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Range of Vertical Hemilaryngectomy by Diode Laser: An Experimental Study in dogs

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ABSTRACT

Recent research on the diode laser has improved hemostatic cutting and an optic fiber delivery system. We explored the range and post-operative recovery of diode laser microsurgery. Three groups dogs: Group A, the right ventricular band and the right vocal fold, the area from the anterior commissure to the medial surface of the thyroid cartilage were cauterized. The VHL for Group B, the excision extended down to the lower margin of the thyroid cartilage and back until it reached the arytenoid cartilage. The additional cauterization of the medial surface of the thyroid cartilage and a 5 x 5 mm window on the anterior commissure to a depth of half the thickness of the thyroid cartilage. The Group C began at the anterior commissure it extended to the right along the thyroid cartilage to remove the right ventricular band, vocal fold, thyroarytenoid muscle and cricoarytenoid muscle. After one week, wounds were completely or partially covered by neo-mucosa. Inflammation and edema were apparent. A pseudomembrane developed in Groups B and C with evidence of infection in Group C. After four weeks the glottal closure and resulting voice was best in Group A and worst in Group C. Likewise, dogs in Group C had a moderate level of hoarseness, while dogs in Group A regained nearly normal voices. Collagen proliferation was most prominent in group C with limited scarring in group A. The diode laser allows a wide surgical range. Postoperative recovery was acceptable. the diode laser shows potential in clinical microsurgery.

Key words: Diode, laser microsurgery, extent, complication

INTRODUCTION

The use of laser surgery for the treatment of laryngeal carcinomas has increased dramatically (Grant et al., 2007; Roedel et al., 2010; Ambrosch and Pazel, 2011; Lim et al., 2011). As compared to conservational surgeries, laser resection preserves a better quality of voice. The procedure is quicker and, in the case of recurrence, provides more salvage options (Motta et al., 1997, 2005; Peretti et al., 2000; Ferri and Armato, 2008; Rubinstein and Armstrong, 2011; Lucioni et al., 2011). Laser surgery is also more cost effective than radiotherapy and when the potential for repeated surgery is considered, has a better cure rate (Motta et al., 2005; Ferri and Armato, 2008; Kujath et al., 2011). Due to its short operative time and limited risk of complications,
laser surgery has begun to be performed as an outpatient procedure further reducing patient inconvenience (Altuna et al., 2005). Many studies have reported cure rates in excess of 80% when laser surgery is applied to T1 and T2 cancers (DeRowe et al., 1998; Peretti et al., 2000; Eckel et al., 2000; Motta et al., 2005; Manola et al., 2008). Comparable cure rates can be achieved in T2 cancers and the procedure has been applied successfully to select T3 cancers with the major contradiction that the carcinoma does not infiltrate the laryngeal cartilage (Peretti et al., 2000; Damm et al., 2000; Motta et al., 2005; Olthoff et al., 2009; Vilaseca et al., 2010; Lopez-Alvarez et al., 2011). Since the inception of laser surgery, the CO₂ laser has been used predominantly. The CO₂ laser emits light in the far red spectrum (Vilaseca et al., 2010). This wavelength interacts strongly with water to vaporize laryngeal tissue (Bajaj et al., 2010). CO₂ lasers penetrate tissue to a depth of only approximately 0.1 mm. This limited penetration reduces the number blood vessels effectively sealed during surgery thus increasing the bleeding observed during CO₂ laser procedures (Vilaseca et al., 2010). Additionally, the use of optic fibers to deliver the energy generated by the CO₂ has yet to be perfected (Zeitels et al., 2006). The laser provides only straight-line delivery making it difficult to treat areas obscured by healthy tissue and reducing the accuracy of resection (Bajaj et al., 2010). The rigid system also limits laryngeal visualization during surgery, increasing the difficulty of performing delicate procedures (Zeitels et al., 2006). Davis et al. (1982) specified the thyroid cartilage as the outward constraint and the cricoid cartilage as the lower boundary of CO₂ laser surgeries. However, these authors noted difficulty in accessing these areas, especially as the excision moved downward, even with excised specimens which provide optimal endoscopic exposure (Davis et al., 1982).

The diode laser emits in the near infrared spectrum (Vilaseca et al., 2010). These wavelengths are absorbed predominantly by melanin, hemoglobin and darker pigments (Sullins, 2002). The concentration of dark pigments determines the penetration extent; however these lasers generally reach depths of 0.3 to 1.0 mm (Sullins, 2002; Vilaseca et al., 2010). Diode lasers demonstrate good hemostatic cutting partially because they can access and seal deeper vessels; they produce a larger thermal damage zone which provides a wider range of coagulation (Zeitels et al., 2006; Robinson et al., 2007; Vilaseca et al., 2010). In addition to reducing operative bleeding, the diode laser may also be administered with an optic fiber (Vilaseca et al., 2010). The optic fiber allows for more precise manipulations and permits access to areas (Bajaj et al., 2010; Sun et al., 2010). These features make the diode laser easier to use and allow for more rapid surgeries (Vilaseca et al., 2010; Bajaj et al., 2010). Additionally, when combined with a flexible laryngoscope the diode laser can facilitate minimally invasive, outpatient surgeries (Zeitels et al., 2006; Vilaseca et al., 2010). Moreover, the diode laser is small, easily transported, relatively inexpensive and requires little day to day maintenance (Sullins, 2002; Ferri and Armato, 2008; Bajaj et al., 2010).

Although promising, the diode laser is a relatively new technology in the field of laryngeal surgery therefore our research aimed to demonstrate the proficient performance of this laser in completing one common procedure, the Vertical hemilaryngectomy (VHL). Three different ranges of VHL were performed on twelve experimental dogs. Post operative observations were recorded to determine if recovery after diode laser surgery was acceptable.

**MATERIALS AND METHODS**

**Experiment animals:** This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use
Committee (IACUC) of Gongli Hospital (Permit Number: 20060828001). Twelve experimental dogs of either sex, weighing between 13-18 kg, were randomly assigned to three surgical groups: A, B and C. Dogs were assigned a number one through four within each group. Dogs were handled under the supervision of the institutional review board of Gongli Hospital.

**Anesthesia:** Prior to surgery the dogs were fasted for eight hours. The animals were anesthetized using 3% sodium pentobarbital with a dose of 30 mg kg⁻¹ administered intravenously. Anesthesia was maintained with additional doses of 3% sodium pentobarbital.

**Surgical equipment:** A semiconductor 805 nm gallium-arsenide-aluminum (GaAsAl) diode laser (Diomed-25; Diomed, Cambridge, UK) was used to perform a Vertical hemilaryngectomy (VHL) with an output set at 10 W. A power output of 10 W was strong enough to readily vaporize soft tissues. In surgical procedures that approached the thyroid cartilage, the cutting power of the laser was reduced by using impulse instead of continuous dosage. The impulses lasted for one second each. The laser was stabilized in a self-designed fiber optic holder (patent coding in China- IP Number: ZL 04 2 0020225.5) which permitted bending to enlarge the visual field during surgery (Fig. 1). A 650 nm semiconducting laser was used for visible targeting and operated at a power of 3 mW with a 400 μm diameter tip. The surgery was monitored with an anterior commissure laryngeal (PuAo, Tonglu, China) and a Sony TV monitor system (Sony, Tokyo, Japan).

**Surgery:** Each group received a slightly different version of the VHL. In Group A the anterior commissure was resected to the intima of the thyroid cartilage and the right vocal fold was excised. This surgery served as a standard to compare with the more aggressive procedures undertaken in groups B and C. The right ventricular band and the right vocal fold, the area from the anterior commissure to the medial surface of the thyroid cartilage were cauterized. A 10 W pulse laser was emitted for 1 s to cauterize the anterior commissure tissue (Fig. 2). The VHL for Group B was more

**Fig. 1:** Digital laryngoscopic image of a dog in group A immediately after surgery. Excisions are covered by yellow-brown burn eschars
Fig. 2: Digital laryngoscopic image of a dog in group B, immediately after surgery. Yellow-brown and black burn eschars cover the surface of the laser excision

Fig. 3: Digital laryngoscopic image immediately after surgery from a dog in group C. Yellow-brown and black burn eschars cover the wound surface

extensive, encompassing the entirety of the region removed in Group A and several additional structures. The resection of the anterior commissure extended into the anterior commissure cartilage. In addition to the right vocal fold, the right ventricular band, the thyroarytenoid muscle and part of the lateral cricoarytenoid muscle were also removed. The excision extended down to the lower margin of the thyroid cartilage and back until it reached the arytenoid cartilage. The additional cauterization of the medial surface of the thyroid cartilage and a 5×5 mm window on the anterior commissure to a depth of half the thickness of the thyroid cartilage (Fig. 3). Cauterization of the anterior commissure and thyroid cartilage was completed with a 10 W pulse laser emitted for 1 s to densely cauterize several points. These points were fused with a transducer.
via the non-surface contact method. The resection in Group C was more extensive still; beginning at the anterior commissure it extended to the right along the thyroid cartilage to remove the right ventricular band, vocal fold, thyroarytenoid muscle and cricoarytenoid muscle. The paraglottic space was remove to the lamina of the thyroid cartilage, the excision then moved down to resect part of the eirothyroid membrane and the upper to the margin of the crecid cartilage then back to remove the entirety of the right arytenoid. With the additional opening of a small window approximately 5×5 mm in size from the anterior commissure through the thyroid cartilage (Fig. 4). In excising anterior commissure and cartilage tissue, a 10 W pulse laser was emitted for one second to densely cauterize several points. these points were fused with a transducer by the non-surface contact method.

**Post-operative:** Dogs one through three of each group was returned to the holding room after regaining consciousness. Dog number four of each group was humanely sacrificed by intravenous injection with 3% sodium pentobarbital (90 mg kg⁻¹) immediately after the operation. This dog underwent gross examination to ensure the resections completed were consistent with the previously described protocol.

The larynges of all twelve animals were photographed immediately after operation using an Olympus P-240 Electronic laryngoscope (Olympus, Tokyo, Japan) under a suspension laryngoscope (FuAo, Tonglu, China) and a Sony TV monitor system (Sony, Tokyo, Japan). The larynges of remaining nine dogs were examined again one week after surgery. A perceptual measure of hoarseness was recorded by experienced clinicians evaluating voluntary phonations observed during the recovery period. Weight change and postoperative complications were recorded for four weeks after surgery. After four weeks the dogs were humanely euthanized. Digital images of the larynges were recorded and gross specimen examination was undertaken.

![Image](image_url)

**Fig. 4:** Digital laryngoscope image taken four weeks after surgery of group A dog. The neo-mucosa covering the wound surface is smooth and forms a new right vocal fold that is slightly thinner and lower that the contralateral, healthy vocal fold.
Histology: Specimens were taken from the anterior commissure 4 weeks after surgery for histological evaluation. Samples were stained with hematoxylin and eosin (HE) and van Gieson stains. Results of these sections were evaluated by a trained pathologist.

Statistical analysis: A two-way ANOVA was employed for comparisons of body weight pre-operation, one week post operation and four weeks post operation for the three operative groups. A p-value of less than or equal to 0.05 was considered statistically significant.

RESULTS
Post operative: Gross examination of the fourth dog of each group post-mortem revealed proper surgical excisions. The range of resection was as anticipated. In group B the muscular excisions and remaining portion of the arytenoid cartilage were identified. In group C, the interior of the thyroid cartilage was visible and the entirety of the arytenoid cartilage was absent. In all three groups the wounds immediately after surgery were covered by yellow-brown burn eschars. Figures two, three and four are digital images captured after surgery for dogs in groups A, B and C, respectively.

One week post surgery: In examinations one week after surgery differences between the three groups became apparent. Wounds in Group A dogs were covered by a neo-mucosa. A moderate edema and inflammation was observed. In Group B, surgical wounds were only partially covered by the neo-mucosa. A moderate level of edema and inflammation was observed. The dogs had also developed a pseudomembrane over parts of the wound surface. Observations of Group C dogs detected partial coverage by neo-mucosa. Edema, inflammation and a pseudomembrane were apparent. Evidence of granulation, extensive exudation and some signs of infection were detected.

Four weeks post surgery: Gross examination after four weeks healing time revealed results consistent with the trend established during the week one observations. As expected, Group A dogs displayed larynges best resembling normal (Fig. 5). The mucosa that had developed over the wound was smooth. A new right vocal fold had formed which, under pathological assessment, was determined to be composed of fibrous, scar-like tissue. This newly formed vocal fold was thinner and located slightly lower in the glottis than the contralateral, healthy vocal fold (Fig. 6). A small opening was reported during glottal closure. Similar to Group A dogs, the dogs in Group B had developed a smooth mucosal covering over the wound area. The external remnants of the vocal fold structure had formed a new vocal fold that was thinner, narrower and located slightly lower than the healthy contralateral vocal fold. Within the glottis, localized granulation had developed (Fig. 7). The glottal closure observed in these dogs was incomplete. Group C dogs displayed a dark red neo-mucosa that covered the entire wound surface (Fig. 8). The thyroid cartilage was thinner on the operative side of the glottis. There was a slight elevation of the mucosa over the location of the original vocal fold, but the glottal closure remained poor. The window cauterized in group C was enclosed completely by neo-mucosa and scar-like tissue and there was no sign of infection (Fig. 9-10). Transillumination of the window showed that the soft tissue at the anterior commissure had been repaired while the cartilage had not (Fig. 10).

In contrast to the observations at one week, there was no evidence of exudation or wound infection.
Fig. 5: Excised larynx specimen from group A dog sacrificed four weeks after surgery. Arrow: Newly formed right vocal fold which is observably thinner and lower than the normal left vocal fold.

Fig. 6: Digital laryngoraphy image of group B dog taken four weeks after surgery. The neo-mucosa forms a smooth covering over the wound surface. The external residue of the right vocal fold formed a new vocal fold like structure that is thinner, narrower and lower that the contralateral fold. Granulation growth can be seen in localized areas.

Van Gieson staining confirmed high collagen content in the scar tissue associated with the lesion in group C with little collagen present in the scar of the subject in group A and moderate collagen proliferation in group B (Fig. 11). HE staining indicated edema in group A, with fissure-like blood vessels present. The group A tissue was otherwise relatively normal. Hyaline degeneration within the mesenchyme was evident in group B, while the group C slide was again dominated by collagen tissue and a large number of fibroblast cells. The group C slide indicated that
Fig. 7: Digital laryngoscopic image after four weeks of surgery of group C dog. The neo-mucosa is dark red in color and covers the entirety of the wound surface. At the location of the excised right vocal fold the neo-mucosa is slightly raised. Evidence of exudation and infection has disappeared. The lamina of thyroid cartilage on the operative side is thinner than that of the contralateral side.

Fig. 8: Larynx from group C dog after four weeks of surgery. The window was enclosed completely by the neo-mucosa, and there was no sign of infection. Submucosal hyperplasia was occurring. A neo-mucosa covered the surface of all lesions; however, the epithelium was histologically abnormal. The cell array was irregular and the mucosal layer was thicker than normal.
Fig. 9: Larynx from group C dog after four weeks of surgery. The lesion surface of the window was enclosed completely by the neo-mucosa and transillumination showed the absence of cartilage at the anterior commissure.

Fig. 10(a-c): Histological sections after four weeks of surgery and stained using van Gieson stain at 100x magnification. (a) Group A; minimal collagen proliferation (collagen stains pick while muscle, cytoplasm RBC and fibrin appear yellow), (b) Group B; more collagen present and (c) Group C; extensive collagen proliferation.

**Hoarseness:** During the course of observation an increased level of hoarseness was noted for all groups one week after surgery. Dogs in groups A and B were mildly hoarse, while those in
Fig. 11(a-c): Histological sections after four weeks of surgery and developed with HE stain, shown at 100x magnification, (a) Group A; marked edema is evident, Fissure-like blood vessels can be observed, (b) Group B dog; collagen proliferation is moderate with hyaline degeneration present in the mesenchyme and (c) Group C; evidence of hyperplasia with a large number of fibroblasts and collagen present.

<table>
<thead>
<tr>
<th>Group</th>
<th>Hoarseness</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-operation</td>
<td>Week 1</td>
</tr>
<tr>
<td>A</td>
<td>None</td>
<td>Mild-moderate</td>
</tr>
<tr>
<td>B</td>
<td>None</td>
<td>Mild-moderate</td>
</tr>
<tr>
<td>C</td>
<td>None</td>
<td>Moderate</td>
</tr>
</tbody>
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*Significant differences, p<0.05

Group C had a moderate level of hoarseness characterized by an audible respiratory sound during vocalization. After four weeks recovery time, dogs in Group A had regained nearly normal vocal quality, Group B dogs had a mild but detectable hoarseness and those in Group C maintained a moderate level of hoarseness (Table 1).

**Weight change and other complications:** Dogs in all three groups showed weight loss during the first week after surgery. The initial weight change was significant for dogs in Groups A and C. Over the course of the next three weeks the dogs regained some of the lost weight; however, at four weeks post surgery, Group C dogs still exhibited a significant difference between their initial and measured weights (Table 1). No other significant post-operation complications were noted in any of the groups.
DISCUSSION

Our study showed that the Diomed-25 semiconductor laser, transmitted by an optic fiber system and in coordination with a suspension laryngorome and a bendable optic fiber holder, is able to reach to a wide operation field. Resection of the anterior commissure was successfully extended forward to the intima of the thyroid cartilage and into the cartilage of the anterior commissure. Using the diode laser we were able to remove the right ventricular band, vocal fold, thyroarytenoid muscle and lateral cricoarytenoid muscle. The resection of the paraglottic space penetrated deep into the median intima of the thyroid cartilage. Unlike the CO₂ laser which struggled to complete excisions in the lower portion of the larynx, the diode laser successfully reached the cricothyroid ligament and the upper margin of the cricoid cartilage (Manola et al., 2008). We found that, by using the bendable optic fiber holder, the anterior commissure is more easily exposed. In our experience, the CO₂ laser is adequate for use when the larynx is well exposed by suspension laryngorome; however, in cases where good exposure is not possible the diode laser represents a valuable surgical option. Additionally, the diode laser avoids the difficulty associated with the straight-line delivery of the CO₂ laser.

The three operation modalities attempted in Groups A, B and C were completed with satisfactory outcomes. Weight changes associated with the surgeries did not deviate greatly from those observed in our past experience with CO₂ laser surgeries and operative bleeding was reduced by using the diode laser. Wound healing and vocal preservation were dependent on the extent of the surgery with the modest excisions of Group A healing the best and maintaining the highest quality voice of the three groups. However, four weeks after surgery the wound healing in all three groups and voice quality were deemed acceptable. Compared to our experience with the CO₂ laser, the diode laser achieves similar post-operative success. Selection of the proper surgical modality is dependent on the range of involvement of the carcinoma. In our experience, patients with T1 carcinomas can be effectively treated with the type of resection completed in Group A. Of the patients undergoing laser microsurgery at our hospital, 60% present with T1 cancers, another 30% are diagnosed with cancers treatable by the procedure completed in Group B. The remaining 10% of patients require more aggressive surgery and would follow the procedure used in Group C.

Past research on the diode laser has isolated several advantages. The low cost, easy transportation and minimal maintenance have attracted clinicians to the diode laser (Sullins, 2002; Robinson et al., 2007; Ferri and Armato, 2008; Riguad et al., 2009; Bajaj et al., 2010; Sun et al., 2010). Additionally, this laser is reported to facilitate shorter surgeries with minimal bleeding. The use of an optic fiber to deliver the laser has improved the ease, accuracy and range of laser microsurgery (Zeitels et al., 2003; Olthoff et al., 2009; Vilaseca et al., 2010; Bajaj et al., 2010; Lopez-Alvarez et al., 2011). As observed in this study, the diode laser reached areas previously inaccessible by the CO₂ laser (Davis et al., 1982). Additionally, our post-operative observations recorded no serious post operative complications and outcomes comparable with those observed with CO₂ surgery (Motta et al., 1997; Peretti et al., 2000; Zeitels et al., 2003; Ferri and Armato, 2008). Owing to these advantages, the diode laser shows potential to supplant the CO₂ laser for use during microsurgery.

When interpreting these results one should note specific differences between the surgery performed in humans and the one we completed in the dogs used in this study. First, incisions in animals may be larger than those performed on humans. Also, the head of a dog can be more overextended thus granting great access to areas within the larynx. We observed that the root of
the tongue in a dog is thinner making it easier for the suspension laryngoronge to expose the whole larynx and anatomically. The ventricular band and vocal cord of a dog are thinner. Nevertheless, the operation procedure is similar both in dogs and in humans.

While our results are promising for the future of the diode laser, additional research is needed to confirm success of the diode laser VHL in human subjects. The use of canine subjects in this study facilitated gross specimen examination to confirm the range of the surgery; however, anatomical difference between humans and canines may affect the feasibility and outcome of the VHL. Future studies may wish perform CO₂ and diode laser mediated surgery on matched patients to facilitate further comparisons between these two lasers.

CONCLUSION

Compared with the conventional open laryngectomy, laser excision is significantly advantageous as it proceeds with minimal resection, results in more rapid wound healing and preserves better laryngeal function. Although the CO₂ laser is currently predominant in the microsurgery field, the diode laser has several advantages. Our results show the diode laser can access a wide surgical range. Post-operative observations reveal outcomes comparable to those achieved with CO₂ lasers. With future investigations on human subjects the diode laser may supplant the CO₂ laser in clinical microsurgery.

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