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Photodynamic therapy and Green Laser blood Therapy

By Zahra Al Timimi, M.S. Jaafar, Mohd Zubir Mat Jafri

Universiti Sains Malaysia, Penang, Malaysia

Abstract – Background: In vitro irradiation of human blood with laser light is under investigation for years, to study the biostimulatory effects on various blood cells. However, any positive effects of this light on the rheology of platelets have not been documented with authenticity due to lack of research. Methods: In our present study, we investigated the influence of different levels of laser on the damage threshold of blood cells. Laser diodes were used as a source of radiation in different levels of irradiation protocol. Blood was taken from one hundred adult patients. After adding anticoagulant (EDTA), the samples were divided into four groups for irradiating with different laser intensities. And each sample was subdivided into two, so that one was irradiated and the other considered as control sample. The samples were made to stand for 30 minutes before determining the change in rheological properties of blood cells. Results: It was established that low level laser therapy when used on human blood in vitro, affects the rheology of erythrocytes and leucocytes. It was observed that it changes the erytherocytatory, leucocytatory, BSR, aggregability indices of blood. Conclusions Thus it was concluded that low level laser therapy can affect the physical as well as chemical properties of blood cells which is not only helpful in preservation of blood but also in revitalizing the physically and chemically stressed erytherocytatory membranes. It was determined that the laser therapy decreases the viscosity of blood thus increasing the electrophoretic mobility of erythrocytes.

Keywords : *Erytherocytatory, Aggregability, leucocytatory, biostimulatory , Laser blood Therapy.*

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PHOTODYNAMIC THERAPY AND GREEN LASER BLOOD THERAPY

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Photodynamic therapy and Green Laser blood Therapy

Zahra Al Timimi^α, M.S. Jaafar^α, Mohd Zubir Mat Jafri^β

Abstract - Background: In vitro irradiation of human blood with laser light is under investigation for years, to study the biostimulatory effects on various blood cells. However, any positive effects of this light on the rheology of platelets have not been documented with authenticity due to lack of research. Methods: In our present study, we investigated the influence of different levels of laser on the damage threshold of blood cells. Laser diodes were used as a source of radiation in different levels of irradiation protocol. Blood was taken from one hundred adult patients. After adding anticoagulant (EDTA), the samples were divided into four groups for irradiating with different laser intensities. And each sample was subdivided into two, so that one was irradiated and the other considered as control sample. The samples were made to stand for 30 minutes before determining the change in rheological properties of blood cells. Results: It was established that low level laser therapy when used on human blood in vitro, affects the rheology of erythrocytes and leucocytes. It was observed that it changes the erythrocytatory, leucocytatory, BSR, aggregability indices of blood. Conclusions Thus it was concluded that low level laser therapy can affect the physical as well as chemical properties of blood cells which is not only helpful in preservation of blood but also in revitalizing the physically and chemically stressed erythrocytatory membranes. It was determined that the laser therapy decreases the viscosity of blood thus increasing the electrophoretic mobility of erythrocytes.

Keywords : Erythrocytatory, Aggregability, leucocytatory, biostimulatory, Laser blood Therapy.

1. INTRODUCTION

The objective of my study is to determine the effects and advantages of green laser pointer 532nm on the rheological properties of human blood in vitro. Researching the bio stimulatory effects of Low level laser therapy on rheological properties of blood cells is an area of great interest for hematologists. Four important effects of low level laser light have already been reported in the scientific literatures which are tissue regeneration, reduction of inflammation, pain relief and immune system enhancement.

The term Photodynamic therapy denotes the in vitro therapy of blood cells which is done to change the rheological properties of blood cells, when preserved for transfusion purposes. The underlying mechanism is that when blood is irradiated with low level laser in an oxygen rich environment, porphyrins absorb energy from

photons and transfer this energy to the surrounding oxygen molecules.

Porphyrins are a component of hemoglobin which carries oxygen to various tissues of the body. When porphyrins are not a component of hemoglobin anymore, as in preserved blood, they absorb light.

Photodynamic therapy involves the use of photoactive drug (photosensitizer) and light which is typically visible or infrared light. When light is absorbed by porphyrin molecules, a chemical reaction is initiated which leads to direct and indirect production of cytotoxic radicals and singlet oxygen (Maiya 2000; Brancalion and Moseley 2002). These toxic chemicals once formed, damage the proteins, lipids, nucleic acids and many other particles of blood without causing any damage to the surrounding irradiated blood components which are PS-free. For example viruses can be killed in whole blood without destroying blood components. (Henderson and Dougherty 1992; Sitnik, Hampton et al. 1998; Maiya 2000; Castano, Mroz et al. 2006; Morton, McKenna et al. 2008; Wilson and Patterson 2008).

Weber in 2005 used a green laser light for the first time for intravascular blood treatment. The basic idea was to increase the energy assimilation of blood by the absorption of green laser light as a complementary color to red light (and red color of erythrocytes). With intravascular positioning of the red light catheter, it was observed that a red spot shines spontaneously through the skin, when the red light was switched on, due to the light reflecting property of hemoglobin. Whereas, no green spot appeared on the skin by switching on a green laser light with a wavelength of 532 nm, as the laser light of this wavelength is almost completely absorbed by hemoglobin. This laser irradiation therapy was introduced for the first time by Weber for the treatment of many diseases. A comparative study between red and green laser light was also conducted, by treating those patients with green laser irradiation who had already been treated with red laser previously.

After this development in the field of low level laser therapy, 20 liver patients and 20 lip metabolism patients were treated with mere green laser light successfully, demonstrating more acceptable results than red light therapy. At that time the effects of green laser on the rheological properties of blood were discovered which were more beneficial than red light. (Weber, Fu ganger -May 2007)

^α Author : School of Physics, Universiti Sains Malaysia, 11800 Penang, Malaysia - MEDICAL LASER RESEARCH GROUP.
Telephone: +6164306900, E-mail : Zahraja2007@yahoo.com

Following effects of green laser irradiation on blood cells have been observed;

- Absorption of the green light quanta by haemoglobin,
- Absorption of the green light by different Cytochromes, Katalases und Peroxidases,
- Stimulation of electric activity of the erythrocyte membrane potential
- Activation of the membrane potential of the mitochondria

There are many different views about the intensity of laser light that is used to treat blood in vitro. The effluence rate of laser which is used to activate the toxic radicals in the blood should certainly be lower than the damage threshold of surrounding vital tissue components. Whereas according to Fischer and Aulmann (1998) most of the time it is desirable to use the highest possible effluence rates in order to achieve maximum effects of photodynamic therapy.

II. MATERIAL AND METHODS

a) *Materials*

During this research diode laser pointer 532 was used as the irradiation source with a wavelength of 532nm and a low power of 100mw. Unlike ordinary light, laser is a high energy device and emits photons on only one direction.

The apparatus used to measure values of the irradiated and non-irradiated blood samples was automate hematology analyzer machine (Sysmex XE - 2100). It is a machine that is used for measuring various chemicals and other properties in many biological samples. It is a quick method and requires almost no individual assistance. This method has many advantages. For example the blood samples can be read in batches or otherwise solely if needed. Thus it assists in research sample readings where a large number of samples are to be read. In blood analysis, the automate hematology analyzer machine is used to measure complete blood count, erythrocyte sedimentation rate and or coagulation profile.

For measurement, dilute samples of blood were passed through an aperture. Electric current was also passing through it. The flow of current brought a variation in the impedance between the ends. Then a lytic reagent for breaking red blood cells was added in the solution. It did not affect the white blood cells and platelets leaving them intact.

b) *Blood collection*

This research was conducted on one hundred blood samples which were collected under the guidelines of National Medical Research from pathology lab in PULAUPINANG GERERAL HOSPITAL .This study was approved by the national institute of health for conducting research in the Ministry of Health Malaysia and also by the Committee of Medical Research and

Ethics. Hundred pathological samples, 5ml each, were obtained from healthy and non healthy adults (all above 18 years age) with different medical histories. The samples were divided into four groups to determine the effect of different levels of laser therapy. After collection of the blood samples, an anti-coagulant potassium ethylenediaminetetraacetic acid (K2/EDTA) (Vacationer, BD Franklin Lakes NJ USA), was added to prevent coagulation. It is a poly amino carboxylic acid which has both in vivo and in vitro applications. It is the most widely used anticoagulant for complete blood count. Each blood sample was further divided into two halves (2.5ml each) and one of them was irradiated whereas the other was kept as control. This control was done to check for blood damage due to the irradiation system (Vacationers, etc.)

c) *Laser Irradiation*

All the four major groups were irradiated with Green diode laser with a wavelength of 532 nm at 100mw in a continuous wave mode, with divergence < 1.5mRad, Beam Mode (TEM₀₀), Beam diameter at aperture ~1.5, Crystal type Nd:VYO4:KTP, Power Source 1 x 3V CR2 Alkaline batteries. The power density was 509.55mW /cm² at a Distance of 6.5 cm from the laser device from blood inside the tube, and diameter of the laser spot was set 0.5 cm. Samples were irradiated in different time periods at energy effluence of 0.5j/ cm², 1.5j/ cm², 3j/ cm² and 5j/cm² for the first, second, third and fourth groups at 1, 3, 6, and 10 sec. respectively.

d) *Method*

A diode laser pointer which was used during research was a laser pointer. All the irradiated and non-irradiated samples of blood were allowed to stand for about 30 minutes at room temperature, before counting was done. Blood counts were then performed both before and after the irradiation.

The method used to measure values of the irradiated and non-irradiated blood samples was automating hematology analyzer machine. It is a machine that is used for measuring various chemicals and other properties in many biological samples. It is a quick method and requires almost no individual assistance. This method has many advantages. For example the blood samples can be read in batches or otherwise solely if needed. Thus it assists in research sample readings where a large number of samples are to be read.

In blood analysis, the automate hematology analyzer machine is used to measure complete blood count, erythrocyte sedimentation rate and or coagulation profile.

e) *Complete blood cell measurements*

For measurement, dilute samples of blood were passed through an aperture. Electric current was also passing through it. The flow of current brought a variation in the impedance between the ends. Then a

lytic reagent for breaking red blood cells was added in the solution. It did not affect the white blood cells and platelets leaving them intact. Then these solutions were passed through another detector this getting the measurements of red blood cells, white blood cells and platelets.

The counter was designed for measuring white blood cells (WBC), red blood cells (RBC), and hemoglobin content (HGB), hematocrit (HCT); mean (red) cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and platelets (PLT), Neutrophil (NEUT), Lymphocytes (LYMPH) and Monocytes (MONO).

- WBC white blood was analyzed by the flow cytometry method using semiconductor laser.
- Red Blood Cell count was analyzed by the RBC detector by Hydro Dynamic Focusing method (DC Detection)
- Hemoglobin (HGB) by the HGB detector based on the SLS hemoglobin detection method
- Hematocrit (HCT) by the RBC cumulative pulse height detection method,
- MCHC was calculated with RBC, HGB and PLT by the Hydro Dynamic Focusing method (DC Detection) or flow cytometry method using semiconductor laser.
- Blood was kept on a shaking device at room temperature 25 c during a sequence of measurements.

III. STATISTICAL ANALYSIS

Statistical analysis was accomplished by using a paired test to analyze the mean and standard deviations of different experimental groups. The null hypothesis was for no statistical difference between the means of the different groups. ($H_0: M_1=M_2=M_3$ where M_1, M_2, M_3 are the mean of the experimental groups). A significant difference was accepted between the means when the P value was less than 5 % ($P < 0.05$)

IV. RESULTS AND FORMATS

The results of our research showed the effect of low level laser light on the rheology of different blood cells as well as a change in the number of cells as below:

a) In Irradiated groups

i. Red blood cells

3, 5 j/cm² irradiation group showed a significant increase in the red blood cells of male patients ($p < 0.05$).

0.5j/cm² irradiation group showed $p = 0.00$

ii. Hemoglobin

3, 5j/cm² irradiation group showed an increase in hemoglobin.

0.5j/cm² irradiation group showed $p = 0.00$

iii. Hematocrit

Only 0.5j/cm² irradiation group showed a significant increase in hematocrit ($p < 0.05$)

Thus it is evident from the above results at very low effluence of 0.5j /cm², only a change in hematocrit is possible while 3 and 5j /cm² increase the red blood cells and hemoglobin.

The test results showed the following changes in irradiated groups:

The increase in white blood cells and red blood cells seen with 3 and 5 j /cm² was two times the increase seen with 0.5 and 1.5j/cm² groups. Similarly HGB increased with 0.5, 3 and 5j /cm² but decreased two times in 1.5j /cm² group and the same change was seen in non-irradiation group.

HCT increased to double in 0.5 and 5j /cm² group where decreased in 3j /cm² group and even more in 1.5j /cm² group.

Neutrophils increased to double in 0.5, 3 and 5j /cm² group but decreased in 1.5j /cm² group.

Lymphocytes increased in 0.5, 3 and 5j /cm² group but double increased in 1.5j /cm² group.

MCV and MCHC increased to double in all the groups.

Platelets double decreased in all groups except in 5j /cm² in which no change was observed.

These results show a positive effect of 5j /cm² effluence power on almost all the cells under investigation, whereas the other three intensities show a variation their effects on different indices.

Irradiation groups with different laser effluence showed the following results with gender differences:

b) In males

In 0.5 j/cm² group HGB, RBC's and HCT increased significantly

In 1.5 j/cm² group HGB, RBC's and HCT increased significantly

In 3j/cm² group HGB and RBC's increased significantly

In 5j/cm² group HGB and RBC's increased significantly.

c) In females

In 0.5 j/cm² group RBC's and HGB increased significantly whereas HCT decreased non-significantly.

In 1.5, 3, 5 j/cm² groups RBC's, HGB and HCT decreased non-significantly.

When the above information was extracted from the results on gender basis, it became evident that some gender difference is also an important factor in determining the efficacy of low level laser therapy on blood.

V. DISCUSSION

The main objective of this study is to explore the bio stimulatory effect of low level laser on human blood

samples. We conducted this research to determine the effect of low-level laser therapy (LLLT) on some rheological properties of human blood in vitro by using laser pointer 532 nm, low power 100mw. It also aims to evaluate the effect of this therapy on reducing inflammation by demonstrating the transformations of blood cells, the effect of LLLT dose response of blood cell and the changes in blood cell counts.

Laser therapy is applied on body tissues which may be cells or culture to bring a change in tissue functions and properties. More than 130 double blinded studies have confirmed the therapeutic benefit of low level laser therapy. Laser therapy is a matter of dose and treatment technology as it is with any other therapy. The power output of laser is important especially for dose calculation. The depth of penetration is dependent on wavelength of light.

The objective of our research was to determine the effects of green laser light on the rheology of different blood cells, in vitro. We evaluated the counts of red and white blood cells, HGB, HCT, MCV, MCHC, PLT and neutrophils.

We demonstrated many beneficial effects of green laser light irradiation on erythrocytatory, leucocytatory, aggregability indices. The bio stimulatory effect of Low level laser therapy on red cells was seen with changes in cell membranes, thus increasing the red cell functionality. The physically and chemically stressed erythrocyte membranes can be revitalized and brought back to functionality for performing its oxophoric function in transfusion reactions.

From the results of our research, we can say that low level laser therapy affects various rheological properties of different blood cells for example red cell deformability, aggregation of cells, critical stress on the cells during preservation time, leucocytatory, erythrocytatory indices, ESR etc.

VI. CONCLUSION

The best effluence power that has a positive effect on almost all blood cells and indices is $5j/cm^2$. It increased white blood cells, red blood cells, hemoglobin with a non-significant decrease in hematocrit. Thus from our research it is proved that low level laser therapy with diode laser 532nm and a high power of 100 mw is advantageous for revitalizing the functional capability of preserved blood and also increases the number of blood cells, thus increasing the function of this blood when injected to recipient.

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