Efficacy of Laser Pulse Frequencies on Blood Flow in Type 2 Diabetic Patients

***Amir Nazih Wadee.**
***Ph. D. P. T. Department of Basic Science for Physical Therapy. Faculty of Physical Therapy. Cairo University.**
Contact phone: 01222367919

Email: anazih@cu.edu.eg

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Abstract

Background: research reports had noted apparent increase in cutaneous and deep blood flow as a result of low intensity laser therapy (LLLT) in normal subjects**. Purpose:** was to investigate the effective laser pulse frequency either (200 or 2000 Hz) on improving blood flow in type 2 diabetic patients. **Subjects:** Forty five diabetic patients selected from out clinic of Kasr El-Aini Hospital, Cairo University assigned randomly into three groups. The blood flow volume, blood flow velocity and caliper of the blood vessel were evaluated before laser application and after twelve sessions using duplex Doppler ultrasound. **Methods:** Combined He-Ne and infrared LILT was administered three times a week for twelve sessions at intensity of 3 J, power 500 mW, 808 nm duration 15 min and pulse frequency 200 Hz for group I, 2000 Hz for group II, and sham LILT for group III on the sural artery at posterior aspect of dominant leg. **Results:** paired t-test revealed that low pulse frequency (200 Hz) LILT produced significant improvement in blood flow volume and blood flow velocity (t= 1.76, p= 0.001 and t= 2.8, p= 0.01) respectively (P<0.05). While there was no significant changes in caliper of the blood vessel of group I, blood flow volume, blood flow velocity or caliper of the blood vessel of group II and group III (t= 2.15, p= 1, t= 2.15, p= 1, t= 1.11 p= 0.31, t= 1.54, p= 0.15, t= 2.51, p= 1, t= 1.21 p= 0.33, t= 1.45, p= 0.15) respectively (P<0.05). ANOVA test in between groups revealed insignificant changes in all pre and post- measures except significant results in blood flow volume and velocity which indicating the superiority of group I on both group II and III by post hoc test. **Conclusion:** low pulse frequency of LILT (200 Hz) improved blood flow volume and velocity than high pulse frequency (2000 Hz) in type 2 diabetic patients.

Key wards: laser, blood flow volume, blood flow velocity, blood vessel caliper.

Introduction:

Purpose of the current study was to investigate the effective laser pulse frequency either low or high pulse frequency (200 or 2000 Hz) on improving blood flow in type 2 diabetic patients. Laser means light amplification by stimulated emission of radiation. The uses of lasers are numerous and wide spread nearly in each field of human attempt in treatment, science and technology (1-3). Number of practical and research works had reported evident increment in cutaneous and profound blood stream subsequently of laser (combined He-Ne and infra red laser) radiation or recommended such putative laser-mediated alteration in blood flow as a mechanism of action for some other clinical or physiological impact. It was accounted for that low intensity laser therapy (LILT) enhanced local microcirculation and it could likewise enhance oxygen supply to hypoxic cells and in the meantime it could evacuate the accumulated waste products (3). But there was a gap found in the literature upon the impact of LILT on blood stream and furthermore inconsistency and conflicting outcomes on the detailed reviews because the exact mechanism was obscure, the powerful parameters were not built up and variable consequences for various blood flow estimations were not concluded (4). Diabetes mellitus influences roughly 100 million people worldwide. 5%-10% have type I (insulin-dependent) and 90% to 95% have type II (non–insulin- dependent) diabetes mellitus, its macrovascular appearances incorporate atherosclerosis and angiopathy. The microvascular consequences, retinopathy and nephropathy, are real reasons for visual deficiency and renal failure (5 and 6).

LILT might enhance microcirculation locally that enhance wound healing, promote oxygen supply to hypoxic cells that improve the metabolic functions of these cells, increment number of white blood cells and antibodies that decline inflammation (7), and in the meantime it could remove the accumulated waste products that diminishing muscle spasm (8, 9), likewise the normalization of the microcirculation might occur because of laser applications and interfere with the " vicious circle " of pain (9). LILT might enhance elasticity of blood vessels without influencing their caliper that improve its accommodation to more blood, that cut the endless loop of pain and influencing the local veins by suction drive thus increment venous return (10-12). Scientists proposed that the physiological reactions to laser were influenced by its pulse frequency which was one of its parameters that ought to be adjusted however the distinctions in change in blood flow in accordance to pulse frequency were not clear yet (13-15). So physiotherapists were still in need to examine and affirm the changes in blood flow after laser application that might guide them to construct the effective parameters of laser so as to gain the most earlier and beneficial results. So, was there a difference in the effect of laser pulse frequency either (200 or 2000 Hz) on improving blood flow in type 2 diabetic patients?

Design of the study: was controlled randomized trial of pre-test post-test control group experimental design. This study was ethically approved from the ethical committee of Faculty of Physical Therapy, Cairo University, and was conducted in the period from January to May 2016. The laser application was conducted in the out clinic of the faculty. The measurements were performed in the diagnostic unit of Kasr El-Aini hospital.

Subjects, materials and methods:

Forty five diabetic patients (type 2) with well controlled blood-suger level for six months prior to the study. They didn't complain from any vascular or blood disease. Patients selected from out clinic of Kasr El-Aini Hospital, Cairo University. Their average age is from 42 to 52 years old with mean age (46.92 $\pm$ 1.5) years, height ranged from (165-174) cm with mean height (174.1 $\pm$ 3.23) cm and weight ranged from (62-80) kg with mean weight (72.09 $\pm$ 6.79) kg. Subjects were randomly assigned into three groups I, II and III.

Group I: fifteen patients had received twelve sessions, every other day; of LILT (combined He-Ne and infrared laser with scanner technique) its intensity was 3 J, power 500 mW, 808 nm duration 15 min and pulse frequency was 200 Hz.

Group II: fifteen patients had received the same but pulse frequency was 2000 Hz.

Group III: control group had received sham LILT.

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) 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.

Instrumentations:

1. **LLLT:** ASA Laser Comby 3 Terza Serie. ASA s.r.l. – Via A. Volta, 9 – 36057 ARCUGNANO (VI) – ITALIA.
2. **Duplex Doppler ultrasound:** MY-A035A Maya MAQ5 digital color duplex Doppler ultrasonic diagnostic- England.

Procedures:

Blood flow measurement procedures: 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 were then completed by the same radiology specialist to register the blood flow volume, blood flow velocity and the caliper of the sural artery (figure 1 and 2).

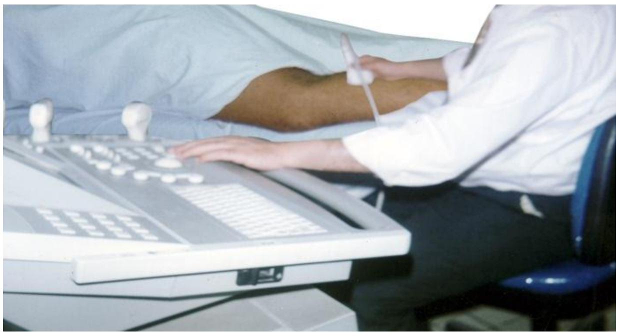

Figure (1): Blood flow measurement by duplex Doppler ultrasound

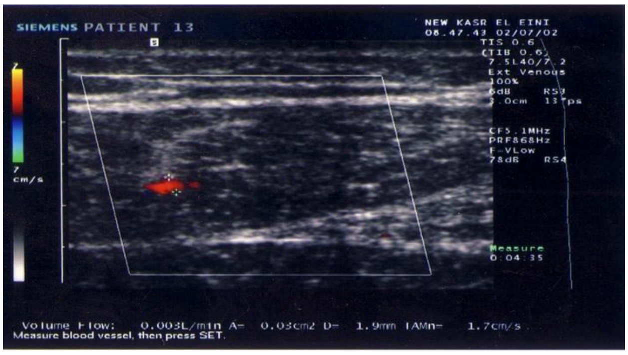

Figure (2): Duplex Doppler ultrasound report

LILT application 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 perpendicular 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 malleoli line. The intensity was 3 J, power 500 mW, 808 nm duration 15 min and pulse frequencies was changed according to the experimental group, either 200 Hz in group I, 2000 Hz in group II or sham LILT in group III. Patients and researcher wear the protective goggles during LILT application. The patients were permitted to have ten minutes rest after laser application. These procedures were repeated for twelve sessions, three sessions per week. 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 (figure 3).

Figure (3): LILT application

Results:

Descriptive statistics (mean and standard deviation) were performed for all measurements. Paired t-test was performed to assess the significance between the pre-test and post-test measures within each group. ANOVA test was performed to evaluate the significance in between groups. Finally, post hoc test was used only when there were significant differences in between groups to test the superiority of one group on the other. The level of significance was 0.05.

I. Effect of LILT pulse frequencies on blood flow volume:

1. Group I (200 Hz): t-test revealed that the mean values of blood flow volume were increased from (2.9 ± 1.51) m.L/min at pre-test to (5.6 ± 1.12) m.L/min at the end of twelve sessions. These changes were significant (t= 1.76 and p= 0.001).

2. Group II (2000 Hz): t-test revealed that the mean values of blood flow volume changed from (4.7 ± 1.81) m.L/min at pre-test to (4.7 ± 1.07) m.L/min at the end of twelve sessions. These changes were insignificant (t= 2.15 and p= 1).

3. Group III (sham LILT): t-test revealed that the mean values of blood flow volume changed from (4.49 ± 1.6) m.L/min at pre-test to (4.5 ± 1.23) m.L/min at the end of twelve sessions. These changes were insignificant (t= 2.51 and p= 1) as shown in table (1) and (figure 4).

Table (1): Blood flow volume changes

Measuring variable	Group	Variables	Blood flow volume (Mean ± S.D) m.L/min	t-test	
				t-value	P-value
Blood flow volume changes	group I	Pre-test	2.9 ± 1.51		
		Post-test	5.6 ± 1.12	1.76	0.001*
	group II	Pre-test	4.7 ± 1.81		
		Post-test	4.7 ± 1.07	2.15	1
	group III	Pre-test	4.49 ± 1.6		
		Post-test	4.5 ± 1.23	2.51	1

*significant

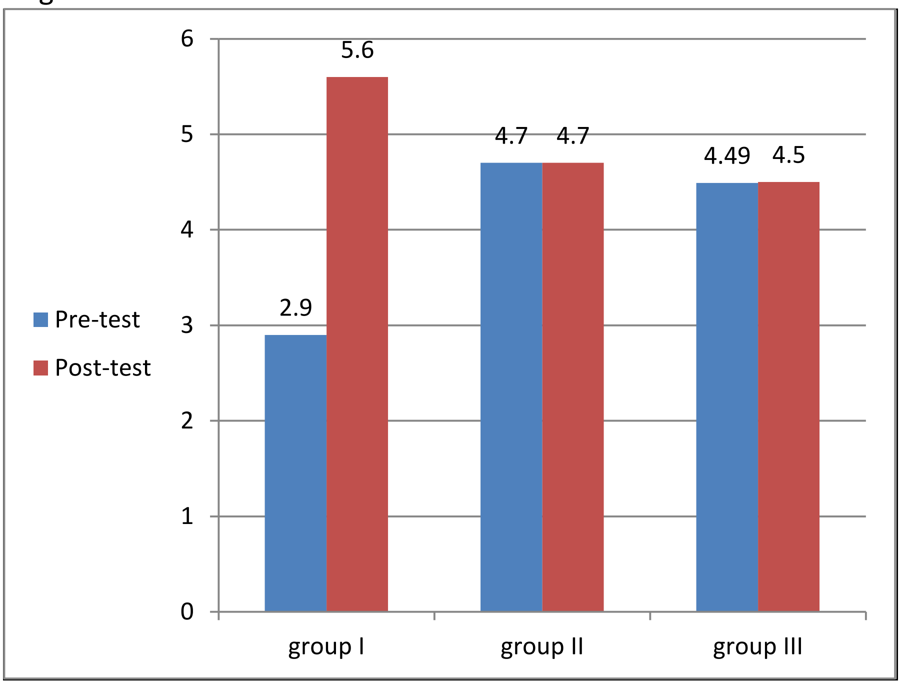

Figure (4): Blood flow volume changes

II. Effect of LILT pulse frequencies on blood flow velocity

1. Group I (200 Hz): t-test revealed that the mean values of blood flow velocity were decreased from (5.02 + 2.87) cm/sec at pre-test to (7.06 + 2.05) cm/sec at the end of twelve sessions. These changes were significant (t= 2.8 and p= 0.01).

2. Group II (2000 Hz): t-test revealed that the mean values of blood flow velocity changed from (4.42 + 2.89) cm/sec at pre-test to (3.55 + 2.65) cm/sec at the end of twelve sessions. These changes were insignificant (t= 1.11 and p= 0.31).

3. Group III (sham LILT): t-test revealed that the mean values of blood flow velocity changed from (3.66 + 1.02) cm/sec at pre-test to (4.21 + 1.01) cm/sec at the end of twelve sessions. These changes were insignificant (t= 1.21 and p= 0.33) as shown in table (2) and (figure 5).

Table (2): Blood flow velocity changes

Measuring variable	Group	Variables	Blood flow volume (Mean + S.D) cm/sec	t-test	
				t-value	P-value
Blood flow velocity changes	group I	Pre-test	5.02 + 2.87		
		Post-test	7.06 + 2.05	2.8	0.01*
	group II	Pre-test	4.42 + 2.89		
		Post-test	3.55 + 2.65	1.11	0.31
	group III	Pre-test	3.66 + 1.02		
		Post-test	4.21 + 1.01	1.21	0.33

*significant

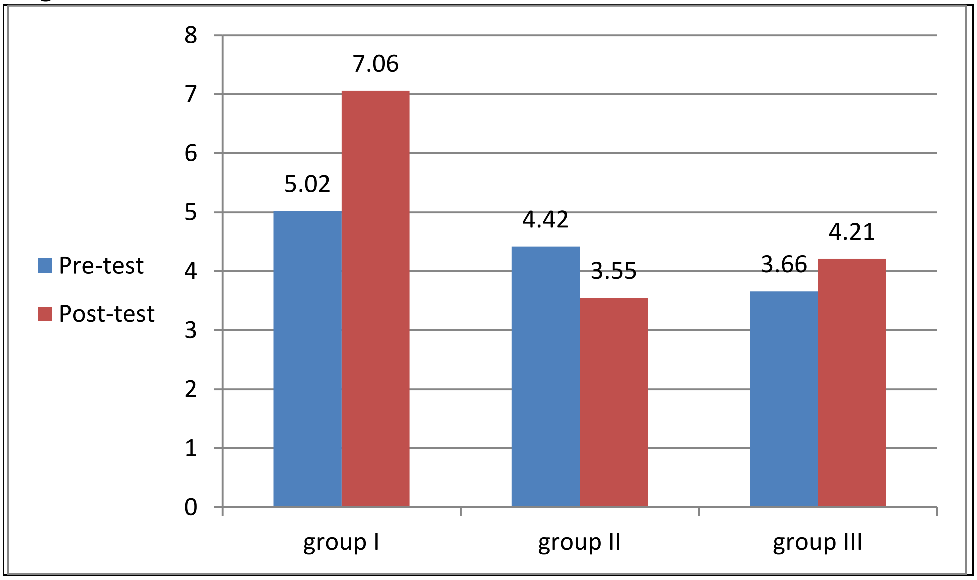

Figure (5): Blood flow velocity changes

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

1. Group I (200 Hz): t-test revealed that the mean values of caliper of the blood vessel changed from (1.49 + 0.29) mm at pre-test to (1.49 + 0.28) mm at the end of twelve sessions. These changes were insignificant (t= 2.15 and p= 1).

2. Group II (2000 Hz): t-test revealed that the mean values of caliper of blood vessel changed from (1.51 + 0.26) mm at pre-test to (1.39 + 0.15) mm at the end of twelve sessions. These changes were insignificant (t= 1.54 and p=0.15).

3. Group III (sham LILT): t-test revealed that the mean values of caliper of blood vessel changed from (1.82 ± 0.03) mm at pre-test to (1.41 ± 0.04) mm at the end of twelve sessions. These changes were insignificant (t= 1.45 and p=0.15) as shown in table (3) and (figure 6).

Table (3): Blood vessel caliper

Measuring variable	Group	Variables	Blood flow volume (Mean ± S.D) mm	t-test	
				t-value	P-value
Blood vessel caliper changes	group I	Pre-test	1.49 ± 0.29		
		Post-test	1.49 ± 0.28	2.15	1
	group II	Pre-test	1.51 ± 0.26		
		Post-test	1.39 ± 0.15	1.54	0.15
	group III	Pre-test	1.82 ± 0.03		
		Post-test	1.41 ± 0.04	1.45	0.15

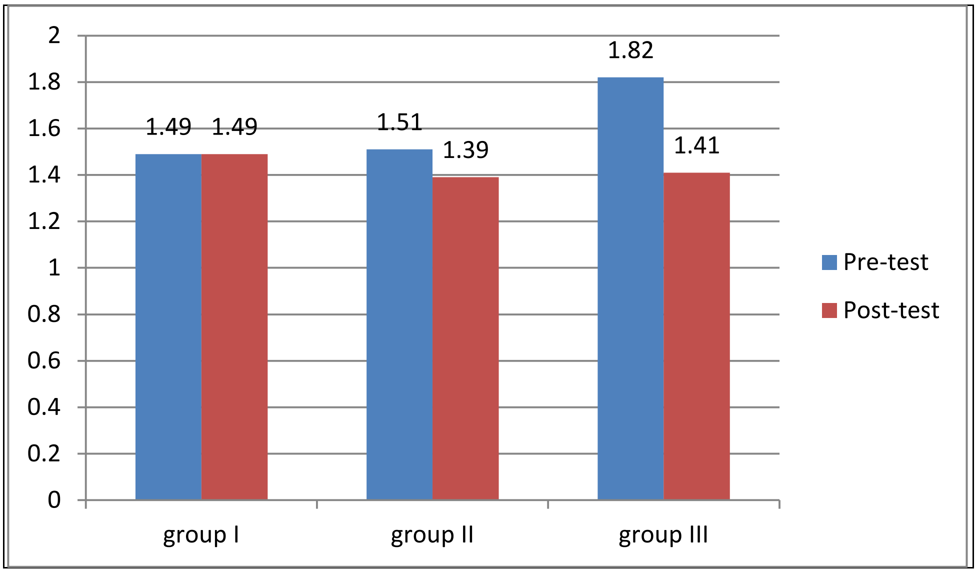

Figure (6): Changes of the blood vessel caliper

IV. Effect of LILT on blood flow in between groups:

1. Blood flow volume: ANOVA test for pre-measurements revealed insignificant changes while post- measurements revealed significant changes (f= 0.48, p= 0.17 and f= 2.15 and p= .04 respectively). Post hoc test of the post results revealed significant changes in between groups I and II, in addition to group I and III, while there was insignificant changes in between group II and III (mid= 0.83, p= 0.02, mid= 0.68, p= 0.02 and mid= 0.02 , p= 0.69 respectively) (table 4).

Table (4): ANOVA and Post hoc tests for blood flow volume

variable	measurement	ANOVA		Post hoc		
Blood flow volume		F	P	Groups	MD	P
	pre	0.48	0.17	I & II	0.83	0.02*
	Post	2.15	0.04*	I & III	0.68	0.02*
				II & III	0.02	0.69

*significant

2. Blood flow velocity: ANOVA test for pre-measurements revealed insignificant changes while post- measurements revealed significant changes (f= 1.9, p= 0.28 and f= 3.74 and p= 0.03 respectively). Post hoc test of the post results revealed significant changes in between groups I and II, in addition to group I and III, while there was insignificant changes in between group II and III (mid= 0.75, p= 0.03, mid= 0.58, p= 0.01 and mid= 0.22 , p= 0.49 respectively) (table 5).

Table (5): ANOVA and Post hoc tests for blood flow velocity

variable	measurement	ANOVA			Post hoc	
		F	P	Groups	MD	P
Blood flow velocity	pre	1.9	0.28	I & II	0.75	0.03*
	Post	3.74	0.03*	I & III	0.58	0.01*
				II & III	0.22	0.49

*significant

2. Blood vessel caliper: ANOVA test for both pre and post- measurements revealed insignificant changes (f= 1.37, p= 0.11 and f= 1.93 and p= 0.34 respectively) (table 6).

Table (6): ANOVA and Post hoc tests for blood flow velocity

variable	measurement	ANOVA	
Blood vessel caliper	pre	1.37	0.11
	Post	1.93	0.34

Discussion:

Within the limitation of the current study, it appeared that the low pulse frequency (200 Hz) produced significant increase ($P<0.05$) in the blood flow volume (t= 1.76, p= 0.001). Gorgey et al 2008 (15) had accounted for that laser application for brief impulses duration emitted in slow recurrences (low pulse frequency) could in reality deposit high quantities of energy without harming tissues, in addition, Stadler et al, 2000 (16) concluded that LILT produced a more profound natural response than long impulses discharged at short repeats (high pulse frequency) and Lins et al, 2010 (17) who said that low pulse frequency had delivered an impact which was near consistent emission. These results had a concurrence with Stadler et al, 2000 (16) who had noticed that the blood stream was significantly increased (0.09 ± 0.006) after laser application. Likewise, Gao and Xing, 2009 (18) reasoned that utilization of LILT (30J/cm2, 30 mW, for 20 min) would bring skin tempertature up in patients with diabetic microangiopathy that showed change in cutaneous microcirculation. Also, Kreisler et al, 2002 and Chen et al, 2008 (19 and 20) found that laser radiation had positively affect the microcirculation of subjects with Raynold's disorder. However, Schindl et al, 2003 and Peplow et al, 2011 (21 and 22) decided that it was hard to give an exact physio-pathological explanation of their outcomes. In addition Desilva et al, 2010 and Peplow et al, 2010 (23 and 24) proposed this accompanying theory to clarify the outcomes: A myolytic activity of laser beam on the blood vessels and notwithstanding an increased cyclic adenosine monophosphate in vessel wall. Hopf and Rollin, 2007 and Minatel et al, 2009 (25 and 26) were adding the reflex activity interceded via autonomic nervous system. Also, they believed an interference with some chemical mediators responsible for the control of vessel tone.

On the same side, it was recorded by Leaper, 2007 and Krug, 2007 (27 and 28) that LILT incremented the number of dermal vessels through a video measuring system for chronic ulcers. It was seen by Sun et al, 2005 and Ring, 2010 (29 and 30) that the quantity of vessels were roughly twice that of control gathering which showed change in local blood flow in transmyocardial laser revascularization. In concurrence with the present study, Sobanko and Alster 2008 (31) concentrated the impact of LILT on the procedure of new formation of blood vessels during recovery in the gastrocnemius muscle utilizing histomorphometric techniques. Also, Stcker et al, 2001 (32) subjected the injured zone to four direct He-Ne laser irradiations (6 mW for 2.3 min) day after day. This review did not compare between various laser parameters and furthermore was dependant on counting the volume thickness of vessels and not on direct measurement strategy of blood stream as Doppler. Niu et al, 2001, Wright et al, 2006 and Kolodyzhniy et al, 2011 (33-35) uncovered the angiogenesis to different chemotactic and growth factors, specifically fibroblast growth factors, lactic acid, biogenic amines. Otah et al, 2005 and Stasinopoulos and Johnson, 2005 (36 and 37) suggested that endothelial cell migration was more imperative than expansion in angiogenesis, so that chemo-attractants, for example, fibronectin, heparin, and platelets- derived variables would assume a noteworthy part in angiogenesis after laser application.

Within the limitation of the present study, doubtlessly the low pulse frequency (200 Hz) LILT delivered significantly increased the blood flow velocity (t= 2.8, p= 0.01). Additionally, these finding affirmed the work by Rabelo et al, 2006 (38) on the in-vitro impact of low-level laser radiation (LLLR) on chose rheologic constants of the human blood that was examined. The varieties of complete blood counting (CBC) parameters to the received dose were resolved, as well as of blood viscosity (an erythrocyte aggregation index), as an exploration technique for some auxiliary modification of blood proteins. This was additionally affirmed by Albertini et al, 2007 (39) who performed the electrophoretic investigation of plasma proteins from the irradiated blood. Furthermore, Dall et al, 2009 (40) studied the impact of LILT on red blood cells and affirmed the non resonant mechanism of this biostimulating effect, by the progressions happening in the cell membrane, by renewing of red cells functional capacities and by a several biochemical impacts at the membrane's level. This diminished the blood viscosity and in turn increased blood flow velocity. Finally, the low pulse frequency that was incorporated into the plan of the present review (200 Hz) produced non-significant difference (P>0.05) in the caliper of the blood vessel. These discoveries affirmed what was discussed by Wigington et al, 2004 (14) that laser irradiation had no impact on the vasomatricity of veins, arteries and lymphatic vessels in type 2 diabetic patients.

Conclusion: LILT with low pulse frequency could enhance blood flow in type 2 diabetic patients while high while high pulse frequency could not influence blood flow.

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كفاءة ترددات نبض الليزر على تدفق الدم في النوع ٢ لمرضى السكري

الخلفية: لاحظت التقارير البحثية الزيادة الواضحة في تدفق الدم الجلدي والعميق نتيجة العلاج بالليزر المنخفض الشدة فى الأشخاص الطبيعين. **الغرض:** كان للتحقيق في كفاءة تردد نبضة الليزر إما (٢٠٠ أو ٢٠٠٠ هرتز) على تحسين تدفق الدم في النوع ٢ لمرضى السكري .**الأشخاص:** خمسة وأربعون من مرضى السكري مختارة من العيادة الخارجية بمستشفى قصر العينى ، جامعة القاهرة. تم تقسيمهم عشوائيا إلى ثلاث مجموعات .و تم تقييم حجم تدفق الدم، و سرعة تدفق الدم ، وحجم الوعاء الدموي قبل تطبيق الليزر وبعد إثني عشر جلسة بإستخدام دوبلكس دوبلر الموجات فوق الصوتية . **الطريقة :** تم التعرض إلى الليزر (هيليوم و نيون و تحت الحمراء) ثلاث مرات في الأسبوع لمدة إثني عشر جلسة كثافتة ٣ جول، و قوته ٥٠٠ ميجا واط، و طوله الموجى ٨٠٨ نانومتر لمدة ١٥ دقيقة و تردد نبضة ٢٠٠ هرتز للمجموعة الأولى ، و ٢٠٠٠ هرتز للمجموعة الثانية، ووهمى للمجموعة الثالثة. في الشريان الربلي في الجانب الخلفي من الساق المهيمن .**النتائج :** كشف إختبار (ت) المذدوج أن تردد النبض المنخفض (٢٠٠ هرتز) لليزر أنتج تحسنا ذو دلالة إحصائية في حجم تدفق الدم وسرعة تدفق الدم (ت = ١.٧٦، و ب = ٠.٠٠١ وت= ٢.٨، و ب= ٠.٠١ على التوالي (ب<٠.٠٥) في حين لم يكن هناك تغيير ذو دلالة إحصائية في حجم الوعاء الدموي فى المجموعة الأولى، وحجم تدفق الدم، وسرعة تدفق الدم أو حجم الوعاء الدموي فى المجموعة الثانية والمجموعة الثالثة (ب = ٢.١٥، و ت = ١، و ب = ٢.١٥، و ت= ١، و ب = ١.١١ ، وت = ٠.٣١، و ب = ١.٥٤، و ت = ٠.١٥، و ب= ٢.٥١، و ت = ١، و ب= ١.٢١ ، و ت = ٠.٣٣، و ب= ١.٤٥، و ت= ٠.١٥) على التوالي. و كشف إختبار أنوفا بين المجموعات أن التغيرات ليست ذات دالة إحصائية في جميع قياسات ما قبل وما بعد بإستثناء نتائج ذات دالة إحصائية في حجم تدفق الدم وسرعتة التي تشير إلى تفوق المجموعة الأولى على كل من المجموعة الثانية والثالثة بوسطة إختبار هوك لما بعد. **الخلاصة:** تردد النبض المنخفض (٢٠٠ هرتز) حسن حجم تدفق الدم وسرعتة عن تردد النبض المرتفع (٢٠٠ هرتز) فى النوع ٢ لمرضى السكرى.

الكلمات الدالة: ليزر، وحجم تدفق الدم ، وسرعة تدفق الدم ، و حجم الوعاء الدموي.