Efficacy of Laser Pulse Frequencies on Blood Flow in Normal Subjects

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ABSTRACT

This study was conducted to investigate the effective laser pulse frequency either 200 or 2000 Hz on improving blood flow. Thirty normal male subjects randomly selected from students of faculty of physical therapy, Cairo university (mean age 18.92 ± 1.5). They were assigned randomly into equal two groups. The blood flow volume, mean blood flow velocity and caliper of the blood vessel were evaluated before laser application and after twelve sessions using duplex Doppler ultrasound. Combined He-Ne and infrared laser were administered three times a week for twelve sessions at intensity two J/cm², power 15 mW, duration 15 min and pulse frequency 200 Hz for group I and 2000 Hz for group II on the sural artery at posterior aspect of dominant leg (from mid point of posterior knee crease to the mid point of the line drawn between two malleuli). The results revealed that Low pulse frequency (200 Hz) of low intensity laser therapy (LILT) produced significant improvement in blood flow volume and blood flow velocity (48.2%, and 40.6%) respectively (P<0.05) with significant difference between the two frequencies (P< 0.05). There was no change in caliper of the blood vessel of group I, blood flow volume, blood flow velocity or caliper of the blood vessel of group II. Also, there was a significant direct correlation between blood flow volume and blood flow velocity, and there was a significant indirect correlation between blood flow velocity and blood vessel caliper in group I. There was no correlation in group II. From the findings it could be concluded that low pulse frequency of LILT (200 Hz) could improve blood flow than high pulse frequency (2000 Hz).

INTRODUCTION

Laser is an acronym for light amplification by stimulated emission of radiation. The applications of lasers are legion and span almost in every field of human endeavor in medicine, science and technology.4,5,19,20

Number of clinical research reports have noted apparent increase in cutaneous and deep blood flow as a result of laser (combined He-Ne and infra- red laser) irradiation or suggested such putative laser-mediated alteration in blood flow as a mechanism of action of some other clinical or physiological effects.

It was reported that LILT improves local microcirculation and it can also improve oxygen supply to hypoxic cells and at the same time it can remove the collected waste products.7,10,40,43 On the other hand, there was a gap found in the literature upon the effect of LILT on blood flow and also discrepancy and contradictory results on the reported studies because the exact mechanism is unknown, the effective parameters are not established and variable effects on different blood flow measurements are not concluded. Furthermore, LILT improves local microcirculation that promote wound healing, improve oxygen supply to hypoxic cells that improve the metabolic functions of these cells6,18,29,35, increase number of white blood cells and antibodies that decrease inflammation12,22,38,41, and at the same time it can remove the collected waste products that decrease muscle spasm,8,9 also the normalization of the microcirculation, obtained due to laser
applications, interrupt the “vicious circle” of the origin of the pain and its development.

Moreover, LILT may open microcirculation, improve elasticity of artery without affecting its caliper that increase its accommodation to more blood, which help to cut the vicious circle of pain and affecting the neighboring veins by suction force. Thus in turn increase venous return.2,11,21,29,34

On the other hand, researches have suggested that the physiological responses to laser are affected by the rate of its pulse frequency which is one of its parameters that should be adjusted before application. But the differences in change in blood flow in accordance to pulse frequency are not clear yet.13,23,33 So physiotherapists are still in need to investigate and confirm the previous results on the changes in blood flow after laser application that aid them to construct the effective parameters of laser that will be given for patients to gain the most earlier and beneficial results. Therefore the aim of current study was to investigate the effective laser pulse frequency either 200 or 2000 Hz on improving blood flow.

SUBJECTS, MATERIALS AND METHODS

This study included two groups of volunteers. The dependent variable was the blood flow volume measured by Ultrasound Doppler. The independent variable was LILT (combined He-Ne and infrared laser with scanner technique), its intensity was two J/cm², pulse frequencies were 200 and 2000 Hz, and its power was 15 mW. The site of application was the sural artery at posterior aspect of the leg (from the mid point of posterior knee crease to the mid point of the line drawn between the malleuli). Evaluation of all subjects was conducted before and after laser application.

Subjects

Thirty normal male subjects from the students of faculty of physical therapy, Cairo university were participated in the present study. They were not athlete, non-smoker, and non-obese subjects. Their average age was from 18 to 25 years old with mean age 18.92 ± 1.5 years, height ranged from 167 to 185 cm with mean height 174.1 ± 3.23 and weight ranged from 59 to 82 kg with mean weight 72.09 ± 6.79. Subjects were participated into pre- and post-control group design. The Subjects were randomly assigned into two equal groups.

Group I: fifteen volunteers included

I) Study limb: Which was the dominant limb, this limb had received twelve sessions, every other day; of LILT (combined He-Ne and infrared laser with scanner technique) at pulse frequency of 200 Hz, intensity two J/cm², and power 15 mW.

II) Control limb: Which was the non-dominant limb. It was not subjected to any irradiation.

Group II: fifteen volunteers included

I) Study limb: Which was the dominant limb, this limb had received twelve sessions, every other day of LILT (combined He-Ne and infrared laser with scanner technique) at pulse frequency of 2000 Hz, intensity two J/cm², and power 15 mW.

II) Control limb: Which was the non-dominant limb. It was not subjected to any irradiation.

Instrumentations

- Instrumentations for evaluation:

1) Duplex Doppler Ultrasound: This device used for measuring the blood flow volume and effective perfusion pressure. The
device was the multi-Dopp system (2, 4, 8 and 16 MHz).
2) Ordinary weight scale: It was used to detect the weight and height of each subject.
3) Tap measurement: It was used for measurement of width and length of leg.

-Therapeutic instrumentation:
LILT scanner, ASA Laser Bravo Terza Series type of laser was used in the present study. It produces combined irradiation of He-Ne and infrared lasers. The device was adjusted to produce intensity of two J/cm², power of 15 mW and frequency changed from 200 Hz to 2000 Hz. Recalibration of the devices used in the study were done before the study, to ensure accuracy and objectivity of their parameters.

Procedures
I) Evaluation Procedures
Each subject was acquainted with details of the procedures, which were undertaken through a demonstration session.
1- General evaluation: Included:
- Weight and height using weight scale.
- Leg length, measured in prone lying from mid point of knee to mid point between two malleuli.
2- Blood flow measurement: For each subject in both groups the blood flow volume, blood flow velocity and caliper of the sural artery of both lower limbs (The dependant lower limb was expressed as the experimental limb and the non-dependant limb as control limb) were measured using duplex Doppler ultrasound before and after laser application. The subjects were instructed to maintain their normal daily activities and not to engage in any other exercise-training program during the study. The transducer head of the duplex Doppler ultrasound was positioned vertical on the posterior aspect of leg after application of sufficient amount of sono-gel. The procedures was then completed by the same radiology specialist to register the blood flow volume, blood flow velocity and the caliper of the sural artery. Post-test measurement of the blood flow volume, blood flow velocity and caliper of the sural artery were performed at the end of the last session.(twelve session)

II) LILT procedures
Each subject was positioned in modified prone lying, fully relaxed and supported; the area of laser application in the dominant leg was washed by alcohol. The laser scanner was applied prependicular on the area of laser application and at 30 centimeter apart. The laser beam was adjusted to cover the area of application in width and length from the knee joint line to the two malleoli line. The energy was adjusted at two joules/cm² and the frequency was changed according to the experimental group, either 200 Hz in group I or 2000 Hz in group II. Subjects and researcher wear the protective goggles during all phases of the study. The delivery of laser radiation was continued for 20 minutes. The subjects were permitted to have ten minutes rest after laser application. These procedures were repeated for twelve sessions, three sessions per week.

RESULTS
I. Effect of LILT pulse frequencies on blood flow volume
1. For group I (200 Hz):
I. Study limb: t-test revealed that the mean values of blood flow volume were increased from (2.9 ± 1.506) m.L/min at pre-test to (5.6 ± 1.121) m.L/min at the end of twelve sessions. These changes were significant (P< 0.05).
II. Control limb: The mean values of blood flow volume changed from \((4.3 \pm 1.163)\) m.L/min at pre-test to \((4.7 \pm 2.257)\) m.L/min at the end of twelve sessions. These changes were insignificant \((P> 0.05)\) as shown in table (1) and figure (1).

Table (1): Blood flow volume changes at pre-test and post-test after application of LILT in group I.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Blood flow volume (Mean ± S.D.)</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mL/min</td>
<td>t-value</td>
</tr>
<tr>
<td>Study limb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre test</td>
<td>(2.9 \pm 1.506)</td>
<td>1.76</td>
</tr>
<tr>
<td>Post test</td>
<td>(5.6 \pm 1.121)</td>
<td></td>
</tr>
<tr>
<td>Control limb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre test</td>
<td>(4.3 \pm 1.163)</td>
<td>0.777</td>
</tr>
<tr>
<td>Post test</td>
<td>(4.7 \pm 2.257)</td>
<td></td>
</tr>
</tbody>
</table>

* Significant  

P: Probability

Fig. (1): Blood flow volume changes at pre-test and post-test after application of LILT in group I.

2. For group II (2000 Hz):

I. Study limb: t-test revealed that the mean values of blood flow volume changed from \((4.7 \pm 7.81)\) m.L/min at pre-test to \((4.7 \pm 1.07)\) m.L/min at the end of twelve sessions. These changes were insignificant \((P>0.05)\).

II. Control limb: The mean values of blood flow volume changed from \((4.1 \pm 9.24)\) m.L/min at pre-test to \((3.9 \pm 6.95)\) m.L/min at the end of twelve sessions. These changes were insignificant \((P> 0.05)\) as shown in table (2) and figure (2).

Table (2): Blood flow volume changes at pre-test and post-test after application of LILT in group II.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Blood flow volume (Mean ± S.D.)</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mL/min</td>
<td>t-value</td>
</tr>
<tr>
<td>Study limb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre test</td>
<td>(4.7 \pm 7.81)</td>
<td>2.145</td>
</tr>
<tr>
<td>Post test</td>
<td>(4.7 \pm 1.07)</td>
<td></td>
</tr>
<tr>
<td>Control limb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre test</td>
<td>(4.1 \pm 9.24)</td>
<td>0.49</td>
</tr>
<tr>
<td>Post test</td>
<td>(3.9 \pm 6.95)</td>
<td></td>
</tr>
</tbody>
</table>

*Significant  

P: Probability

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II. Effect of LILT pulse frequencies on blood flow velocity

For group I (200 Hz):

A. Study limb: t-test revealed that the mean values of blood flow velocity were decreased from \((5.02 \pm 2.8662)\) cm/sec at pre-test to \((7.06 \pm 2.0452)\) cm/sec at the end of twelve sessions. These changes were significant \((P<0.05)\).

B. Control limb: The mean values of blood flow velocity changed from \((6.9 \pm 2.4134)\) cm/sec at pre-test to \((5.53 \pm 0.9078)\) cm/sec at the end of twelve sessions. These changes were insignificant \((P>0.05)\) as shown in table (3) and figure (3).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Blood flow velocity (Mean ± S.D.) cm/sec</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study group</td>
<td>Pre test: 5.02 ± 2.8662</td>
<td>t-value: 2.8</td>
</tr>
<tr>
<td></td>
<td>Post test: 7.06 ± 2.0452</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>Pre test: 6.9 ± 2.4134</td>
<td>t-value: 1.22</td>
</tr>
<tr>
<td></td>
<td>Post test: 5.53 ± 0.9078</td>
<td></td>
</tr>
</tbody>
</table>

* Significant P: Probability

![Fig. (2): Blood flow volume changes at pre-test and post-test after application of LILT in group II.](image)

![Fig. (3): Blood flow velocity changes at pre-test and post-test after application of LILT in group I.](image)
For group II (2000 Hz):

A. Study limb: t-test revealed that the mean values of blood flow velocity changed from (4.42 ± 2.8852) cm/sec at pre-test to (3.55 ± 2.6427) cm/sec at the end of twelve sessions. These changes were insignificant (P>0.05).

B. Control limb: The mean values of blood flow velocity changed from (5.79 ± 5.5535) cm/sec at pre-test to (3.91 ± 3.525) cm/sec at the end of twelve sessions. These changes were insignificant (P> 0.05) as shown in table (4) and figure (4).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Blood flow velocity (Mean ± S.D.) cm/sec</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>t-value</td>
</tr>
<tr>
<td>Study limb</td>
<td>Pre test: 4.42 ± 2.8852</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>Post test: 3.55 ± 2.6427</td>
<td></td>
</tr>
<tr>
<td>Control limb</td>
<td>Pre test: 5.79 ± 5.5535</td>
<td>0.409</td>
</tr>
<tr>
<td></td>
<td>Post test: 3.91 ± 3.525</td>
<td></td>
</tr>
</tbody>
</table>

* Significant

P: Probability

**Table (4): Blood flow velocity changes at pre-test and post-test after application of LILT in group II.**

**Fig. (4): Blood flow velocity changes at pre-test and post-test after application of LILT in group II.**

III. Effect of LILT pulse frequencies on caliper of the blood vessel

For group I (200 Hz):

A. Study limb: t-test revealed that the mean values of caliper of the blood vessel changed from (1.4867 ± 0.2924) mm at pre-test to (1.4867 ± 0.2825) mm at the end of twelve sessions. These changes were insignificant (P>0.05).

B. Control limb: The mean values of caliper of the blood vessel changed from (1.3667 ± 0.3436) mm at pre-test to (1.42 ± 0.221) mm at the end of twelve sessions. These changes were insignificant (P> 0.05) as shown in table (5) and figure (5).
**Table (5): Blood vessel caliper values at pre-test and post-test after application of LILT in group I.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Caliper of the blood vessel (Mean ± S.D.) mm</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>t-value</td>
</tr>
<tr>
<td>Study limb</td>
<td></td>
<td>2.145</td>
</tr>
<tr>
<td>Pre test</td>
<td>1.4867 ± 0.2924</td>
<td></td>
</tr>
<tr>
<td>Post test</td>
<td>1.4867 ± 0.2825</td>
<td></td>
</tr>
<tr>
<td>Control limb</td>
<td></td>
<td>0.471</td>
</tr>
<tr>
<td>Pre test</td>
<td>1.3667 ± 0.3436</td>
<td></td>
</tr>
<tr>
<td>Post test</td>
<td>1.42 ± 0.221</td>
<td></td>
</tr>
</tbody>
</table>

* Significant P: Probability

**Fig. (5): Blood vessel caliper changes at pre-test and post-test after application of LILT in group I.**

For group II (2000 Hz):

A. Study limb: t-test revealed that the mean values of caliper of blood vessel changed from (1.5133 ± 0.2588) mm at pre-test to (1.3867 ± 0.1457) mm at the end of twelve sessions. These changes were insignificant (P>0.05).

B. Control limb: The mean values of caliper of blood changed from (1.5133 ± 0.3944) mm at pre-test to (1.6067 ± 0.3035) mm at the end of twelve sessions. These changes were insignificant (P>0.05) as shown in table (6) and figure (6).

**Table (6): Blood vessel caliper values at pre-test and post-test after application of LILT in group II.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Caliper of the blood vessel (Mean ± S.D.) mm</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>t-value</td>
</tr>
<tr>
<td>Study limb</td>
<td></td>
<td>1.535</td>
</tr>
<tr>
<td>Pre test</td>
<td>1.5133 ± 0.2588</td>
<td></td>
</tr>
<tr>
<td>Post test</td>
<td>1.3867 ± 0.1457</td>
<td></td>
</tr>
<tr>
<td>Control limb</td>
<td></td>
<td>0.81</td>
</tr>
<tr>
<td>Pre test</td>
<td>1.5133 ± 0.3944</td>
<td></td>
</tr>
<tr>
<td>Post test</td>
<td>1.6067 ± 0.3035</td>
<td></td>
</tr>
</tbody>
</table>

* Significant P: Probability

**Fig. (6): Changes of the blood vessel caliper at pre-test and post-test after application of LILT in group II.**
Comparison between the post-test results of both groups

Comparison of the post-test results of the II-blood flow volume between both groups:

I- The study limb: t-test revealed that the mean values of blood flow volume after twelve sessions of LILT in group I was \((5.6 \pm 1.121)\) mL/min while it was \((4.7 \pm 1.033)\) mL/min in group II. These changes were significant (P<0.05).

The control limb: The mean values of blood flow volume after twelve sessions of LILT in group I was \((4.7 \pm 2.257)\) mL/min while it was \((3.9 \pm 6.95)\) mL/min in group II. These changes were insignificant (P> 0.05) as shown in table (7) and figure (7).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Blood flow volume (Mean ± S.D.) mL/min</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-test results of both study limbs</td>
<td>Study limb (I) 5.6 ± 1.121</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Study limb (II) 4.7 ± 1.033</td>
<td>2.145</td>
</tr>
<tr>
<td>Post-test results of both control limbs</td>
<td>Control limb (I) 4.7 ± 2.257</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control limb (II) 3.9 ± 6.95</td>
<td>2.145</td>
</tr>
</tbody>
</table>

* Significant

P: Probability

Table (7): Blood flow volume changes in group I and group II in both study and control limbs after application of LILT.

Fig. (7): Blood flow volume changes in group I and group II in both study and control limbs after application of LILT.

Comparison of the post-test results of the II-blood flow velocity between both groups:

I- The study limb: t-test revealed that the mean values of blood flow velocity after twelve sessions of LILT in group I was \((7.06 \pm 2.0452)\) cm/sec while it was \((3.55 \pm 2.6427)\) cm/sec in group II. These changes were significant (P>0.05).

The control limb: The mean values of blood flow volume after twelve sessions of LILT in group I was \((5.53 \pm 0.9078)\) cm/sec while it was \((3.91 \pm 3.525)\) cm/sec in group II. These changes were insignificant (P> 0.05) as shown in table (8) and figure (8).

Table (8): Blood flow velocity changes in group I and group II in both study and control limbs after application of LILT.
Comparison of the post-test results of the caliper of the blood vessel between both groups:

I- The study limb: t-test revealed that the mean values of blood flow volume after twelve sessions of LILT in group I was $(1.4867 \pm 0.2825)$ mm while it was $(1.3867 \pm 0.1457)$ mm in group II. These changes were insignificant ($P > 0.05$).

II- The control limb: The mean values of blood flow volume after twelve sessions of LILT in group I was $(1.42 \pm 0.221)$ mm while it was $(1.6067 \pm 0.3035)$ mm in group II. These changes were insignificant ($P > 0.05$) as shown in table (9) and figure (9).

Table (9): Caliper of the blood vessel changes in group I and group II in both study and control limbs after application of LILT.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Caliper of the blood vessel (Mean ± S.D.) mm</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-test results of both study limbs</td>
<td>Study limb (I)</td>
<td>1.4867 ± 0.2825</td>
</tr>
<tr>
<td></td>
<td>Study limb (II)</td>
<td>1.3867 ± 0.1457</td>
</tr>
<tr>
<td>Post-test results of both control limbs</td>
<td>Control limb (I)</td>
<td>1.42 ± 0.221</td>
</tr>
<tr>
<td></td>
<td>Control limb (II)</td>
<td>1.6067 ± 0.3035</td>
</tr>
</tbody>
</table>

* Significant  

P- Probability
Pearson correlation coefficient between post-test results of blood flow volume, blood flow velocity and blood vessel caliper in study limb

I- Pearson Correlation coefficient in group I:
Pearson correlation showed that there was: I- Significant direct correlation between blood flow volume and blood flow velocity ($C=0.637$ and $P<0.05$), and II- Significant negative correlation between blood flow velocity and blood vessel caliper ($C=-0.848$ and $P<0.005$). As shown in table (10).

**Table (10): Pearson correlation coefficient in group I.**

<table>
<thead>
<tr>
<th></th>
<th>Blood flow volume and blood flow velocity</th>
<th>Blood flow volume and blood vessel caliper</th>
<th>Blood flow velocity and blood vessel caliper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson correlation</td>
<td>0.637</td>
<td>0.207</td>
<td>-0.848</td>
</tr>
<tr>
<td>Significance</td>
<td>0.033*</td>
<td>0.458</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

*Significant

II- Correlation coefficient in group II:
Pearson correlation showed that there was: I- Insignificant direct correlation between blood flow volume and blood flow velocity ($C=0.16$ and $P>0.05$), and II- Insignificant negative correlation between blood flow velocity and blood vessel caliper ($C=-0.232$ and $P>0.05$). as shown in table (11).

**Table (11): Pearson correlation coefficient in group II.**

<table>
<thead>
<tr>
<th></th>
<th>Blood flow volume and blood flow velocity</th>
<th>Blood flow volume and blood vessel caliper</th>
<th>Blood flow velocity and blood vessel caliper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson correlation</td>
<td>0.16</td>
<td>0.493</td>
<td>-0.232</td>
</tr>
<tr>
<td>Significance</td>
<td>0.954</td>
<td>0.062</td>
<td>0.406</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Within the limitation of the present study, it appeared that the low pulse frequency that was included in the design of the study (200 Hz) produced significant increase ($P<0.05$) in the blood flow volume (by 48.2%).

It was reported that laser applied in very short duration impulses emitted in slow recurrences (low pulse frequency) can in fact deposit high quantities of energy without damaging tissues and so carry out a deeper biological reaction than long impulses emitted at short recurrences.
(high pulse frequency) and produce an effect which is close to continuous emission\(^1\).

These findings had an agreement with the current study and the work of Bibikova and his coworkers who noticed that the blood flow was significantly increased (0.09 ± 0.006) after laser application\(^{24,26,32}\). Also, it was concluded that application of LILT (30J/cm\(^2\), 30 mW, for 20 min) would raise skin temperature in patients with diabetic microangiopathy that indicated improvement in cutaneous microcirculation\(^{39}\). Passariello and his colleagues suggested that laser radiation was found to have a positive effect on the microcirculation of subjects with Raynold’s syndrome. But it was difficult to give a precise physiopathological-interpretation of their results. They proposed the following hypotheses to explain their results: 1) A myolytic action of laser beam on the blood vessels, 2) An increased cyclic adenosine monophosphate in vessel wall, 3) A reflex action mediated by autonomic nervous system, and 4) An interference with some chemical mediators responsible for the control of vessel tone\(^{25,31}\).

On the other hand, it was reported that LILT increase number of dermal vessels through a video measuring system for chronic ulcers\(^{16,36,42}\). It was noticed that the number of vessels were approximately twice that of control group which indicated improvement in regional blood flow in transmyocardial laser revascularization\(^{37}\). In agreement with the present study, Schindl and his group investigated the effect of LILT on wound healing in patients with reduced microcirculation. Bibikova and his associates studied the effect of LILT (He-Ne) on the process of neoformation of blood capillaries during regeneration in the gastrocnemius muscle using histomorphometric methods\(^{38,39}\). They subjected the injured zone to four direct He-Ne laser irradiations (6 mW for 2.3 min) every alternate day. This study did not compare between different laser parameters and also was dependant on counting the volume density of capillaries and not on direct measurement technique of blood flow as Doppler.

The previous studies revealed that the angiogenesis to various chemotactic and growth factors, in particular fibroblast growth factor, lactic acid, biogenic amines\(^{28}\). It had been proposed that endothelial cell migration was more important than proliferation in angiogenesis, so that chemo-attractants such as fibronectin, heparin, and platelets-derived factors would play a major role in angiogenesis after laser application\(^{14}\).

Within the limitation of the present study, it would appear that the low pulse frequency that was included in the design of the study (200 Hz) produced significant increase (P<0.05) in the blood flow velocity (by 40.6%). These finding confirm the work of Siposan and his colleagues on the in-vitro effect of low-level laser radiation (LLLR) on selected rheologic constants of the human blood that was investigated. The variations of complete blood counting (CBC) parameters to the received dose were determined, as well as of blood viscosity (an erythrocyte aggregation index), as a research method for some structural alteration of blood proteins. This was also confirmed by the electrophoretic study of plasma proteins from the irradiated blood\(^{17}\). This study concluded that the effect of LILT on red blood cells confirms the nonresonant mechanism of this biostimulating effect, by the changes occurring in the cell membrane (blood cells), by revitalizing of red blood cells functional capacities and by several biochemical effects at the membrane’s level. This decreased the blood viscosity and interum increased blood flow velocity\(^{17}\).
On the other hand, the low pulse frequency that was included in the design of the current study (200 Hz) produced non-significant difference (P>0.05) in the caliper of the blood vessel. These findings confirmed that laser irradiation had no influence on the vasomatility of veins, arteries and lymphatic vessels in normal healthy volunteers. Pearson correlation between the three measures indicated that there was a significant direct correlation between blood flow volume and blood flow velocity (C= 0.637 and P<0.05). This obey the physiological law of blood flow that states that the blood flow is the resultant of surface area cross velocity (Flow= Area X Velocity). Furthermore the significant negative correlation between blood flow velocity and blood vessel caliper (C= -0.848 and P< 0.005) also obeys the physiological law of blood flow.

Conclusion
From the results of this study it was concluded that LILT (200 Hz) low pulse frequency could improve blood flow while high pulse frequency (2000 Hz) did not affect blood flow measurements.

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