

1 Photodynamic therapy and Green Laser blood Therapy

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5

6 Abstract

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

25

26 **Index terms**— Erytherocytatory, Aggregability, leucocytatory, biostimulatory, Laser blood Therapy.

27 1 INTRODUCTION

28 he objective of my study is to determine the effects and advantages of green laser pointer 532nm on the rheological
29 properties of human blood in vitro. transfusion purposes. The underlying mechanism is that when blood
30 is irradiated with low level laser in an oxygen rich environment, porphyrins absorb energy from photons and
31 transfer this energy to the surrounding oxygen molecules.

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

34 Photodynamic therapy involves the use of photoactive drug (photosensitizer) and light which is typically visible
35 or infrared light. When light is absorbed by porphyrin molecules, a chemical reaction is initiated which leads
36 to direct and indirect production of cytotoxic radicals and singlet oxygen (Maiya 2000;Brancaleon and Moseley
37 2002) . These toxic chemicals once formed, damage the proteins, lipids, nucleic acids and many other particles
38 of blood without causing any damage to the surrounding irradiated blood components which are PS-free. For
39 example viruses can be killed in whole blood without destroying blood components. ?? Weber in 2005 used a
40 green laser light for the first time for intravascular blood treatment. The basic idea was to increase the energy
41 assimilation of blood by the absorption of green laser light as a complementary color to red light (and red
42 color of erythrocytes). With intravascular positioning of the red light catheter, it was observed that a red spot

5 B) BLOOD COLLECTION

43 shines spontaneously through the skin, when the red light was switched on, due to the light reflecting property of
44 hemoglobin. Whereas, no green spot appeared on the skin by switching on a green laser light with a wavelength of
45 532 nm, as the laser light of this wavelength is almost completely absorbed by hemoglobin. This laser irradiation
46 therapy was introduced for the first time by Weber for the treatment of many diseases. A comparative study
47 between red and green laser light was also conducted, by treating those patients with green laser irradiation who
48 had already been treated with red laser previously.

49 After this development in the field of low level laser therapy, 20 liver patients and 20 lip metabolism patients
50 were treated with mere green laser light successfully, demonstrating more acceptable results than red light therapy.
51 At that time the effects of green Researching the bio stimulatory effects of Low level laser therapy on rheological
52 properties of blood cells is an area of great interest for hematologists. Four important effects of low level laser light
53 have already been reported in the scientific literatures which are tissue regeneration, reduction of inflammation,
54 pain relief and immune system enhancement.

55 The term Photodynamic therapy denotes the in vitro therapy of blood cells which is done to change the
56 rheological properties of blood cells, when preserved for Following effects of green laser irradiation on blood cells
57 have been observed;

58 ? Absorption of the green light quants by haemoglobin, ? Absorption of the green light by different
59 Cytochromes, Katalases und Peroxidases, ? Stimulation of electric activity of the erythrocyte membrane potential
60 ? Activation of the membrane potential of the mitochondria There are many different views about the intensity
61 of laser light that is used to treat blood in vitro. The effluence rate of laser which is used to activate the toxic
62 radicals in the blood should certainly be lower than the damage threshold of surrounding vital tissue components.
63 Whereas according to Fischer and Aulmann (1998) most of the time it is desirable to use the highest possible
64 effluence rates in order to achieve maximum effects of photodynamic therapy.

65 2 II.

66 3 MATERIAL AND METHODS

67 4 a) Materials

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

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

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

81 5 b) Blood collection

82 This research was conducted on one hundred blood samples which were collected under the guidelines of National
83 Medical Research from pathology lab in PULAUPINANG GERERAL HOSPITAL .This study was approved
84 by the national institute of health for conducting research in the Ministry of Health Malaysia and also by the
85 Committee of Medical Research and Ethics. Hundred pathological samples, 5ml each, were obtained from healthy
86 and non healthy adults (all above 18 years age) with different medical histories. The samples were divided into
87 four groups to determine the effect of different levels of laser therapy. After collection of the blood samples,
88 an anti-coagulant potassium ethylenediaminetetraacetic acid (K2/EDTA) (Vacationer, BD Franklin Lakes NJ
89 USA), was added to prevent coagulation. It is a poly amino carboxylic acid which has both in vivo and in vitro
90 applications. It is the most widely used anticoagulant for complete blood count. Each blood sample was further
91 divided into two halves (2.5ml each) and one of them was irradiated whereas the other was kept as control. This
92 control was done to check for blood damage due to the irradiation system (Vacationers, etc.) c) Laser Irradiation
93 All the four major groups were irradiated with Green diode laser with a wavelength of 532 nm at 100mw in a
94 continuous wave mode, with divergence < 1.5mRad, Beam Mode (TEMoo), Beam diameter at aperture ~1.5,
95 Crystal type Nd:VYO4:KTP, Power Source 1 x 3V CR2 Alkaline batteries. The power density was 509.55mW
96 /cm² at a Distance of 6.5 cm from the laser device from blood inside the tube, and diameter of the laser spot
97 was set 0.5 cm. Samples were irradiated in different time periods at energy effluence of 0.5j/ cm², 1.5j/ cm², 3j/
98 cm² and 5j/cm² for the first, second, third and fourth groups at 1, 3, 6, and 10 sec. respectively.

99 6 d) Method

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

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

108 In blood analysis, the automate hematatology analyzer machine is used to measure complete blood count,
109 erythrocyte sedimentation rate and or coagulation profile. lytic reagent for breaking red blood cells was added in
110 the solution. It did not affect the white blood cells and platelets leaving them intact. Then these solutions were
111 passed through another detector this getting the measurements of red blood cells, white blood cells and platelets.

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

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

122 III.

123 7 STATISTICAL ANALYSIS

124 Statistical analysis was accomplished by using a paired test to analyze the mean and standard deviations of
125 different experimental groups. The null hypothesis was for no statistical difference between the means of the
126 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
127 difference was accepted between the means when the P value was less than 5 % ($P < 0.05$) IV.

128 8 RESULTS AND FORMATS

129 The results of our research showed the effect of low level laser light on the rheology of different blood cells as
130 well as a change in the number of cells as below: a) In Irradiated groups i. Red blood cells 3, 5 j/cm^2 irradiation
131 group showed a significant increase in the red blood cells of male patients ($p < 0.05$). 0.5 j/cm^2 irradiation group
132 showed $p = 0.00$

133 ii. Hemoglobin 3, 5 j/cm^2 irradiation group showed an increase in hemoglobin.
134 0.5 j/cm^2 irradiation group showed $p = 0.00$

135 iii. Hematocrit Only 0.5 j/cm^2 irradiation group showed a significant increase in hematocrit ($p < 0.05$)

136 Thus it is evident from the above results at very low effluence of 0.5 j/cm^2 , only a change in hematocrit is
137 possible while 3 and 5 j/cm^2 increase the red blood cells and hemoglobin.

138 The test results showed the following changes in irradiated groups:

139 The increase in white blood cells and red blood cells seen with 3 and 5 j/cm^2 was two times the increase seen
140 with 0.5 and 1.5 j/cm^2 groups. Similarly HGB increased with 0.5, 3 and 5 j/cm^2 but decreased two times in 1.5 j/cm^2
141 group and the same change was seen in non-irradiation group.

142 HCT increased to double in 0.5 and 5 j/cm^2 group where decreased in 3 j/cm^2 group and even more in 1.5 j/cm^2
143 group.

144 Neutrophils increased to double in 0.5, 3 and 5 j/cm^2 group but decreased in 1.5 j/cm^2 group.

145 Lymphocytes increased in 0.5, 3 and 5 j/cm^2 group but double increased in 1.5 j/cm^2 group.

146 MCV and MCHC increased to double in all the groups.

147 Platelets double decreased in all groups except in 5 j/cm^2 in which no change was observed.

148 These results show a positive effect of 5 j/cm^2 effluence power on almost all the cells under investigation,
149 whereas the other three intensities show a variation their effects on different indices.

150 Irradiation groups with different laser effluence showed the following results with gender differences: b) In
151 males In 0.5 j/cm^2 group HGB, RBC's and HCT increased significantly In 1.5 j/cm^2 group HGB, RBC's and
152 HCT increased significantly In 3 j/cm^2 group HGB and RBC's increased significantly In 5 j/cm^2 group HGB and
153 RBC's increased significantly.

154 9 c) In females

155 In 0.5 j/cm^2 group RBC's and HGB increased significantly whereas HCT decreased non-significantly.

156 In 1.5, 3, 5 j/cm^2 groups RBC's, HGB and HCT decreased non-significantly.

15 CONCLUSION

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

162 V.

163 13 DISCUSSION

164 The main objective of this study is to explore the bio stimulatory effect of low level laser on human blood samples.
165 We conducted this research to determine the effect of low-level laser therapy (LLLT) on some rheological properties
166 of human blood in vitro by using laser pointer 532 nm, low power 100mw. It also aims to evaluate the effect of
167 this therapy on reducing inflammation by demonstrating the transformations of blood cells, the effect of LLLT
168 dose response of blood cell and the changes in blood cell counts.

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

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

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

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

185 14 VI.

186 15 CONCLUSION

187 The best effluence power that has a positive effect on almost all blood cells and indices is 5j /cm². It increased
188 white blood cells, red blood cells, hemoglobin with a non-significant decrease in hematocrit. Thus from our
189 research it is proved that low level laser therapy with diode laser 532nm and a high power of 100 mw is
190 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. 1 2 3 4 5 6



Figure 1:

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²Volume XI Issue V Version I © 2011 Global Journals Inc. (US) 2011 December e) Complete blood cell measurementsFor 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

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