blade scalpel.

*Measurement of lengths*

Following resection, the specimen was laid flat on a microscope slide. The length of the resected specimen, both with cold steel and laser resection, was measured immediately in order to prevent the specimen drying and changing size. All measurements were recorded by the same person in the dissection laboratory with digital callipers to the nearest 0.01 mm, using the microscope for visualisation. There was no blinding to resection technique during measurement. All measurements were taken three times, and the average measurement recorded. For specimens with permission for photography, a photograph was taken using a tripod-mounted camera, with a scale placed below the microscope slide. The size of the specimen was measured again from the photograph using ImageJ software (Wayne Rasband, previously National Institutes of Health). The specimen was then fixed to cucumber using Loctite Super Glue and the length of the specimen was measured again, both with digital callipers and ImageJ software if permission for photography was available. Finally, the defect sizes in both the true and false vocal cords were measured using the digital callipers, while visualising the larynx through the microscope.

*Data analysis and sample size calculation*

A sample size calculation was performed which required a group size of at least three specimens per group. Data were recorded in Microsoft Excel (Microsoft, Washington, United States). Statistical analysis was performed in GraphPad Prism 8 (GraphPad Software, San Diego, United States). The specimen size and defect size measurements were all assumed to be normally distributed. Descriptive statistics were performed on each data set. The paired samples t test was used to compare tissue shrinkage between sites (true and false vocal cord), between resection methods (cold steel and laser), before and after application to cucumber, between defect sizes at different sites (true and false vocal cord) and between defect sizes depending on resection method (cold steel and laser). A *p* value of <0.05 was taken to represent a statistically significant result.

*Ethics and consent*

The project was granted ethical approval from the Keele University School of Medicine Student Project Ethics Committee. The dissection laboratory operates under the license from the Human Tissue Authority. All cadaveric material had consent for use in teaching and research.

**Results and Analysis**

A total of 28 larynxes were made available for this study. The basic characteristics and descriptive statistics are displayed in Table 1. Twenty-five specimens had permission for photography and were able to have size measurements recorded using the ImageJ software.

There was shrinkage of all resected specimens, regardless of the location (true or false vocal cord), resection type (cold steel or laser) or measurement technique (digital callipers or ImageJ software) (Table 2, Figures 2 and 3). For cold steel control resection, shrinkage varied between 8-14%; for laser resection, shrinkage varied between 35-45%. The greatest shrinkage was noted for specimens from the false vocal cord with laser resection, mounted on cucumber and measured with the ImageJ software. In this group, the average specimen size measured 5.49 mm, a shrinkage of 45.06% from the original 10 mm marked lesion.

Tissue shrinkage was significantly higher in specimens undergoing laser resection, compared to those with cold steel resection (*p* <0.0001) (Figures 2 and 3). For example, when measured with digital callipers at the true vocal cord without cucumber, shrinkage increased from 9.43% with cold steel to 35.41% with laser resection. Tissue shrinkage with laser resection was significantly higher than with cold steel resection in all cases, irrespective of the location of the specimen (true or false vocal cord), whether or not the specimen was mounted on cucumber or whether the size was recorded with digital callipers or ImageJ software.

Mounting the specimen onto cucumber significantly reduced the specimen size (*p* <0.0001 for most scenarios) (Figures 2 and 3). The *p* value was higher (although still <0.05) for cold steel resections measured with digital callipers at both the true and false vocal cord sites.

Comparisons were made for tissue shrinkage between the true and false vocal cord sites. For cold steel dissection, average tissue shrinkage was greater at the true vocal cord compared to false vocal cord. Conversely, with laser resection, tissue shrinkage was greater at the false vocal cord compared to true vocal cord. These differences were not all statistically significant.

The defect sizes in the larynx following resection of the specimen were all greater than 10 mm, ranging from an average of 10.37 mm in the true vocal cord cold steel resection (smallest average defect) to 11.27 mm in the true cord laser resection (largest average defect) (Table 3, Figure 4). There was no significant difference with either cold steel or laser resection when comparing the difference in defect sizes between the true and false cord specimens. Laser resection resulted in a larger defect compared to cold steel resection; this was significantly larger at the true vocal cord site (11.27 mm compared to 10.37 mm, *p* = 0.0002).

**Discussion**

In oncological surgery, obtaining clear surgical margins which are free from cancer is crucial. Unsatisfactory surgical margin is an independent risk factor for recurrence free survival as well as overall survival.14 The head and neck surgeon may find that the margin reported by the histopathologist is significantly less than the anticipated margin from the surgical resection. This discrepancy is due to shrinkage of the resected specimen which can affect the size of the margin. Additionally, the effect of thermalisation from the CO2 laser may further hinder accurate reporting of the surgical margin, compounding the effect of tissue shrinkage.

A study from 1986 reported on the shrinkage of the oesophagus after resection for carcinoma.15 The upper and lower margins of the specimen were reduced to 44% and 54%, respectively, of their in-situ lengths. This provided early documentation for shrinkage of surgical specimens which may explain the discrepancy claimed by surgeons and pathologists regarding the length of the margins.

In 1991, a melanoma group in New York studied the shrinkage of cutaneous surgical specimens from 199 malignant melanomas.16 They were able to devise a formula to calculate the in vivo (pre-excision) specimen diameter from the in vitro (fixed-tissue) specimen diameter. They group validated their findings in 1992.17 Four hundred and seven patients with malignant melanoma were included in the prospective study which measured pre-excision surgical margins and compared with fixed-tissue (contracted) margins. The overall shrinkage of specimens was around 20%. They found that the shrinkage was higher in younger patients (25% in patients aged less than 50 years, 20% in patients aged 50 to 59 years, and 15% in patients aged 60 years and older).

In head and neck surgery, using a canine model, one group attempted to quantify the change in size of the mucosal and muscle surgical margins following excision, formalin fixation and slide preparation of tongue and labiobuccal tissue.18 In the study from 1997, ten mongrel dogs underwent excision around custom-made discs and the excised specimens were measured immediately after excision, after formalin fixation and after slide preparation. The mean shrinkage from initial resection to final microscopic assessment was 30.7% at the lingual surface mucosal margins, 34.5% at the deep tongue margin and 47.3% at the labiobuccal mucosal margin. With this study, in order to obtain a 5 mm pathologically clear margin at histological assessment, the surgical margin needed to be at least 8-10 mm.

In humans, 27 patients with carcinoma of the tongue and buccal mucosa, mucosal margins were assessed prior to resection and half an hour after excision.11 The mean shrinkage of the tongue margins was 23.5% compared to 21.2% for buccal mucosal margins. This paper found that the shrinkage for T1/T2 tumours (25.6%) was significantly more compared to T3/T4 tumours (9.2%). The study suggested that the shrinkage should be considered to prevent concerns with positive post-operative margins when assessed by the histopathologist.

In 2014, another prospective study looked at 10 excised larynxes from patients with advanced laryngeal cancer who had undergone laryngectomy, with a view to assessing the thermal-effect caused by surgical incisions using scalpel, CO2 laser, harmonic scalpel and electrocautery.19 Incisions were made on macroscopically healthy laryngeal mucosa, some distance from the primary tumour. Using the scalpel incision as a control, the study quantified the amount of shrinkage for each of the other three resection techniques. The CO2 laser resulted in the largest amount of shrinkage, with a mean shrinkage of 2.91 mm compared to the surgical margin with the scalpel. The site of the incisions was not reported as being consistent between specimens and it was not possible to determine the amount of shrinkage which may also have been as a result of the scalpel incision. The larynx is divided into three regions (supraglottic, glottis and subglottis) with different histology and structures; it is possible that the shrinkage profile may be different between regions. It was not clear that this study allowed for this as the excisions were not consistently taken from the same locations on each larynx.

One study looked at the effect of formalin fixation on specimen size. In 100 head and neck cancer specimens excised during surgery, the specimens were measured immediately after resection and again after formalin fixation.20 The specimens decreased in size by 4-6% after fixation. The authors commented that care must be taken when interpreting specimen sizes so as to not underestimate the true size of the tumour.

TLM using the CO2 laser is a popular technique for management of early stage laryngeal cancer. This study has quantified the shrinkage of laryngeal specimens taken from both the true and false vocal cords, comparing laser resection to cold steel resection. We have tried to minimise discrepancy in size measurements by measuring all specimens three times with digital callipers and recording the average value, while viewing the specimen through the operating microscope to improve accuracy. For those specimens with permission for photography, the measurements were taken a second time using digital analysis to reduce subjective changes in measurement technique. All specimens were taken from the same location on each specimen and the study performed with both supraglottic (false vocal cord) and glottic (true vocal cord) tissue to account for any possible differences in shrinkage profiles in different areas of the larynx.

Our results demonstrate shrinkage of all specimens, regardless of their original location (true or false vocal cord), resection technique (cold steel or laser) or mounting technique (with or without cucumber). Laser resection resulted in average shrinkage between 35-45%, which was significantly more than tissue shrinkage with cold steel resection (8-14%). Mounting the specimens on cucumber also significantly increased shrinkage.

The present study was performed on fresh frozen larynxes. The effect of freezing and rewarming may affect the water content of tissues, compared to in vivo samples. We hypothesise that the frozen larynx will hold more water than in vivo. This is likely to result in a reduced thermalisation effect from the laser, possibly leading to an underestimate of the degree of tissue shrinkage in our study.

The results have implications for surgical practice. Assuming average shrinkage of 40% for laser resection, a 1 mm margin measured at the time of surgery will measure only 0.6 mm when reported by the histopathologist and will be described as a close margin, potentially prompting discussions about further treatment. Awareness of this shrinkage is important to help inform management plans. Conversely, the surgeon would need to remove a specimen using the laser with a margin of 1.7 mm to allow for a 1 mm margin as measured by the pathologist.

**Conclusion**

Early stage laryngeal cancer can be treated with TLM with the CO2 laser. The human larynx is a relatively small anatomical structure and excess removal of normal, healthy tissue during cancer surgery should be minimised. This study demonstrates that tissue specimens from the larynx shrink after excision. Laser resection results in significantly more tissue shrinkage compared to cold steel resection. Surgeons and pathologists should be aware of this shrinkage when considering positive and close margins, to ensure that future treatment plans for the patient are appropriate.

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**Ethical Standards**

The authors assert that all procedures contributing to this work comply with the ethical standards of the Keele University School of Medicine Student Project Ethics Committee and with the Helsinki Declaration of 1975, as revised in 2008. The dissection laboratory operates under the license from the Human Tissue Authority. All cadaveric material had consent for use in teaching and research.

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**Tables**

**Table 1:** Basic characteristics and descriptive statistics of the larynxes included in the study.

|  |  |  |
| --- | --- | --- |
| **Basic characteristics** | | **Number of specimens (%)** |
|  | *Sex* | |
|  | – Male | 22 (79) |
|  | – Female | 6 (21) |
|  | *Photography permission* | |
|  | – Yes | 25 (89) |
|  | – No | 3 (11) |
| **Descriptive statistics** | | **Mean average ± SD** |
|  | Age (years) | 81 ± 10 |
|  | Height (m) | 1.73 ± 0.12 |
|  | Weight (kg) | 68 ± 18 |
|  | Time in freezer (months) | 28 ± 8 |
| SD: Standard deviation. | | |

**Table 2:** Specimen size and specimen shrinkage at both the true and false vocal cord sites, with both cold steel and laser resection, measured with both digital callipers and ImageJ software.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Specimen size measured with digital callipers (n=28)** | | **Specimen size measured with ImageJ software (n=25)** | |
| **Cold steel resection** | **Laser resection** | **Cold steel resection** | **Laser resection** |
| *Specimen on microscope slide* | | | | |
| True vocal cord specimen size ± SD (mm) | 9.06 ± 0.49 | 6.46 ± 1.06 | 8.86 ± 0.47 | 6.18 ± 1.01 |
| True vocal cord specimen shrinkage ± SD (%) | 9.43 ± 4.94 | 35.41 ± 10.62 | 11.45 ± 4.72 | 38.23 ± 10.13 |
| False vocal cord specimen size ± SD (mm) | 9.15 ± 0.42 | 6.15 ± 0.97 | 8.96 ± 0.44 | 5.69 ± 1.04 |
| False vocal cord specimen shrinkage ± SD (%) | 8.50 ± 4.16 | 38.50 ± 9.68 | 10.42 ± 4.37 | 43.06 ± 10.38 |
| *Specimen mounted on cucumber* | | | | |
| True vocal cord specimen size ± SD (mm) | 8.89 ± 0.50 | 6.17 ± 1.10 | 8.61 ± 0.46 | 5.94 ± 1.06 |
| True vocal cord specimen shrinkage ± SD (%) | 11.08 ± 5.04 | 38.26 ± 10.98 | 13.92 ± 4.59 | 40.62 ± 10.61 |
| False vocal cord specimen size ± SD (mm) | 8.97 ± 0.42 | 5.88 ± 0.94 | 8.74 ± 0.38 | 5.49 ± 1.06 |
| False vocal cord specimen shrinkage ± SD (%) | 10.31 ± 4.18 | 41.18 ± 9.43 | 12.61 ± 3.75 | 45.06 ± 10.59 |
| Sizes are given as mean averages. SD: Standard deviation. | | | | |

**Table 3:** Defect sizes in the true and false vocal cord specimens with both cold steel and laser resection.

|  |  |  |
| --- | --- | --- |
|  | **Resection type** | |
| **Cold steel resection** | **Laser resection** |
| True vocal cord defect size ± SD (mm) | 10.37 ± 0.54 | 11.27 ± 1.09 |
| False vocal cord defect size ± SD (mm) | 10.49 ± 0.47 | 10.62 ± 1.98 |
| Sizes are given as mean averages. SD: Standard deviation. | | |