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Histopathological effects of laser fiber diameter in different laser modulations: an experimental exvivo study

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Abstract

We aimed to compare the histopathological effects of different laser fiber diameters on kidney tissue using different modulations of holmium laser. An ex vivo trial was performed employing veal kidneys. A 100 W HoYAG laser device (Cyber Ho, Quanta System, Samarate, Italy) was employed, accompanied by 550 µm and 272 µm laser fibers for all experimental procedures. Incision depth (ID), vaporization area (VA), coagulation area (CA), surface section (SS), lateral effect (LE) and total laser area (TLA, calculated as VA+CA) were assessed. Histopathological measurement results of different laser fiber diameters were compared in Standard mode (SM) and Virtual Basket™ (VB) mode. In SM and VB, a statistically significant difference was found in the mean CA values between 272 (µm) and 550 (µm) laser diameters (p: 0.02 and p<0.001, respectively). In SM, the lowest and highest mean CAs were observed with the 272 (µm) lasers at 0.5 J-40 Hz and 1.5 J-40 Hz laser configurations (0.263±0.003 mm^2 and 1.353±0.015 mm^2, respectively). In VB, the lowest and highest mean CAs were observed with the 550 (µm) at 0.5 J-20 Hz and 1 J-20 Hz laser settings (0.987±0.014 mm2 and 1.969±0.029 mm^2, respectively). We found that both SM and VB modulations have higher CA values, a measure of hemostasis, in 550 µm fibers compared to 272 µm fibers.

Keywords: Ho:YAG, laser effect, laser fiber diameter, laser modulations

Introduction

Various types of lasers serve diverse purposes in different branches of medicine [1-3]. In urology, it has become an indispensable surgical tool, especially as endoscopic procedures have replaced open surgery over the years. When we consider of lasers in urology, the holmium:yttrium–aluminum–garnet (Ho:YAG) laser comes to mind, which can be used in meany surgical procedures such as stone surgery, benign prostatic hyperplasia, and bladder cancer with its simple and effective structure that allows for versatile use [4-6]. Although the Ho:YAG laser was previously used only in stone surgery, in recent years it has been used extensively in Holmium Laser Enucleation of the Prostate (HoLEP) surgery [7].

After the widespread use of the HoYAG laser in surgeries such as HoLEP, intensive research and development studies have been conducted to make the holmium laser more effective in soft tissues. In this context, the more effective use of holmium laser with different modifications and technical features has been the most important method used by the industry. Virtual Basket™ (VB) (Quanta System, Samarate, Italy) has come to the forefront as a modulation that helps in a more effective dissection plan and effective stone crushing by creating two consecutive bubbles [8,9]. In HoLEP surgery, VB modulation has been suggested to have advantages such as better enucleation and less

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bleeding [9]. Although many studies have been conducted on VB modulation, which has attracted attention for these features, in stone surgery, there are limited studies on its effect on tissues [10,11]. The use of holmium lasers for many different purposes has raised the question of which fiber size is more appropriate. Although there are studies on which laser fiber diameter is safe and suitable for stone surgery with holmium lasers, there are no similar studies on HoLEP or other soft tissues [12,13]. Our trial objectived to compare the histopathological effects of different laser fiber diameters on kidney tissue using different modulations of holmium laser.

Material and Methods

Experimental setup

Ethics committee approval is not required for this study. The manuscript contains no clinical studies or patient data, nor does it contain any studies with human or animal subjects performed by the any of the authors.

Veal kidneys were used in an ex vivo trial due to their availability of smooth and flat soft tissue, which is conducive for histological examination, as well as their multilocular anatomical structure. The veal kidneys were obtained directly from fresh cadavers at the slaughterhouse and stored in 0.9% saline at 2–6°C until laser activation. In the course of each experiment, the veal kidneys were meticulously sectioned into blocks measuring 3×2×2 cm. These blocks were then securely affixed onto the experimental table (Figure 1). Employing the minirobot, the laser fiber was systematically guided along the X–Y plane at a consistent rate of 2 mm/sec. Maintaining a distance of 1 mm from the tissue, the laser fiber induced a 20 mm fissure within the kidney tissue (Figure 2).

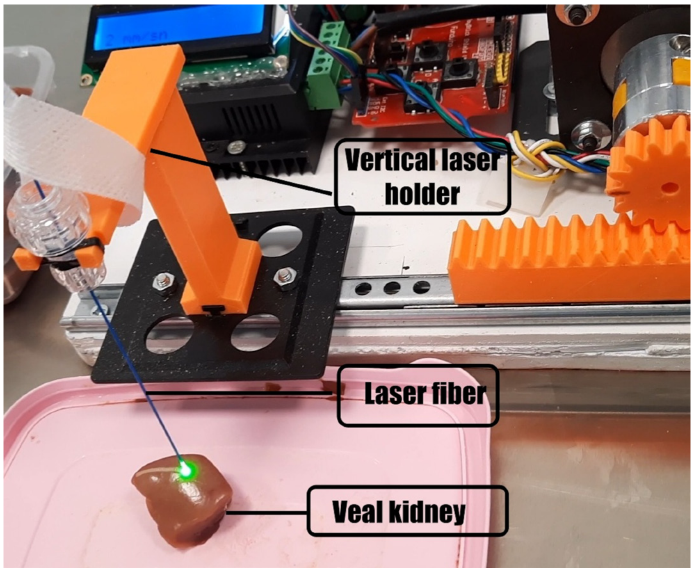

Figure 1. A compact laser robotic plotting system with a laser holder capable of automatic linear movement along a single axis (X-Y)

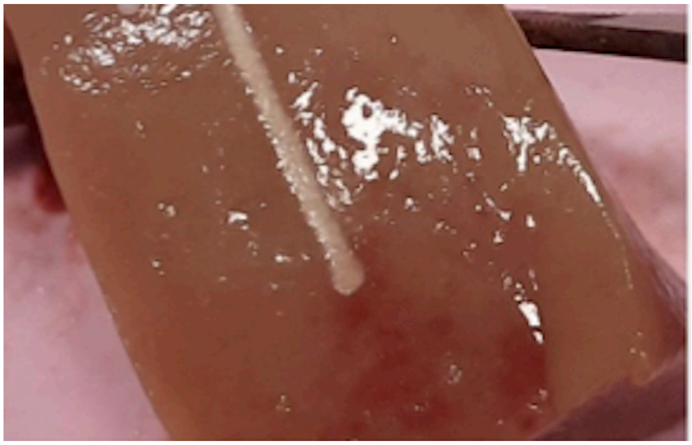
Figure 2. Kidney tissue with 20 mm fissure formed by laser

To ensure procedural accuracy, the fiber ends were consistently renewed prior to each experiment by means of precision ceramic scissors.

A 100 W Ho:YAG laser device (Cyber Ho, Quanta System, Samarate, Italy) was employed, accompanied by 550 µm and 272 µm laser fibers for all experimental procedures. The laser settings were configured based on the laser mode capabilities, encompassing a laser power ranging from 10 to 60 W, a frequency ranging between 20 and 40 Hz, energy levels spanning 0.5 to 1.5 J, and a medium pulse duration of 600 µsec (with a minimum of 50 µsec and a maximum of 1100 µsec). Standard modulation (SM), and VB pulse modulations of Ho:YAG lasers were utilized for the experiments.

A mini laser plotter robot was designed and used specifically for this study. The technical characteristics of this robot are described in detail in the article by Sirin et al. [11].

Histological assessment

Following the conclusion of each trial, the samples underwent a series of procedures. They were first photographed, meticulously catalogued, appropriately labeled, and then carefully preserved in a solution containing 10% formalin solution. The laser-induced fissures within each samples were meticulously sectioned both vertically and horizontally. Subsequently, these sections were divided into two segments and incorporated in paraffin-embedded specimens. The paraffin-embedded specimens were skillfully sliced into five sets of sections, each measuring 50 microns in thickness. These delicate sections were then delicately affixed onto glass slides and subjected to haematoxylin–eosin staining. For an unbiased assessment, an independent pathologist (referred to as M.O.) meticulously analyzed the breadth, profoundness and area of laser penetration. This evaluation was conducted using an electronic light microscope (specifically the OLYMPUS BX53-SC 100 model by Europa SE&Co). The parameters assessed included: Surface section (SS): This refers to a slender horizontal slice of the uppermost tissue layer influenced by the laser.

Lateral effect (LE): Representing the greatest horizontal distance extending from the center of the evaporation interval to

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the normal tissue, inclusive of the coagulation area.

Incision depth (ID): Indicating the depth of the laser incision.

Vaporization area (VA): Denoting the region of tissue subjected to vaporization due to the laser treatment.

Coagulation area (CA): Signifying the area of tissue that experienced coagulation due to the laser.

Total laser area (TLA): Calculated by combining the vaporization area (VA) with the coagulation area (CA).

These various measurements were collected in a blinded manner by the pathologist (M.O.) for each individual laser incision made during the experiments. The specialized cellSens imaging software (developed by Olympus LS-Life Science, Europa SE&Co) was employed for accurate measurement and analysis (Figure 3).

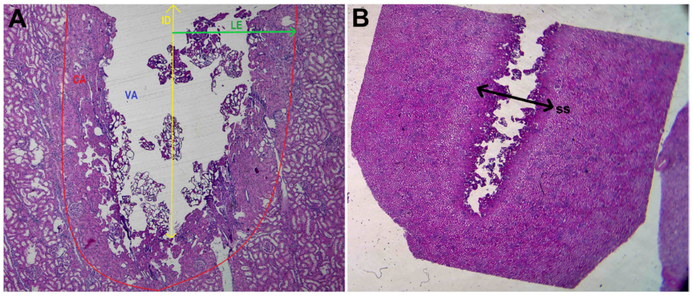

Figure 3. A) lateral effect (LE) (Representing the greatest horizontal distance extending from the center of the evaporation interval to the normal tissue, inclusive of the coagulation area), coagulation area (CA), vaporization area (VA), incision depth (ID), and total laser area (TLA, calculated as VA+CA), **B)** Surface section (SS) (This refers to a slender horizontal slice of the uppermost tissue layer influenced by the laser)

Statistical analysis

The research data were subjected to statistical analysis using the Statistical Package for the Social Sciences (SPSS), version 22.0 (SPSS Inc., Chicago, USA), computer software. The data were expressed as mean±standard deviation (SD). The comparison of parameter means was conducted through the utilization of the Mann–Whitney U test. A significance level of p values under 0.05 were deemed statistically significant.

Results

Every experimental data was replicated three times, resulting in a total of 72 experiments. The descriptive features of the Ho:YAG laser settings during experiments are shown in Table 1.

The mean VA, CA, TLA, ID, LE and SS histopathological dimensions and statistical comparison of 272 (μm) and 550 (μm) laser diameters in Ho:YAG laser SM and VB modulations according to the energy configurations used in air environment are presented in Figure 4 and 5. In SM and VB, a statistically significant difference was found in the mean CA values between 272 (μm) and 550 (μm) laser diameters (p: 0.02 and p<0.001, respectively).

In SM the lowest and highest mean VAs were observed with the 272 (μm) at 0.5J-40 Hz and 1.5 J-40 Hz laser settings (0.31±0.009 mm^2 and 3.263±0.019 mm^2, respectively), and with the 550 (μm) at 1 J-40 Hz and 0.5 J-20 Hz laser configurations (0.127±0.005 mm^2 and 1.713±0.016 mm^2, respectively). In VB, the lowest and highest mean VAs were observed with the 272 (μm) at 1.5 J-20 Hz and 1.5 J-40 Hz laser configurations (0.39±0.007 mm^2 and 3.263±0.018 mm^2, respectively), and with the 550 (μm) at 0.5J -20 Hz and 1 J-40 Hz laser settings (0.473±0.011 mm^2 and 2.763±0.014 mm^2, respectively) (Figure 4).

In SM, the lowest and highest mean CAs were observed with the 272 (μm) laser at 0.5 J-40 Hz and 1.5 J-40 Hz laser configurations (0.263±0.003 mm^2 and 1.353±0.015 mm^2, respectively), and with the 550 (μm) at 0.5 J-40 Hz and 1 J-20 Hz laser settings (0.690±0.009 mm^2 and 1.240±0.026 mm^2, respectively). In VB, the lowest and highest mean CAs were observed with the 272 (μm) at 0.5 J-20 Hz and 0.5 J-40 Hz laser configurations (0.573±0.016 mm^2 and 1.157±0.01 mm^2, respectively), and with the 550 (μm) at 0.5J-20Hz and 1J-20 Hz laser settings (0.987±0,014 mm^2 and 1.969±0.029 mm^2, respectively) (Figure 4).

In SM, the lowest and highest mean TLAs were observed with the 272 (μm) at 0.5 J-40 Hz and 1.5 J-40 Hz laser configurations (0.577±0.012 mm^2 and 4.617±0.033 mm^2, respectively), and with the 550 (μm) at 1 J-40 Hz and 0.5 J-20 Hz laser settings (0.873±0.002 mm^2 and 2.673±0.026 mm^2, respectively). In VB, the lowest and highest mean TLAs were observed with the 272 (μm) at 0.5 J-20 Hz and 1.5 J-40 Hz laser settings (1.000±0.01 mm^2 and 4.330±0.013 mm^2, respectively), and with the 550 (μm) at 0.5 J-20 Hz and 1 J-40 Hz laser settings (1.460±0.004 mm^2 and 3.943±.017 mm^2, respectively) (Figure 4).

In SM, the lowest and highest mean IDs were observed with the 272 (μm) at 0.5 J-40 Hz and 1.5 J-40 Hz laser configurations (0.357±0.015 mm^2 and 1.947±0.016 m mm^2, respectively), and with the 550 (μm) at 1 J-40 Hz and 0.5 J-20 Hz laser configurations (0.473±0.003 mm^2 and 1.977±0.016 mm^2, respectively). In VB, the lowest and highest mean IDs were observed with the 272 (μm) at 1 J-20 Hz and 1.5 J-40 Hz laser settings (0.380±0.01 mm^2 and 1.573±0.021 mm^2, respectively), and with the 550 (μm) at 0.5 J-20 Hz and 1.5 J-20 Hz laser settings (0.573±0.004m mm^2 and 2.690±0.045 mm^2, respectively) (Figure 5).

In SM, the lowest and highest mean LEs were observed with the 272 (μm) at 1 J-20 Hz and 1 J-40 Hz laser configurations (0.420±0.01 m mm^2 and 0.787±0.015 mm^2, respectively), and with the 550 (μm) at 0.5 J-40 Hz and 1 J-20 Hz laser settings (0.490±0.012 mm^2 and 0.983±0.007 mm^2, respectively). In VB, the lowest and highest mean LEs were observed with the 272 (μm) at 1 J-20 Hz and 1.5 J-20 Hz laser configurations (0.327±0.014 mm^2 and 0.680±0.019 mm^2, respectively), and with the 550 (μm) at 1.5 J-20 Hz and 1 J-40 Hz laser configurations (0.450±0.017 mm^2 and 0.640±0.01 mm^2, respectively) (Figure 5).

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In SM, the lowest and highest mean SSs were observed with the 272 (µm) at 0.5 J-40 Hz and 1.5 J-40 Hz laser settings (1.362±0.023 mm^2 and 2.780±0.04 mm^2, respectively), and with the 550 (µm) at 0.5 J-40 Hz and 1.5 J-40 Hz laser settings (1.270±0.021m mm^2 and 1.55±0.029 mm^2, respectively). In VB, the lowest and highest mean SSs were observed with the 272 (µm) at 0.5 J-20 Hz and 1.5 J-40 Hz laser settings (1.128±0.014 mm^2 and 2.503±0.041 mm^2, respectively), and with the 550 (µm) at 1 J-20 Hz and 1.5 J-20 Hz laser configurations (0.847±0.015 mm^2 and 2.340±0.026 mm^2, respectively) (Figure 5).

Table 1. The descriptive features of the HoYAG laser and the experimental setup details using mini laser plotter robot system

Laser device	100W Ho:YAG (Cyber Ho, Quanta System, Samarate, Italy)
Pulse modulation	Standard mode (SM), Virtual Basket™ (VB)
Emission power (Watts)	10-20-30-40-60
Pulse energy (J)	0.5-1-1.5
Frequency (Hz)	20-40
Pulse duration	Medium pulse, 600 µsec each pulse
Laser fiber diameter (µm)	272 and 550
Automatic robotic arm speed	2mm/sec
Distance of fiber to the tissue	1mm
Tissue fissure length	20mm

Figure 4. VA (vaporization area), CA (coagulation area) and total laser area (TLA, calculated as VA+CA) values of different laser settings of 272 (µm and 550 (µm) laser diameters in Ho:YAG laser SM and VB modes

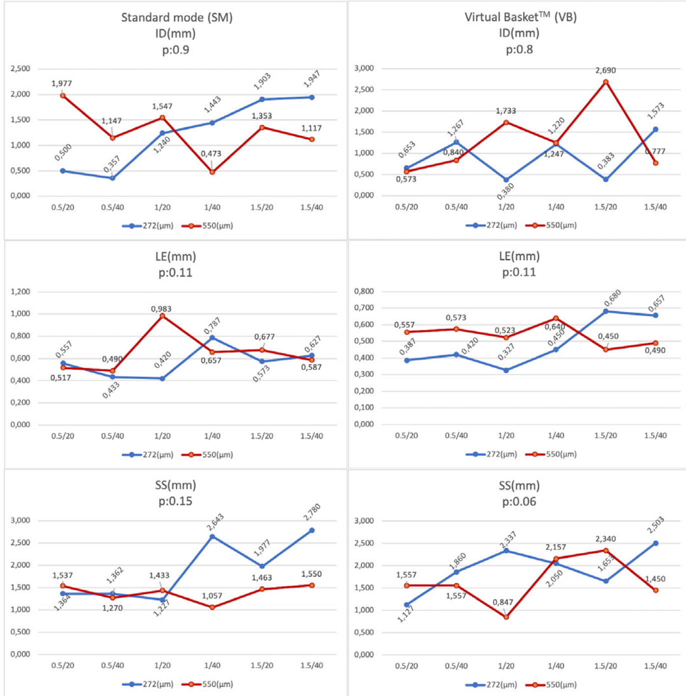

Figure 5. ID (incision depth), LE (lateral effect), and SS (surface section) values of different laser settings of 272 (µm) and 550 (µm) laser diameters in Ho:YAG laser SM and VB modes

Discussion

In recent years, the Ho:YAG laser has become an indispensable surgical tool in endourology. While it was previously used only in stone surgery, it is now used in many procedures, especially in prostate surgery [14,15]. During this expansion, it has acquired innovative technical and physical properties, and maintained its efficacy in various fields. While VB modulation provides effective stone crushing by reducing the retropulsion of the stone in stone surgery, it enables to dissect two different tissue plans with two consecutive bubble effects in soft tissue[9,16]. In addition, VB modulation has become a point of interest after Bozzini et al. showed that VB contributed to less bleeding and better dissection in their series of HoLEP operations with VB [9].

In HoLEP surgery, laser settings such as 1J-40Hz are more frequently used [9]. Although each surgeon uses laser modifications suitable for his surgical technique over time, the two main effects of laser soft tissue surgery such as prostate are gaining importance. These are VA values that simulate the tissue dissection effect and CA values that simulate the hemostasis effect during surgery. İn the present study, we observed that that as higher wattages are reached in VA in standard modulation, the values in 272 µm fibers are higher than 550 µm fibers, while in VB modulation, the values in 550 µm fibers are higher than 272 µm fibers. These results show that the better dissection effect of VB with successive bubbles is more evident in thicker laser fibers. Although there are no studies comparing the effect of different laser fibers on tissue, Assmus et al. compared the degradation degree of 550 µm and 1000 µm fiber thicknesses after Moses™ modulation on HoLEP surgery and showed that 1000 µm fibers less degraded [17].

As in all surgeries, hemostasis is very important in laser surgery. When we look at the CA values that best simulate the hemostasis effect experimentally, we see that the 550 µm fiber has higher CA values than the 272 µm fiber in both SM and VB. Therefore, we can conclude that relatively thicker fibers can achieve a better hemostasis effect in both modulations. When we look at the values expressed as TLA, which is the sum of VA and CA, we see that the values are higher in the 272 µm fiber in SM and higher in the 550 µm fiber in VB. However, these two values were not found to be statistically significant. In other words, the significant difference in the CA values in favor of the 550 µm fiber was balanced by the VA values, and no statistical significance was found in the TLA values.

Although there was a difference in ID, which is the measure of the depth effect of the laser, between the various Joule and Hertz values, this difference was not found to be statistically significant. Even in VB, the ID values at 1J- 40 Hz were found to be the same for 272 µm and 550 µm fibers. In other words, the depth of the laser is not determined by the thickness of the laser fiber, but by the value of the laser energy and power. LE, which is the measure of the lateral effect of the laser, and SS, which is the most superficial value of this effect, were not found to be statistically significant with different laser fiber thicknesses for both VB and SM modulation.

There are specific limitations in our study that should be pointed out. First, since the kidney model we used was not perfused, the coagulation, vaporization, and heat absorption effects of the laser, as in living tissue with blood supply, could not be observed in our experiment. Furthermore, due to the inherent limitations of the laser device's diverse pulse modulation capacities, we could only incorporate a restricted array of laser settings within the experiment. We also exclusively utilized the moderate pulse duration to constrain the quantity of variables. Another point that may make the comments in our study controversial is that while operations are performed in an environment with serum physiologic conditions, our experiment was performed in the air.

Conclusion

Despite the limitations of this experimental study, we found that both SM and VB modulations have higher CA values, a measure of hemostasis, in 550 µm fibers compared to 272 µm fibers. Also, unlike SM, VA values in VB are higher in thick fibers. In our study, contrary to expectations, values such as ID and LE are not affected by the thickness of the laser fiber. We consider that the outcomes of our study should be assisted by clinical trials on living tissues in order to obviously indicate the effects of different fiber thicknesses in different laser modulations.

Conflict of Interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical Approval

Not applicable for this section. The manuscript does not contain clinical studies or patient data. This article does not contain any studies with human subjects performed by the any of the authors.

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