

## The Role of Anticoagulant Type in Modulating the Effect of Laser Irradiation on Blood Rheology

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Received Date: March 25, 2017; Accepted Date: March 28, 2017; Published Date: March 31, 2017

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### Abstract

#### Background

Evaluating blood optical and rheological considerations plays a role vital to various routine clinical investigations and therapeutic processes, and such evaluation is a superlative model for identifying the interaction mechanisms between biological tissues and low levels of laser irradiation. The purpose of this research was to investigate the in-vitro influence of laser radiation on certain blood indices.

#### Material and Methods

Blood samples were obtained from 40 healthy volunteers, and 30 samples were divided into 4 groups, 1 of which was the control and the other 3 of which were exposed to 3 doses of green laser while using ethylene diamine tetra acetic acid (EDTA) as an anticoagulant. The remaining 10 samples were anticoagulated using heparin and grouped into 2 aliquots, 1 of which was the control and the other of which was exposed to a single dose of green laser radiation. The test achieved a wavelength of 532 nm and a beam-spot diameter of 4 mm. The irradiation times used were 1.8, 3.7, and 6.2 sec, resulting in irradiation doses of 1.5, 3 and 5 J/cm<sup>2</sup> respectively.

#### Results

Green laser irradiation promoted significant alterations in the mean corpuscular volume (MCV) of erythrocytes regardless of the type of anticoagulant. It also increased the white blood cell (WBC) count when EDTA was used as the anticoagulant, but not when heparin was used.

#### Conclusion

The type of anticoagulant used may alter the effect of lasers on certain blood parameters.

**Keywords:** Hematological parameters; Low level laser; Mean cell volume; Heparin

### Introduction

In biomedical studies, laser-tissue interaction is of great interest because it has significant applications in both diagnostics and treatment. Major aspects of laser-tissue interaction that must be considered include the thermal properties of the tissue and the thermal changes caused by the interaction of light and tissue [1]. These interactions are determined by the power density of the wavelength and the exposure time [2]. The effects of green-laser radiation on blood is determined by several factors, including absorption of the green light's quanta by hemoglobin and various cytochromes, peroxides, and catalases; stimulation of the red blood cell (RBC) membrane potential; and motivation of the mitochondria membrane potential. Low-level laser therapy can disturb the physical as well as chemical characteristics of blood cells. It has been confirmed that laser therapy decreases the viscosity of blood and consequently enhances the electrophoretic motion of the RBCs [3]. In addition, laser radiation encourages conformational alterations of RBC membranes, which are

related to alterations in the structural states of both RBC-membrane proteins and the lipid bilayer, causing modifications in the motion of ion pumps [4].

Principally because of their comparatively simple structure and availability, RBCs have been preferred as the ideal model for studying the effect of laser radioactivity on cell membranes. In addition, alterations in RBC osmotic fragility and deformability would certainly be distinguished if laser irradiation induced any variation in the functional properties of RBC membranes [5].

### Materials and Methods

All participants were instructed about the purpose of the study and verbal consent was taken from all candidates. The study was approved by scientific committee of Department of physiology, college of medicine, Almustansyria University.

## Participants

The study used 40 apparently healthy individuals who had no previous history of chronic illness and who were not regularly taking any medications. Participants were informed of the aims of the research, and all gave their verbal consent to participation before being categorized into 2 groups. Group 1 included 30 subjects, 18 males and 12 females, who had a mean age of  $26.96 \pm 5.06$  and underwent complete blood count (CBC) testing that used ethylene diamine tetra acetic acid (EDTA) as the anticoagulant. Group 2 included 10 subjects, 8 males and 2 females who had a mean age of  $24.9 \pm 3.96$  and underwent CBC testing with heparin used as the anticoagulant.

## Blood samples

From each participant, about 10 ml of blood was collected under aseptic conditions by means of venipuncture into tubes containing EDTA in the amount of 1.3 mg/ml of blood as an anticoagulant. Immediately after collection, each sample was divided into 4 equal aliquots, 1 to be used as a non-irradiated control and the other 3 to be exposed to various laser doses using a continuous ND: YAG laser. Another 5 ml of blood was collected into tubes containing heparin in the amount of 0.2 mg/ml of blood. Each of these samples was divided into 2 equal aliquots, 1 to be used as a non-irradiated control and the other to be irradiated with a laser dose of  $3 \text{ J/cm}^2$ , which was the dose that showed the best response in the experiments using EDTA.

## Laser specifications

As an irradiation source, the study used a diode green laser pointer with a wavelength of 532 nm and a low power of 100 mW when using a continuous wave mode. The crystal type of this source was Nd:VVO4:KTP. The diameter of the beam spot was 0.4 cm.

## Sample irradiation

From each tube, 2.5 ml of the sample blood was exposed to the green laser beam. For the exposed sets, the doses were 1.5, 3 and  $5 \text{ J/cm}^2$  and the exposure times were 1.8, 3.7 and 6.2 seconds, respectively. The laser beam was consistently focused on the test tube's midpoint, and the irradiation was performed at room temperature ( $18\text{-}25^\circ\text{C}$ ).

For both irradiated and non-irradiated samples, the study analyzed complete blood counts, including white blood cell (WBC), RBC, mean corpuscular volume (MCV) mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width (RDW), using a hematology analyzer machine (Swelab Alfa Standard; Sweden). All investigations were prepared and conducted at a lab at The National Center of Hematology Specialty Center for Blood Diseases.

## Statistical evaluation

The data were analyzed using Excel software, and the values were expressed as mean  $\pm$  standard deviation. The differences among the irradiated and control samples were estimated by applying a paired t-test. The p value was determined according to the analysis of the significance of the difference. A p value less than 0.05 were considered significant.

## Results

As Table 1 shows, increasing laser doses significantly changed both the WBC count and the MCV. When EDTA was used as anticoagulant.

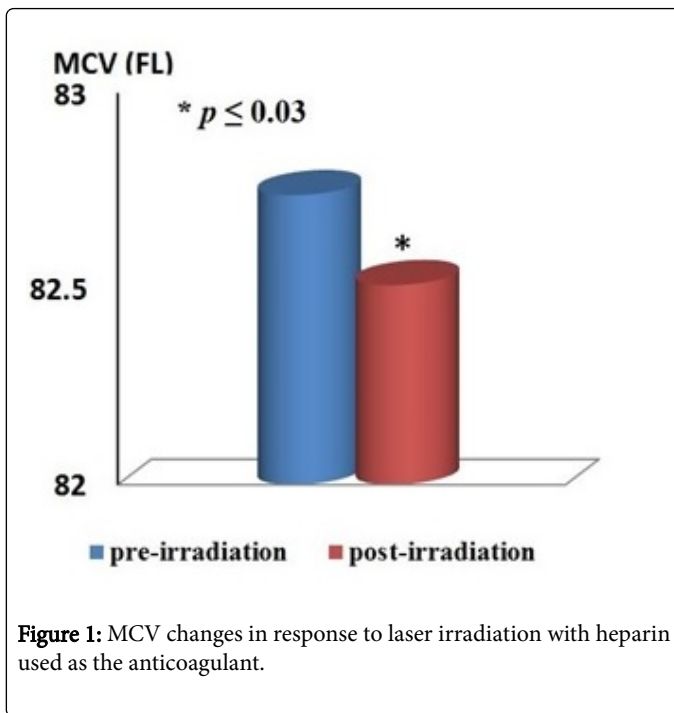
However, when heparin was used as the anticoagulant, the results showed that the in-vitro exposure of blood samples to laser therapy induced significant changes in the MCV ( $p < 0.03$ ) only Figure 1, with no significant changes to other hematological indices Table 2.

Parameter	Pre-irradiation Mean $\pm$ SD	1.5 J/cm <sup>2</sup> Mean $\pm$ SD	p value	3 J/cm <sup>2</sup> Mean $\pm$ SD	p value	5 J/cm <sup>2</sup> Mean $\pm$ SD	p value
WBC	7.8 $\pm$ 2.2	7.8 $\pm$ 2.0	0.3	7.9 $\pm$ 2.1	0.03	7.9 $\pm$ 2.1	0.04
RBC	5.0 $\pm$ 0.4	4.9 $\pm$ 0.4	0.3	4.9 $\pm$ 0.4	0.3	4.9 $\pm$ 0.3	0.2
MCHC	32.9 $\pm$ 1.7	32.9 $\pm$ 1.4	0.3	33.0 $\pm$ 1.5	0.1	33.0 $\pm$ 1.3	0.1
RDW	11.7 $\pm$ 0.6	11.7 $\pm$ 0.7	0.4	11.7 $\pm$ 0.7	0.4	11.7 $\pm$ 0.7	0.4
MCV	86.5 $\pm$ 4.9	86.3 $\pm$ 4.8	0.02	86.2 $\pm$ 4.8	0.0003	86.1 $\pm$ 4.9	0.001

**Table 1:** The effect of various laser doses on certain hematological parameter when EDTA was used as the anticoagulant.

Parameter	Mean pre-irradiation	Mean post-irradiation	p value
WBC	7.8 $\pm$ 1.5	8.0 $\pm$ 1.2	0.2
RBC	4.9 $\pm$ 0.3	5.2 $\pm$ 0.4	0.3
MCHC	34.1 $\pm$ 0.7	34.0 $\pm$ 0.8	0.3
RDW	11.6 $\pm$ 0.3	11.6 $\pm$ 0.4	0.5

**Table 2:** Comparison of pre and post irradiated blood samples with heparin used as the anticoagulant.



## Discussion

Anticoagulation is achieved either by binding calcium ions, as EDTA and citrate do or by inhibiting thrombin, as heparin does. Heparin is the preferred anticoagulant for most clinical chemistry analyses, for blood gas analysis and for measuring some trace elements, ammonia and blood pH. EDTA is particularly useful for hematological examination. However, these commonly used anticoagulants have been reported to have varying effects on blood components. Therefore, blood handling and the choice of an anticoagulant may affect the quality of data and potentially result in analytical bias [6]. To evaluate the effects of laser irradiation per se and exclude the possible effect of EDTA on RBC morphology, which may induce anisocytosis [7] and WBC count and morphology [8], we reinvestigated the role of laser irradiation on various hematological parameters when heparin was used as an anticoagulant.

Normal WBC values range from (4000-11000 cell/ $\mu$ l) [9] and can be increased both physiologically and pathologically. In the present study, when EDTA was used as an anticoagulant, the mean value of total WBC showed a substantial increment after irradiation, which is in agreement with the results of Siposan and Lukacs [10], who noted that WBC counts changed significantly, by 5.26%. Conversely and interestingly, Rathore and Ali [11] noted a significant decrease in WBC counts due to laser exposure, which may have been caused by cellular perturbations and membranous alterations. The differences between the results of their study and those of the present study may be due to differences in the length of exposure to the laser irradiation, which was 30 minutes in their study. That study did not mention the total energy dose and spot diameter used.

Unlike with EDTA, with heparin, the WBC count did not show significant alterations after irradiation. The reason for this is unclear; nevertheless, using EDTA has been reported as sometimes associated with platelet clumping [12]. Because electronic counting instruments are based on a specific size range, clumps that exceed the size-exclusion

limit for platelets may instead be counted as white cells. Such a phenomenon may reach a significant level when enhanced by laser irradiation, which induces platelet aggregation. However, the present study found that when EDTA was used as an anticoagulant, lasers could potentially stimulate mitochondrial alterations, leading to the splitting of mononuclear cells [13], but this result could not be verified when heparin was used, possibly because of the smaller sample size (only 10) for heparin. To clarify this point, further studies are needed, particularly those using flow cytometry to confirm exactly which types of cells increase.

In addition, the present study found that the MCV showed significant alterations after irradiation, even when heparin was used as the anticoagulant. Because heparin has minimal chelating properties, minimal effects on water shifts, and relatively low cation concentration compared with EDTA [14], we concluded that laser radiation encouraged conformational transitions of the erythrocyte membrane that were related to changes in the structural states of both its proteins and the lipid bilayer. This resulted in modulating the membrane's functional properties, including changes in the activity of its ion pumps and, thus, changes in its ion flows [15].

To our knowledge, there have been no prior studies of the effects of anticoagulant type on modulating the effects of laser irradiation on blood rheology. The present study provided data on such effects, and these data warrant further evaluation and reproduction to clarify them.

## Conclusion

Within the limits of this study, low level laser therapy at a wavelength of 532 nm and 3 J/cm<sup>2</sup> showed significant rheological changes to blood in vitro, which could be considered for clinical application, especially for managing ischemic diseases. Green laser irradiation at a wavelength of 532 nm and up to 5 J/cm<sup>2</sup> demonstrated protective effects on RBC count, RDW, and MCHC. The type of anticoagulant used may alter the effect of a laser on certain blood parameters.

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