

# INVESTIGATION OF GREEN TURTLE (*CHELONIA MYDAS*) CUTANEOUS FIBROPAPILLOMATOSIS RECURRENCE RATES FOLLOWING DIODE LASER SURGERY



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## Abstract

A pilot study was performed to investigate fibropapillomatosis (FP) recurrence rates after 940 nm-diode laser surgeries. Fifteen animals had a total of 274 tumors removed by diode laser. Cutaneous FP recurrence rates were evaluated during a period of 1-year. According to tumor sizes classification, 166 tumors (60.6%) were <1 cm, 67 tumors (24.5%) between 1-4 cm, 31 tumors (11.3%) >4-10 cm and 10 tumors (3.6%) >10 cm. Seven animals (46.6%) were lightly afflicted, 4 (26.7%) moderately, and 4 (26.7%) heavily afflicted. Thirteen animals (86.7%) did not present tumor regrowth and 2 (13.3%) presented small tumors after 1-year. Copyright 2018 Elsevier Inc. All rights reserved.

**Key words:** Laser surgery; marine turtles; veterinary medicine

**F**ibropapillomatosis (FP) is an infectious disease that affects green turtles (*Chelonia mydas*), but it is sometimes found in other species.<sup>1,2</sup> The benign neoplasms are linked to a herpes virus etiology, and can be found in the form of a single or multiple lesions of varying sizes, with different pigmentation, and location.<sup>1,2</sup>

This disease develops in young and adult animals, not being described in newborns.<sup>1</sup> Despite the benign behavior, it can directly compromise sight, swimming, prehension of food and, consequently, the animal's life. They also affect internal organs, impairing their function.<sup>3</sup> It is believed that the occurrence of this disease is linked to anthropogenic actions, e.g., polluted coastal areas, contaminated with agricultural, domestic, industrial waste or marine biotoxins, among other factors.<sup>4</sup> At the moment, there is no description of an effective conservative treatment,

although surgical excision is the most recommended procedure. However, the regrowth of the tumors eventually occurs after the surgery.<sup>4,5</sup>

High-power diode lasers in surgical procedures are under investigation in various other areas of health sciences.<sup>6,7</sup> The absorption of electromagnetic energy by the target tissue is quickly converted into heat, leading to photo thermal effects as carbonization, vaporization, and coagulation/degradation, or simply denaturation of the constituent proteins.<sup>7</sup> Literature shows growing reports of lasers in veterinary medicine,<sup>8,9</sup>

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and substantial information provides by human medicine encourages its use, which includes reduction of bleeding and decrease of the tumor recurrence rates.<sup>6,10</sup> The purpose of this pilot study was to evaluate recurrence of green turtle cutaneous FP following diode laser surgery.

## MATERIAL AND METHODS

Fifteen green turtles (*C. mydas*) presenting with FP were admitted at a rehabilitation center located on southeast coast of Brazil, between 2013 to 2014. FP diagnosis was confirmed based on clinical manifestations and histopathological analysis. After supportive treatments and restoration of body condition, we elected to perform surgical interventions to remove the tumors. Prior to surgery, the tumors were classified individually by size according to Work et al.<sup>11</sup> (A < 1 cm; B = 1 to 4 cm; C > 4 to 10 cm; D > 10 cm diameter). Tumor score reflected the spectrum of severity of FP in green turtles ranging from nonafflicted (0) to lightly (1), moderately (2), and heavily (3) afflicted according to Balazs.<sup>12</sup>

All animals were anesthetized with ketamine (5 mg/kg IM), midazolam (2 mg/kg IM), and morphine sulfate (0.5 mg/kg IM) as preanesthetic medication, followed by propofol (5 mg/kg IV). General anesthesia was achieved with endotracheal intubation and maintenance with sevoflurane (3% to 5%). Local anesthesia was performed by intratumoral infiltrative blockage with lidocaine hydrochloride 2% diluted in physiological saline solution, not exceeding the toxic dose of 7 mg/kg.

Surgical excision was performed with a diode laser (Medilaser, DMC Group, São Carlos, SP, Brazil) emitting at  $\lambda = 940$  nm with 10 W of optical power in pulsed mode (repetition rate of 10 Hertz and 50 milliseconds of pulse width) coupled to a 400  $\mu$ m-optical fiber diameter. For all tumors, an

incision was made around the base of the tumor with surgical margins of 1 cm. Subsequently, after tumor removal, the adjacent tissue was carbonized by defocusing the laser, i.e., distancing the optical fiber from the target tissue at approximately 5 cm (Fig. 1A–C).

Skin sutures in A and B tumor's site, as well as for carapace and plastron tumors were not performed. If profuse bleeding occurred in large tumors (C and D), we controlled the hemorrhage by clipping the vessel followed by tumor removal, and then ligating the vessel with suture.

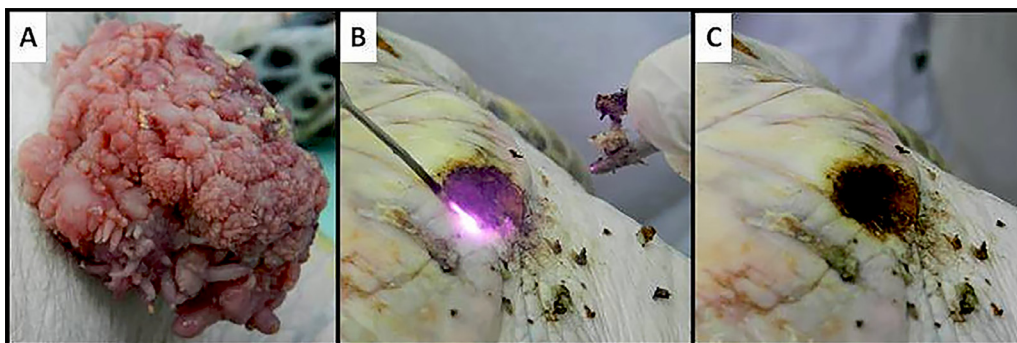
After the surgery, the animals were kept out of the water on soft, moist surfaces for 48 hour to recovery and observation. Antimicrobial therapy was started with ceftazidime (22 mg/kg IM q.72 hour) and amikacin sulfate (5 mg/mL IM q.72 hour), and continued for 30 days.

Antiinflammatory therapy was pursued using meloxicam (0.2 mg/kg IM q.24 hour) for the course of 5 days in captivity.

The animals were followed for 1-year for clinical evaluation according to the following organizations recommendations: National Parks and Wildlife Service,<sup>13</sup> Florida Fish and Wildlife Conservation Commission<sup>14</sup> and Wider Caribbean Sea Turtle Conservation Network.<sup>15</sup> Time to complete wound healing, presence or absence of secondary infection in postoperative period and recurrence of tumors were assessed and recorded.

## RESULTS

Most rescued animals showed weakness and improper body condition (anemia, cachexia, and parasitosis). After support treatment, we removed 274 tumors distributed on the dorsal and ventral fins, carapace and plastron, head and cervical region. The occurrence of tumors classified as A, B, C, and D was 166 (60.5%), 67 (24.5%), 31 (11.5%), and 10 (3.5%), respectively (Table 1).



**FIGURE 1.** Representative images of FP surgery using a diode laser. Nonpigmented tumor classified as C according Work et al.<sup>11</sup> before surgery procedure (A); Diode laser surgery (B); Carbonized adjacent tissue after tumor resection (C).

**TABLE 1. Number of occurrences, tumor size, and location, tumor score, and regrowth found in sea turtles with FP**

Turtle ( <i>C. mydas</i> )	N	Tumors Location (N)				Tumors Size (N)				Tumor Score	Regrowth (N/size & score)
		H/N	F	C	P	A	B	C	D		
1	28	2	21	1	4	20	6	1	1	3	-
2	49	9	36	0	4	18	16	11	4	3	-
3	17	3	12	0	2	6	8	3	-	2	-
4	16	4	10	1	1	12	3	1	-	2	-
5	2	0	2	0	0	2	-	-	-	1	-
6	28	4	20	1	3	23	5	-	-	2	-
7	8	2	6	0	0	4	4	-	-	1	-
8	3	1	2	0	0	-	3	-	-	1	-
9	5	2	3	0	0	2	3	-	-	1	-
10	4	1	2	0	0	4	-	-	-	1	-
11	30	3	22	1	4	8	10	9	3	3	3/A1
12	15	2	10	0	3	4	3	6	2	3	-
13	60	10	45	1	4	58	2	-	-	2	2/A1
14	5	1	3	0	1	3	2	-	-	1	-
15	4	1	3	0	0	2	2	-	-	1	-
Total	274	45	197	5	27	166	67	31	10	n.a	n.a
%	100	16	72	2	10	60.5	24.5	11.5	3.5	n.a	n.a

N: number of tumors.

n.a: not applied.

Individual tumors size: A < 1 cm; B = 1-4 cm; C > 4-10 cm; D > 10 cm diameter.

Tumor score: nonafflicted (0) to lightly (1), moderately (2), and heavily (3) afflicted.

Tumor location: H/N = Head and/or Neck; F = Flippers; C = Carapace; P = Plastron.

According to severity score, 7 animals (46.6%) were lightly afflicted, 4 (26.7%) moderately, and 4 (26.7%) heavily afflicted. Periocular tumors were found in 1 animal (6.6%), but we did not perform surgery due to risk of ocular injury secondarily. The maximum number of tumors in a single animal was 60 and the minimum was 2. Additionally, about 70% of tumors were pigmented, while the other 30% were nonpigmented.

The surgical procedure did not require primary closure for hemostasis in A and B tumor's site owing to photothermal effect. In the C and D tumor sites, profuse bleeding occurred in 3 C (3/31, 9.7%) and 5 D (5/10, 50%) tumors due to the presence of large vessels, however it was easily controlled by clipping the vessels and subsequently suture.

The average time to complete healing was 28 days for sutured and nonsutured A and B skin tumors due to small sizes; and 35 days for C and D skin tumors. On the other hand, approximately 60 days were required for complete healing process of nonsutured lesions located on the carapace and plastron. No infections were observed. Interestingly, only 2/15 animals (13.3%) presented with tumor recurrence, while the other 13/15 animals (86.7%) did not present with FP during the follow-up period.

It is noteworthy to remark that regardless of size tumor or location, we observed that pigmented tumors were more easily removed than nonpigmented tumors. In addition, no bleeding from pedunculated tumors was noticed. In contrast, the time of surgery and bleeding during removal of sessile tumors in the plastron (a region with little or no pigmentation) were higher compared to pedunculated tumors.

No adverse effects and postoperative complications were observed, even though a large number of tumors were removed.

## DISCUSSION

In this study, the diode laser surgery showed to be an attractive option to remove FP with no side effects postsurgery. Although there is no consensus to treat FP, some therapeutic approaches include photodynamic therapy,<sup>16</sup> electro-chemotherapy,<sup>17</sup> and cryosurgery.<sup>18</sup> Despite the promising results, the large number of tumors found sometimes in sea turtles could limit those procedures. Therefore, surgical removal becomes more suitable to perform.

Few studies have investigated the recovery and rehabilitation time (1-year following surgery with

no FP recurrence)<sup>13-15</sup> until the release back into the sea.<sup>5</sup> According to Wyneken et al.<sup>5</sup> the recovery time needed is inaccurate mainly due to immune factors. The authors describe successive recurrences in animals with more than 10 years of captivity preventing the release.

In a study by Page-Karjian et al.<sup>3</sup> the authors investigated the factors influencing survivorship of rehabilitating green turtles with FP and reported the use of CO<sub>2</sub> laser to remove 23 FP lesions in 13 green turtles. The procedure was promising, however, 5/13 animals (38.5%) presented relapses in an approximated period of 36 days, and at the end of the study, 14/23 of the tumors (60.9%) regrown. In contrast, our results showed FP recurrence in 13.3% (2/15) of the animals. In fact, it is difficult to compare both techniques since in their study, no information is provided regarding laser protocol. These controversial results could be explained by the different light parameters employed as wavelength, optical power, and pulse repetition rate.

Another point to be highlighted is that tumors have a high viral load.<sup>19</sup> Knowing that viral agents are involved in the FP genesis, discussions about the use of vaccines and antiviral drugs have taken place.<sup>20</sup> Nonetheless, clinical trials of any modality have not been carried out until now. In our study, when the adjacent tissue was carbonized after tumor removal, reduction of viral load may have occurred in the tumor's area preventing tumor regrowth.

Diode lasers have distinguished themselves in soft tissue surgeries for causing tissue coagulation, avoiding the use of sutures, surgical time reduction, and decreased risk of postoperative infections owing to lower bacterial contamination promoted by heat production.<sup>21</sup> This study aimed to demonstrate the use of a diode laser in surgical treatment of FP in green turtles. The procedure was considered effective, reinforcing previous results that demonstrated the benefits of diode laser to others soft tissue surgeries.<sup>6,7</sup>

Since we did not observe any adverse effects, and FP remains noncurable, treatment with diode laser showed to be a promising approach for tumor removal with limited recurrence of FP. The limitations of this technique include eyelids tumors due to possible ocular damage caused by photothermal effects, laser safety, operator skill sets, equipment cost, and availability.

In summary, diode laser surgery shows promise as a treatment option for cutaneous FP in green turtles. This method should be considered a new option for FP resection with the benefits of absence of secondary infections and promotion of quick

recovery with a low recurrence rate after 1-year, and low complications. The clinical observations reported in this pilot study encourage us to recommend this technique for surgical resection of FP in sea turtles. Furthermore, new insights suggest that FP shares genomic drivers and therapeutic vulnerabilities with human cancers, becoming FP amenable to treatment with human anticancer therapeutics.<sup>22</sup> Thus, further directions could encompass the association of anticancer drugs and diode laser surgery.

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