Effect of Ho-YAG laser therapy on Complete Blood Count for Lithotripsy (in vitro)

Ferial. Y. Nazal *Mohammed K. Hamid **Hazim R. Akal

Dept. of Medical Physics - College of Medicine - University of Thi-Qar
*Department of urology surgery, Al-Hussain Teaching Hospital, Nassyriah
**Dept. of urology surgery - College of Medicine - University of Thi-Qar

Emial: Ferial.nazal@yahoo.com
Mobile: 07804128861

Abstract:

Background :
The purpose of this study is to investigate the in vitro effect of Ho – YAG laser on Complete Blood Picture in blood sample from ureteroscopic lithotripsy (patients with urinary stones). The study is conducted to evaluate the effect of Ho-YAG laser on Complete Blood Count (CBC) was estimated before and after exposure in vitro. The study was conducted on Complete Blood Picture, the taken number of (10) parameters was estimated before and after exposure. Result showed that there was a significant decreased in the percentage of Lymphocyte, PLT, HCT, RBC, HBG, MCHC, after exposure to 2100 nm (Ho-YAG) laser. It is found that (Ho-YAG) laser increase some parameters of WBCs, MCV, MONO and MCH and after exposure.

Keywords: (2100 nm) holmium - YAG laser, Complete Blood Count (CBC).

Introduction:
For several years it was holmium laser available for endourological applications, because it was considered the main application in lithotripsy result of a typical repetition rates (< 12 Hz) are low and the values of energy (< 2 J) many tissue related applications with the laser systems (Werner Falkenstein, 2004). a physiological type of cell death distinguish by certain morphological nuclear and biochemical changes (Rowaida A. Al-khazragi et al., 2008).

The holmium laser, Ho: YAG, has an infrared wavelength of 2100 nm. It is a perfect lithotripter, suitable for all types of fragmentation of urinary stones due to the impact of the strong photo acoustic effect and high peak power. The optical penetration is up to 0.4 mm, but because of higher absorption through the water (Piotr Humański, 2010). Holmium laser at the wave length of 2100 nm has clinically very important physical properties owing to a balance between the coefficients of absorption and scatter. The property of a laser to be absorbed by the incident target gives it the ability to cut sharply, and is described as the coefficient of absorption.

Scattering be important to ensure seal blood into the surrounding tissue to maintain the cease hemorrhage. The Cutting and coagulation characteristics of the laser is also based on the survival of the pulse duration and the pulse length so is the idea way to divide and eradication of a member of hemorrhage position at the shed on thin damaged tissue. Laser power capacity reached effectively through flexible thin silica fiber have beneficial use through flexible miniature endoscopes to deal with the damaged tissue of the flexible urinary system to be effective with minimal damage.

Most of the authors have recommended the use of 0.2 - 0.8 joules at 10 -20 pulses per second. Holmium laser at the wave length of 2100 nm has clinically very important physical properties owing to a balance between the coefficients of absorption and scatter. The property of a laser to be absorbed by the
incident target (in the present context human tissue) gives it the ability to cut sharply, and is described as the coefficient of absorption. Carbon dioxide laser happens to be on one end of the spectrum with one of the sharpest cutting lasers (Rajesh Taneja, 2011).

During the past few decades have witnessed surgical treatment of urinary tract due to improvements in technology (Papatsoris AG et al., 2011).

Currently ureteroscopy (URS) is a precise, minimally invasive surgical intervention that can assess the entire collecting system to treat a stone using intracorporeal lithotripsy (Athanasios G et al., 2012). Ho: YAG laser lithotripsy has become the reference standard for both rigid and flexible URS (Turk C et al., 2011). The Ho-YAG laser is a solid state, pulsed laser that emits light at 2100 nm. The optical absorption coefficient for water at this wavelength is approximately 40 cm\(^{-1}\) so that the holmium wavelength is absorbed significantly by water. Since tissues are composed mainly of water, the majority of the holmium energy is absorbed superficially and this results in superficial cutting or abrating only (Lt Col AS Sandhu et al., 2007).

Whole blood is an excellent model for studying a systemic human organ, because it permits one to study the interaction of its constituents after laser light irradiation at different wavelengths (Istvan Stadler et al., 2000).

METHODS AND MATERIALS:

Fresh whole blood was obtained from Al-Hussein Teaching hospital department of urology user to lithotripsy from 30 patients. The present study was undertaken to examine the effect of (2100nm) Ho-YAG laser irradiation with power of (2.12 µw).

Typical pulse duration (100 ns-250 µs) interaction (thermal + mechanical) absorption coefficient (30 cm\(^{-1}\)) and Tissue penetration < 0.5 mm (KARL Storz – Endoscope, Germany) on whole blood. Lymphocytes from this blood can be considered to be immunologically intact, and the other hematologic parameters were normal in these samples due serial phlebotomy of these patients at regular intervals.

The human blood samples are obtained from patients. The samples of blood 6 ml were obtained immediately treated with EDTA anticoagulant from Sigma Company and each sample was divided into two aliquots samples and inserted into tubes of 1cm diameter (one control and the anther for irradiation with Ho- YAG laser at maximum output power (1.2-0.5 J) and control. To assure its homogeneity, the blood was stirred gently before and during irradiation in all samples at 3 min intervals approximately. The laser was continuous wave type, who were referred for various blood analyses Ruby hematology analyzer (RUBY EC – REP, Diagnostics Division, Germany) for measurements of complete blood count (CBC), to assess the effect of holmium laser irradiation on CBC. This irradiation administer was over to be the laser energy per volume unit (J/cm\(^3\)) rather than the laser energy per area surface unit (J/cm\(^2\)). The laser output power is 0.8 J, irradiation time is 3 min and the fluence is 48 J/cm\(^3\).

For HO- YAG laser the equation will be:

\[
f = \frac{u(\omega)A}{\nu} \quad \text{................. (Yousry M Mustafa et al., 2013)}
\]

Statistics analysis: In this work, Use statistical analysis was made using SPSS, version 23 to determine the effect of HO-YAG laser on the complete blood count the most crucial concern is the difference between the control and laser irradiation thus a paired t-test \(P<0.01\) was used to evaluate the difference between the irradiated samples and non-irradiated control. For those with significant difference, the percentage of relative variance (R.V.) was calculated to evaluate the extent of the relative change between irradiated and non-irradiated samples. All values are means ± SD.

Results:

The results of the effects Ho-YAG laser on human blood in vitro for CBC parameters of 30 patients before and after irradiation are shown in the following tables (1). A positive results in which the relative variation (R.V.) of a given blood parameter is the difference between its value after irradiation and before irradiation, divided by the value before irradiation.

\[
R.V = \frac{\Delta X}{X} \quad \text{(where } \Delta X \text{ is difference between before and after irradiation)}
\]

In this study we measure the effect of Ho-YAG laser on CBC the following parameters: CBC measurements include 10 parameters these are white blood cells, or leucocytes.

WBC: Leukocyte count; normal values ranging between 4and 11 thousands /mm\(^3\). Experimental data (Table 1) indicated that the relative variations (R.V.) of WBC ranged between -41.9% and 17.9%. In 17 cases
out of 30, the variations were positive; they were negative in 13 cases.

The results showed a significant increase (P<0.01) in the White blood cell count of (9.7093 ±3.52423 cell/mm³).

RBC: Erythrocytes count; normal values ranging between 4 and 6.5 millions /mm³. the relative variations (R.V.) of the RBC ranged between -11.8 % and 6.7% . In 11 cases out of 30, the variations were positive and in 19 case were negative. The results showed a significant decrease (P˂0.01) in RBCs irradiated (4.6767±0.62133 cell/mm³).

HBG: Hemoglobin concentration, normal values ranging between 11 and 16 gm/dl. the relative variations (R.V.) of HBG ranged between -5.5 % and 10.3 % (P-value = 0.01) . In 9 cases out of 30, the variations were positive, and in 21 case it was negative. The results showed a significant decrease (P<0.01) in Hb irradiated (13.5083±1.64064g/dl).

HCT or packed cells volume PCV: Hematocrit, normal values ranging between 36 and 54%. the relative variations (R.V.) of HCT ranged between -2.1 % and 11.22 % . In 8 cases out of 29 , the variations were positive, and in 21 case it was negative . The results showed a significant decrease (P<0.01) in Hematocrit irradiated (38.7803±6.32727 %).

MCV: Mean corpuscular volume, normal values ranging between 75 and 97 μm³. the relative variation (R.V.) of MCV ranged between -5.6% and 21.1% . In 27 cases out of 30, the variation were positive, and 3 cases they were negative . The results showed a significant increase (P<0.01) in the MCV irradiated (80.522 ±9.04412 Fl).

MCH: Mean corpuscular hemoglobin, normal values ranging between 25 and 33 picograms. The relative variations of the MCH ranged between -13.3% and 11.8% . In 24 cases out of 29, the variations were positive, and 5 cases they were negative. And in one case no effect . The results showed a significant increase (P<0.01) in the MCHC irradiated (33.9437±2.25414pg).

MCHC: Mean corpuscular hemoglobin concentration, normal value ranging between ( 29.5 and 35.9 g/dl ) the relative variations of MCHC ranged between -9 % and 9.2 . In 3 cases out of 30, the variations were positive, and 27 cases they were negative . showed a significant decrease (P<0.01) in MCHC irradiated (33.9437±2.25414g/dl).

PLT: Platelet or thrombocytes count, normal values ranging between 141 and 450 thousands/mm³. the relative variation of the PLT ranged between - 20% and 27.5 % . In 4 cases out of 30, the variations were positive, and in 26 cases they were negative. showed a significant decrease (P<0.01) in Platelet irradiated (299.433±79.9558 cell/mm³).

Lymph: Lymphocyte number, in concentration, normal values ranging between 10 and 60 % . the relative variations of the lymphocyte number ranged between -26.8% and 54.3 % . In 14 cases out of 30, the variations were positive, and in 16 cases they were negative . showed a significant decrease (P<0.01) in Lymphocytes irradiated (2.757±0.86603%).

Mono: Monocytes number, in concentration, normal values ranging between 1 and 15%) the relative variations of the Monocytes ranged between -18.1% and 13.9 % . In 23 cases out of 30, the variations were positive, and in 7 cases they were negative . The results showed a significant increase (P<0.01) in the Monocytes irradiated (0.504±.15165 %).
Figure 3: Effect of HO-YAG laser on HCT in cases of control and irradiation

Figure 4: Effect of HO-YAG laser on MCV in cases of control and irradiation

Figure 5: Effect of HO-YAG laser on platelet in cases of control and irradiation

Figure 6: Effect of HO-YAG laser on MCH in cases of control and irradiation

Figure 7: Effect of HO-YAG laser on MCHC in cases of control and irradiation

Figure 8: Effect of HO-YAG laser on Monocyte in cases of control and irradiation
Table (1): The effect of HO-YAG laser on Complete blood picture after 3 min irradiation (irradiance 48 J/cm²)

| XN | RBX | RBY | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX | RX |RX
Discussion:

Physical properties: laser tissue interaction

Absorption: the most important factor in laser-tissue interaction. Its direct effect is the generation of heat in the tissue. Depending on the amount of heat generated, the result will be either coagulation or vaporization. As the laser beam penetrates an absorbing medium, its intensity decreases exponentially. In urological applications of laser technology, the two chromophores available in the human body are water and hemoglobin.

Reflection: percentage of the laser beam that is reflected upon interacting with tissue. This causes the beam to lose efficiency and raises the risk to the surrounding tissue, which could suffer an unwanted rise in temperature. Reflection of a given laser depends on the optical properties of the tissue and the irrigation fluid.

Dispersion: from an optical point of view, body tissue is not homogeneous, causing dispersion when the laser beam interacts with it. This dispersion depends on the size of the tissue particles and the wavelength of the laser. Lasers with shorter wavelengths tend to exhibit more dispersion.

The effect of HO-YAG laser irradiation on the organism has several clinical and biological effects, above anti-inflammatory, immunostimulatory, neurotropic, analgesic, desensitizing, antiemetic, normalizing. The blood rheology and hemodynamics means (depending the break off of the patient and the pathology). The tangible test study was undertaken to assay the positive of HO-YAG laser on human blood in vitro and despite divagate induced modifications on different rheological constants of the blood and in addition to evaluate the appropriately of these modifications. The results of this interpret take a crack at incomparably demonstrated drift the irradiation of blood at low doses and gifts densities leads to the following effects without causing crass centre stall injury (Iijima K et al., 1991). which display that low powered HO-YAG laser irradiation revive parental carry off on RBC membranes, reducing hypotonic hemolysis and stabilizing the cell membrane.

RBC, HGB and HCT were strongly influenced by HO-YAG laser action due to the strong absorption of irradiation standpoint, but bid deadly the RBCs. The mature red blood cell is a relatively simple structure whose defray action is down reference to packaging hemoglobin molecules efficiently, performance them from the lungs to the microcirculation and back always 11 minutes, and keeping them in a effect assert for 120 day. Busy measure of RBCs depends on its acutely hurtful become available impeach (derived from surface glycoproteins) which permits it to bring down rotation circulating cells, thereby interdiction “clumping;” its desolate doughnut-like shape, which is archeologically scale predisposed to and permits trough go about of the cells browse capillaries; and its proficiency to expect oxidative set off to the hemoglobin molecule, thereby living the four iron atoms on at all times hemoglobin molecule in the ferrous (Fe2+) state, in which configuration they are able to merge oxygen reversibly. Since the cell contains no nucleus and has no capacity to mingle proteins, weak molecules cannot be replaced on the red blood cells long lifespan. The shape of the cell is maintained.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Irradiation</th>
<th>P value</th>
<th>Mean R.V.(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>9.692±3.29533</td>
<td>9.709±3.54242</td>
<td>&lt;0.01</td>
<td>3.176±0.91521</td>
</tr>
<tr>
<td>RBC</td>
<td>4.500±0.52521</td>
<td>4.676±0.62133</td>
<td>&lt;0.01</td>
<td>0.701±0.32333</td>
</tr>
<tr>
<td>HBG</td>
<td>13.403±1.51145</td>
<td>13.508±1.64644</td>
<td>&lt;0.01</td>
<td>1.206±0.43512</td>
</tr>
<tr>
<td>HCT</td>
<td>38.837±6.26408</td>
<td>38.780±6.32727</td>
<td>&lt;0.01</td>
<td>0.835±0.42560</td>
</tr>
<tr>
<td>MCV</td>
<td>84.441±8.28450</td>
<td>80.522±9.04412</td>
<td>&lt;0.01</td>
<td>4.840±0.89740</td>
</tr>
<tr>
<td>MCH</td>
<td>28.346±3.20428</td>
<td>27.996±3.32238</td>
<td>&lt;0.01</td>
<td>1.430±0.25678</td>
</tr>
<tr>
<td>MCHC</td>
<td>33.676±2.55839</td>
<td>33.943±2.25414</td>
<td>&lt;0.01</td>
<td>0.380±0.30852</td>
</tr>
<tr>
<td>LYMPH</td>
<td>2.789±0.85865</td>
<td>2.757±0.86603</td>
<td>&lt;0.01</td>
<td>3.600±1.82231</td>
</tr>
<tr>
<td>MONO</td>
<td>0.504±0.14251</td>
<td>0.504±0.15165</td>
<td>&lt;0.01</td>
<td>3.056±0.55454</td>
</tr>
<tr>
<td>PLT</td>
<td>294.866±79.57852</td>
<td>299.433±79.95589</td>
<td>&lt;0.01</td>
<td>1.423±1.00967</td>
</tr>
</tbody>
</table>

Table 2 : Mean and Standard deviation of complete blood count in case of control and irradiated of HO-YAG laser P < 0.01 N : Number of samples.
the cell’s volume is even, and hemoglobin and other important molecules in the cell (such as membrane lipids and structural proteins) are protected from oxidation by enzyme systems that are driven by glucose catabolism. The normal red blood cell is fragment impassable crescent disc which maintains osmotic control with the concerning activity. As the surrounding medium becomes hypotonic, running grit be feigned into the cell to convince sheet anchor. In the course of time lower than certainly hypotonic conditions the cell will fill to power and infringement. The irradiation of red blood cells by HO-YAG laser for 48 J/cm³ makes the red blood cells have increased skills to destroy and agree to bear nearby insufferable hypotonic solutions that lyses un-irradiated normal blood cells (Chludzinska L et al., 2005) who reported that low powered lasers stabilized stored erythrocytes in hypotonic satisfy and reduced the turnover in deformability for stored erythrocytes (Iijima K et al., 1993).

**Conclusion:**

The exposure of the blood to HO-YAG laser improve its rheological properties. It was observed that the therapy with HO-YAG laser has been more efficient than that with laser in the improvement of properties of human blood cells in vitro. In this study we measure the following parameters: CBC measurements include 10 parameters these are white blood cells, or leucocytes number (WBCs), red blood cells or erythrocytes count (RBCs), quantity of hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Platelet or thrombocytes count (PLT), Lymphocyte number (LYMPH) and Monocytes number (MONO).

**Reference:**


Piotr Humański MD, FEBU, Department of Urology, Specjalista Hospital, Kutno, Poland, Holmium : YAG laser : an obviously necessary piece of equipment for an outpatient urological surgery, 2010, VOL.5(1) 55-58.


Rowaida A. Al-khazragi MSc, Israa F. Al-Samaraee MSc, PhD. Effect of Laser light on lymphocyte Apoptosis, IRAQI J MED SCI, 2008; VOL.6 (1):45-51.

