

The effect of low level laser therapy on platelet-related coagulation factors (VIII and vWF)

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ABSTRACT

Low level laser therapy (LLLT) empirically used in the treatment of various medical conditions stimulating certain intracellular biological activities resulting in acceleration of wound healing due to various biostimulatory effects. It has been widely tested on cell culture studies; the mechanism of action is complex, in general it involves absorption of light on subcellular level particularly the electron chain system in the mitochondria resulting in the biostimulatory effect.

The chronic inflammatory process have been shown to be reduced in periodontal tissue by LLLT with subsequent release of metalloproteinase's and collagenases resulting in improvement in tissue healing and wound remodeling. It has been also shown to cause smooth muscle relaxation and subsequent vasodilatation. Several studies *in vitro* have shown that LLLT at certain wavelengths may stimulate fibroblast proliferation. Furthermore; (He-Ne) LLLT has been shown to have stimulatory effect on cultured osteoblast resulting in enhancement of proliferation, differentiation and calcification. Other empirical uses of laser therapy include analgesia, antimicrobial activity, laser curettage and relieving trigeminal neuralgia.

Objectives and Results: This study was designed to measure two of the coagulation factors of whole blood samples obtained from 25 healthy male and female adult volunteers before and after LLLT using three different wavelengths (650, 532 and 405 nm), namely factor VIII and von-Willebrand factor; both of these factors are available within platelets granules. The mean concentration of VIII and vWF in all samples were 1.15 and 4.46 ng/ml respectively. The mean concentration and the percent change in individual samples of VIII and vWF after LLLT are listed respectively as follows: 650 nm (0.84, 6.23 and 4.42, 1.48), 532 nm (0.97, - 8.23 and 4.17, -4.24), and 405 nm (0.91, 5.81 and 4.33, -1.42). Paired T-test showed no significant change on VIII including the concentration and percent. The percent change was in VIII concentration was in general towards a negative value for both 650 and 532 nm LLLT, while LLLT with 405 nm resulted in a slight increase in overall samples.

KEY WORDS: Biostimulation, Low Level Laser Therapy, VIII, vWF.

INTRODUCTION

Low level laser therapy (LLLT) has been used with visible, infrared, and ultraviolet (UV) light, but the most effective results have come from using visible spectrum, ranging from (600-700 nm) (Schindl, 2000). LLLT is also known as laser phototherapy (LPT), biostimulative therapy (BT), and low-intensity laser therapy (LILT) (Diessehf, 2008). The energy range emitted by Laser beams can be of benefit in medical application to use for its welding /cutting activity, inducing cell necrosis and tissue breach, this capability has used widely in microsurgery and correction of corneal refractive index. In many other situations, LLLT using low power Lasers has been investigated for the proposed beneficial effects on cellular levels, some has reported the usefulness of Laser therapy on normal cells in cell culture inducing these cells for expression of certain biological activities, other have proposed a good value for induction of cancer cells to switch their genetic material to indulge in the programmed cell death (apoptosis) (Wasik, 2003).

Biological effects of LLLT using for the examining of numerous contexts, stimulation of cells with LLLT. LLLT stimulates wound healing, nerve regeneration, enhanced remodeling collagen synthesis and repair of bone, restoration of normal neural function following injury, normalization of abnormal hormonal function, pain attenuation, stimulation of endorphin release, and modulation of the immune system (Luger, 1998). The chemical energy within the cell converted by effect of photonic energy, in the form of Adenosine-Triphosphate (ATP), which leads to normalization of cell function, pain relief, and healing. Cell membrane permeability is altered, followed by physiological changes in the target cells (Huang, 2013). The effects of LLLT on wound healing are often attributed to increased cell proliferation Hawkins, 2005.

Effects of different LLLT wavelengths on cellular function: For different types *in vitro* studies it was observed that (860 nm) laser light stimulates cellular proliferation (Di Giacomo, 2013). Laser light with wavelength (660 nm) up regulates the production of basic fibroblast growth parameters (Shadab Bagheri-Khoulenjani, 2014) and (632.8 nm) laser light transforms fibroblasts into my fibroblasts in cultured fibroblasts (Deppe, 2007). It was also observed that cellular proliferation increased by (632.8 nm) laser light used in cultured keratinocytes Somayeh (Dastanpour, 2015). Stimulate the release of IL-1 and IL-8. And increase the motility rate Schindl, 2003. Macrophages are activated by (632.8 nm) Laser light and various Laser wavelengths reportedly increase growth factor secretion from cultured macrophages (Moore, 2005). The effects of low –intensity Laser irradiation on vascular endothelial cells

have a very rare studies. However, *in vitro* an increased vascular endothelial cell proliferation has been described (Tsen, 2007). It has also been found that the proliferation rate light dependent, at a maximum in the presence of (665 nm) and (675 nm) light, while (810 nm) light inhibits the proliferation of cultured fibroblasts Almeida –Lopes, 2001. The increased of the proliferation, migration, or adhesion of cultured keratinocytes or fibroblasts have been failed by using low intensity Laser irradiation from a gallium-aluminum-arsenide Pereira, 2002. In addition, the *vitro* biostimulation dependent on many factors, including laser irradiation parameters such as wavelength, fluence, Laser output power and energy density have been investigated by many reported (Kreisler, 2003; Gigo-Benato, 2010). Parameters that are helpful for increasing proliferation rates can sometimes have adverse effects on protein synthesis (Tsen, 2011). Therefore, it is crucial to know the correct combination of parameters (wavelength, power density, and fluence) to arrive at the maxim proliferation rate of cells.

Effects on Blood Cells: The blood is a tissue that is composed of cells (erythrocytes, leukocytes and platelets), flowing within an extracellular matrix (plasma) the major constituent of which is water and about 10% of solutes composed of proteins, minerals, amino acids, vitamins and other constituents. *In vivo*, the blood is normally kept in a fluid state (anti-coagulated) because of the normal function of endothelial cells lining the blood vessel lumen secreting several anticoagulants (heparin and anti-thrombin III) and materials counteracting the normal function of platelets(nitric oxide and prostacycline) (Marques, 2004).

Coagulation factors: Factor VIII is a large complex glycoprotein that is deficient in hemophilia A. It has a domain organization consisting of A1-A2-B-A3-C1-C2 where the B domain is a heavily glycosylated region that is dispensable for procoagulant activity. Within the secretory pathway, factor VIII is processed to a heterodimer of the heavy chain (domains A1-A2-B) in a metal ion association with the light chain (domains A3-C1-C2). Upon secretion from the cell, von-Willebrand factor binds the light chain of factor VIII and stabilizes the factor, preventing degradation, after injury factor VIII is activated and separates from von Willebrand factor. Factor VIII is produced in liver sinusoidal cells and endothelial cells outside of the liver throughout the body. Factor VIII is also found in alpha granules of platelets along with other coagulation factors like V, fibrinogen, fibronectin and PDGF, while platelets Delta granules, or dense bodies, contain ADP, calcium, serotonin, which are platelet-activating mediators.

Aims of Study: Evaluate the alterations in platelet-related coagulation factors in response to LLLT with different wavelengths and powers.

2. MATERIALS AND METHODS

Twenty five blood samples were collected from non-smoker healthy adult volunteers of an age range between 20-35 years old, divided into two containers; trisodium citrate 3.8% in a ratio of 9:1 and lithium-heparin. Each individual tube was then fractionated into four separate tubes of (1 ml). And immediately subjected to LLLT for exactly 30 seconds in different wavelengths of 650 nm (15.6 J/cm²), 532 nm (14.4 J/cm²), and 405 nm (13.2 J/cm²). All samples were then incubated in shaking water bath at 37°C for exactly 30 min. Centrifugation was done for 5 min. at 5000 rpm top separate the plasma.

Plasma samples were tested for the following parameters; factor 8 (VIII) and von-Willebrand Factor (vWF), using ELISA kits purchased from Elabscience corp. (China).

Types of laser utilize wavelengths and powers:

Output power of Laser (405 nm) = (110) mW.

Output Power of Laser (532 nm) = (120) mW.

Output Power of Laser (650nm) = (130) mW.

Beam Spot Size for each one = (0.25) cm.

3. RESULTS

Factor 8 (VIII) concentrations was estimated using ELISA kit purchased from Elabscience, the means of the test results are shown in table(1), the concentration is estimated in ng/ml. The percent of increment or decrement in VIII concentration for individual samples, as well as for vWF was calculated according to the following equation:
 $\% \text{ increment or decrement} = \{\text{Concentration after LLLT} - \text{Concentration before LLLT}\}/\text{Concentration before LLLT} \times 100\%$.

The percent change (increment or decrement) was estimated for individual samples and the mean percent change was estimated finally. Since VIII concentration in plasma is widely variable in different populations, the normal value of this factor should always be estimated in different populations. According to table (1), our study clearly states that the normal value is 1.15 ± 1.04 ng/ml in the studied group.

Table 1. Shows the mean concentration of VIII and vWF in test samples before and after LLLT. The p value was calculated with Spearman's paired-T test

Wavelength	VIII			vWF		
	Before (ng/ml) Mean \pm SD	After (ng/ml) Mean \pm SD	P-value	Before (ng/ml) Mean \pm SD	After (ng/ml) Mean \pm SD	P-value
650 nm	1.15 \pm 1.04	0.84 \pm 0.70	0.196	4.46 \pm 1.32	4.42 \pm 1.12	0.889
% 650 nm	1.15 \pm 1.04	6.23 \pm 86.43	0.773	4.46 \pm 1.32	1.48 \pm 19.19	0.459
532 nm	1.15 \pm 1.04	0.97 \pm 1.05	0.335	4.46 \pm 1.32	4.17 \pm 0.99	0.133
% 532 nm	1.15 \pm 1.04	-8.23 \pm 51.29	0.372	4.46 \pm 1.32	4.24 \pm 14.31	0.008*
405 nm	1.15 \pm 1.04	0.91 \pm 0.79	0.213	4.46 \pm 1.32	4.34 \pm 1.25	0.547
% 405 nm	1.15 \pm 1.04	5.81 \pm 43.86	0.439	4.46 \pm 1.32	1.48 \pm 17.53	0.113

Significant p-values were regarded to be less than 0.05. It is obvious that there is a significant correlation only with LLLT of 532 nm, resulting in an increase in the mean concentration of vWF.

Figure (1) shows that there is an obvious negative correlation between the concentration of VIII before and after LLLT in the three used wavelengths for each individual sample, while Figure (2) shows that there is a variable correlation between the percent of change in VIII concentration after LLLT in the three used wavelengths, clearly illustrated in table (1), with no significant correlation after applying paired-T test. These results can be summarized in Figure (3), which refers to the random distribution of individual samples in correlation with the three wavelengths, showing that there is a significant negative correlation only with 532 nm, while the other wavelengths were insignificantly positively and negatively correlated with the original VIII concentration.

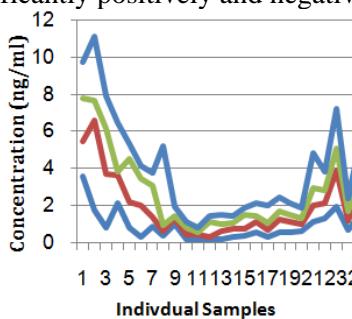


Figure 1. Shows the concentration of VIII Before and after LLLT for individual sample

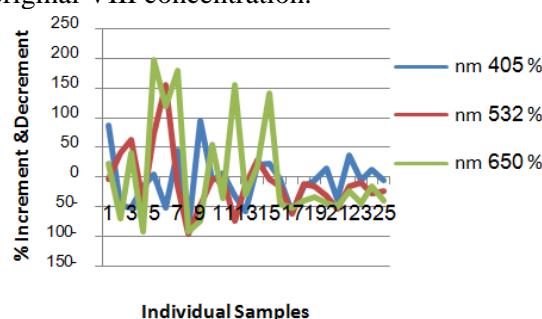


Figure 2. Shows the percent of increment or decrement In VIII concentration for individual samples

Also paired-T test showed that there is no significant correlation using different wavelengths, and it could predict that there is a negative correlation for both 650 and 532 nm, and a positive correlation only for 405 nm that resulted in a total increment in VIII concentration in about 5.81 ± 43.86 , as shown in Figure (3).

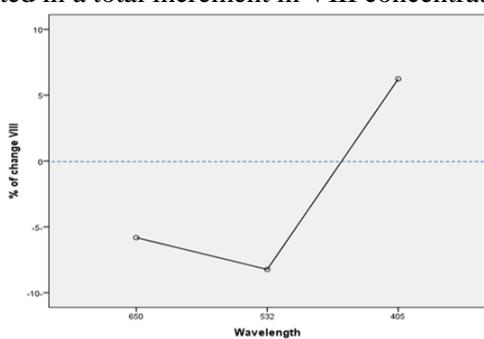


Figure 3. Shows the percentage of increment and decrement in VIII concentration for individual samples

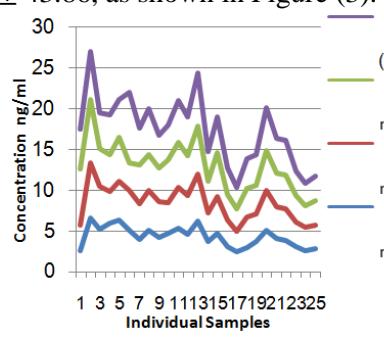


Figure 4. Shows the concentration of vWF before and after LLLT for individual samples

In contrast to VIII, there was a net increment in vWF concentration after LLLT with the three used wavelengths, as shown in Figure (6), but this increment remains only significant with 532 nm (see table (2)).

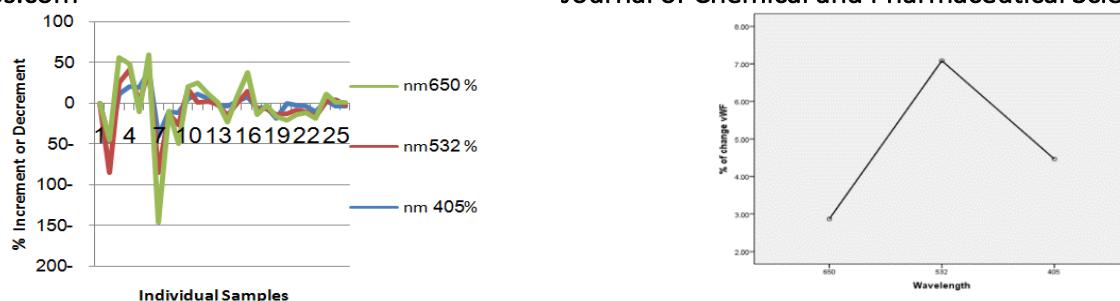


Figure 5. Shows the percent of increment or decrement in vWF concentration for individual

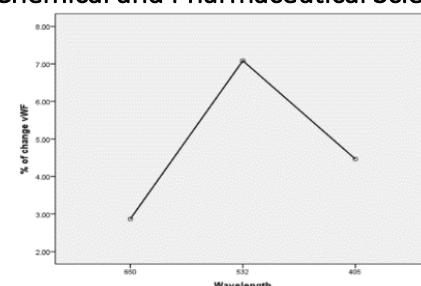


Figure 6. Shows the percentage of increment and decrement in vWF concentration for individual samples

Table 2. Correlations coefficient and the P-value of VIII and vWF level in test samples after LLLT in different wavelengths (650, 532, 405nm).

Before (ng/ml)	After (ng/ml) 650 nm	% After 650 nm	After (ng/ml) 532 nm	% After 532 nm	After (ng/ml) 405 nm	% After 405 nm
VIII						
p(Correlation Coefficient)	0.211	-0.098	-0.192	0.234	-0.036	-0.299
P value	0.311	0.643	0.358	0.26	0.866	0.147
vWF						
p(Correlation Coefficient)	0.557**	0.38	0.322	0.614**	0.423*	0.148
P -value	0.004*	0.061*	0.117	0.001*	0.035*	0.481

p: Spearman's correlation coefficient.

Because there is a non-parametric correlation between VIII concentrations before and after LLLT, it would be easier to express the results using the Spearman's T- test, to be shown in Figure (7), both the correlation coefficient and the p-values are expressed accordingly with each graph. In Figure (8), the Spearman's T- test more reliably expresses the relationship between VIII concentration before and after LLLT expressed as a percent change, and it is obvious that there is a negative relationship in all wavelengths used.

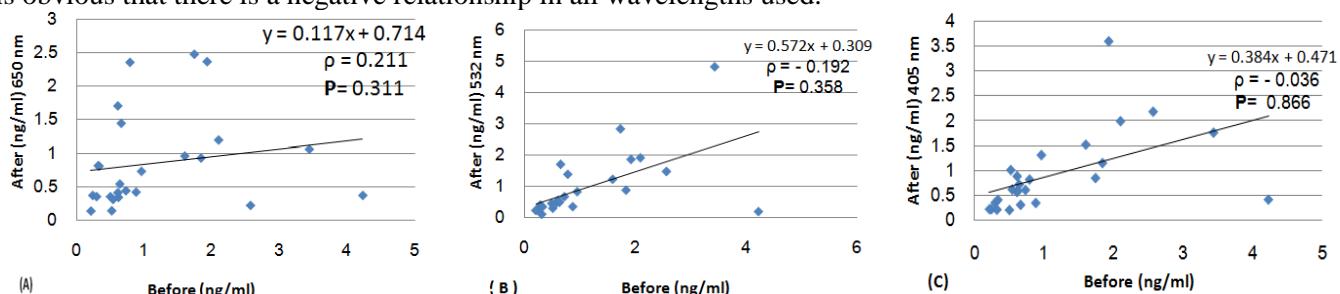


Figure 7.A, B, C: Correlations coefficient and P-value of VIII level after LLLT with three wavelengths (650, 532, and 405) nm

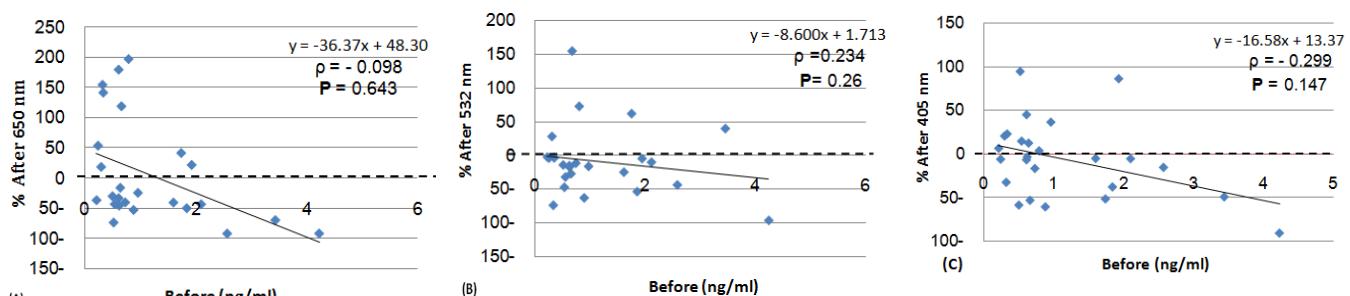


Figure 8.A, B, C: Correlation coefficient and P-value for VIII of the percent change for individual cases after LLLT with different wavelengths (650, 532, 405) nm

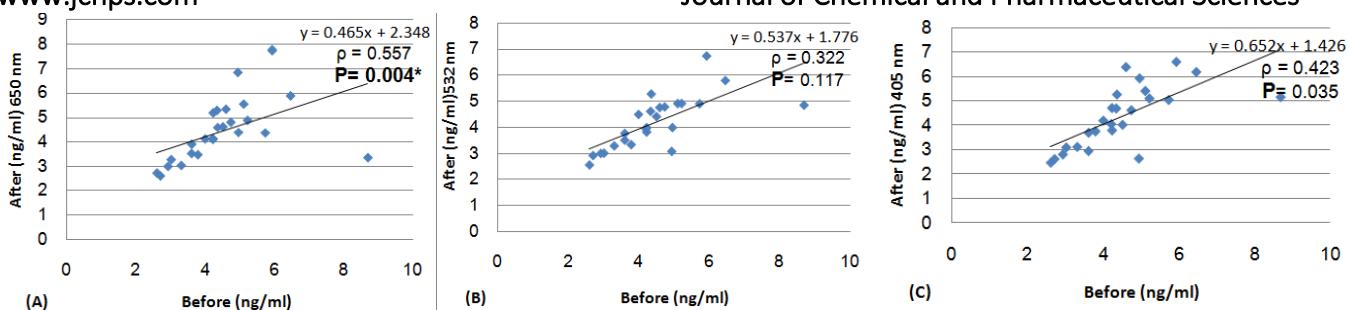


Figure 9.A,B,C: Correlation coefficient and P-value of vWF level after LLLT with three wavelengths (650, 532, 405nm)

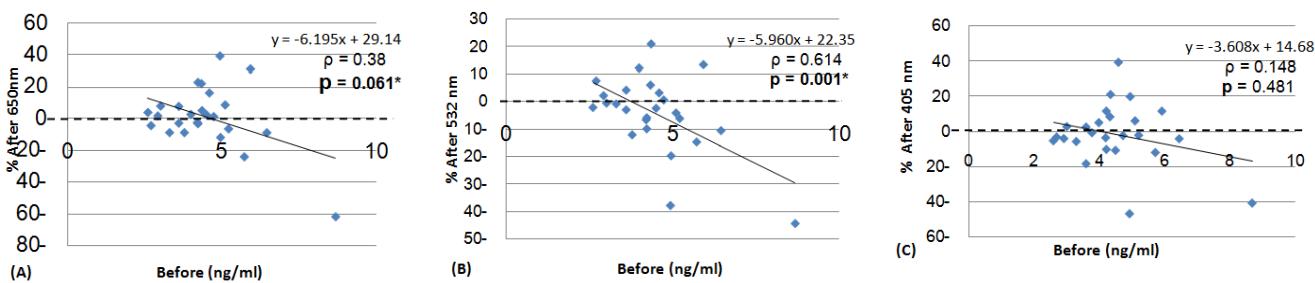


Figure 10.A,B,C: Correlations coefficient and P- value of vWF of the percent change for individual cases after LLLT with different wavelengths (650, 532 and 405) nm

DISCUSSION

Factor VIII is regarded as one of the coagulation factors that play a key role in the activation of the intrinsic pathway, its deficiency results in bleeding tendency as a result of inherited genetic defect of VIII gene located on X-chromosome, a disease known as hemophilia A. Von-Willebrand factor is regarded as an essential cofactor for VIII activation, its deficiency results in von-Willebrand disease characterized by easy bleeding from soft tissues. This study provides evidence that the only effect of LLLT is with using 532 nm that contributes to slight increase in vWF level. It is possible that the source of increased vWF is platelets, because the main site of vWF synthesis is the liver, endothelial cells and is stored in platelets alpha granules, while there is a net decrease in VIII concentration in tested samples after LLLT, and it is partially explained by the possible in vitro activation and exhaustion of VIII in collected blood, but with no evidence of gross blood clot.

Brill (2007) revealed that at high and low rate of shift the red light decreases adhesion and aggregation of blood platelets both respectively. Infrared laser radiation is effective only at high rate of shift leading to increase of adhesion and decrease of aggregation of blood platelets. In small doses only and at low rate of shift Blue laser is effective which leads to decrease of adhesion while at high rate it provokes increase of adhesion (Brill, 2007). Likewise, Burduli (2006) studied the aggregation properties of platelets in patient with exacerbating peptic ulcers in a randomized clinical trial, using intravenous and cutaneous irradiation, and revealed a significant activation of aggregation (Burduli, 2006).

The effects of laser light on platelet activation and reactivity were significant over a wide range of applied energies these results confirmed by Gresner (2004); low and medium laser light energies (18 and 54 J) increased platelet activation, the irradiation with a high-energy laser light (108 J) resulted in depressed platelet reactivity and attenuated platelet response to activators. They also concluded that the formation of platelet microparticles in either resting or ADP-activated platelets have been significantly influenced by laser light irradiation, while in collagen-activated platelets no significant effect was observed. On the other hand, laser light irradiation significantly increased the formation of platelet aggregates both in resting and agonists-activated platelets (Gresner, 2004). Topaz *et al* tested platelet aggregation after LLLT with pulsed ultraviolet laser source using different energy exposure; they concluded that there is a significant decrease in ADP and collagen-induced platelet aggregation with a steady increase in CD43 (glycoprotein IIb/IIIa) expression with increasing energy of exposure, while expression of P-selectins (CD62) remained constant (Topaz, 2000).

Similarly, Brill *et al* have tested the effect of He-Ne laser (632.8 nm) and they have concluded that there is a decrease in platelets aggregation and adhesion on extracellular matrix, the inhibitory effect of LLLT has continued for up to 1 hour. In another work, the level of thromboxane A₂ has been shown to increase after LLLT of platelet-rich plasma (Brill, 1993; Alkaim, 2014; Algubili, 2015; Ahmad, 2015; Al-Robayi, 2014; Alkaim, 2015).

A quick overview to our abovementioned results indicates that there is no significant change in the concentration of VIII after LLLT with the three used wavelengths. Although there is an apparent net increase in the

concentration of VIII expressed by the mean percent change (650 nm: 6.23 ± 86.43 , 532 nm: -8.23 ± 51.29 , 405 nm: 5.81 ± 43.86), there is an obvious non-parametric distribution of individual tests expressed by the Spearman's test, indicating that there is a net decrease in the levels of VIII in individual test samples (see Figure 8), but with no statistical significance for all wavelengths employed in this work.

On the contrary, the level of vWF has shown a net increase in the means of percent change with the three wavelengths of LLLT (650 nm: 1.48 ± 19.19 , 532 nm: 4.24 ± 14.31 , 405 nm: 1.48 ± 17.53), but the Spearman's test shows that there is no statistical significant change in vWF concentration for individual test samples (see Figure 10), except for LLLT with 532 nm (p-value of 0.001).

To our best knowledge, this is the first study to evaluate the effect of LLLT with different wavelengths on the level of factor VIII and vWF. The overall results refer that there is no significant effect on the level of both these factors, and that LLLT is safe to be used in vivo for the treatment of different disease conditions without an effect on coagulation status.

CONCLUSION

Our results were designed to measure two of the coagulation factors of whole blood samples obtained from 25 healthy male and female adult volunteers before and after LLLT. Paired T-test showed no significant change on VIII including the concentration and percent.

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