Jurnal Sains Kesihatan Malaysia **9** (2) 2011: 9-13

Kertas Asli/*Original Article*

Influence of Low Power He-Ne Laser Irradiation on Hemoglobin Concentration, Mean Cellular Volume of Red Blood Cell, and Mean Cellular Hemoglobin

(Pengaruh Sinaran Kuasa Rendah He-Ne Terhadap Konsentrasi Hemoglobin, Min Isi Padu Sel Darah Merah dan Min Hemoglobin Sel)

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ABSTRACT

The effect of low power 0.95 mW He-Ne laser irradiation (λ = 632.8 nm) on the subpopulations of human blood parameters such as hemoglobin concentration (HGB), mean cellular volume of red blood cell (MCV), and mean cellular hemoglobin (MCH) were investigated by electronic sizing at the Wellness Centre of Universiti Sains Malaysia (USM). These parameters were correlated with human characteristics such as age, gender, ethnic, and blood types. The correlations were obtained by finding patterns in changes of blood parameters after radiation, non-parametric tests using SPSS version 11.5, centroid and peak positions, and flux variations. The analysis revealed significant changes according to human characteristics, for age ($p = 0.067$), gender ($p = 0.044$), ethnic ($p = 0.094$), and blood types ($p = 0.099$). This finding shows that the centroid and peak positions, flux peak and total flux, were highly correlated with human characteristics and can become a significant indicator for blood analysis. Furthermore, the encircled flux analysis demonstrated a good future prospect in blood research, thus leading the way as a vibrant diagnosis tool to clarify diseases associated with blood.

Keywords: He-Ne laser irradiation, erythrocytes, hemoglobin, mean cellular volume, mean cellular hemoglobin.

ABSTRAK

Kesan penyinaran laser He-Ne kuasa rendah ke atas subpopulasi parameter darah manusia seperti kepekatan hemoglobin (HGB), isipadu sel min (MCV) (bagi sel darah merah (RBC), dan hemoglobin sel min (MCH) diselidiki dengan menggunakan pensaizan elektronik di Pusat Sejahtera, Universiti Sains Malaysia (USM). Parameter tersebut dikorelasikan dengan ciri-ciri manusia seperti umur, jantina, etnik, dan jenis darah. Korelasi diperolehi dengan mencari corak dalam perubahan parameter darah selepas radiasi, ujian tak parameter menggunakan SPSS versi 11.5, kedudukan sentroid dan puncak, serta ubahan fluks. Analisis menunjukkan perubahan signifikan mengikut ciri manusia, bagi umur ($p = 0.067$), jantina ($p = 0.044$), etnik ($p = 0.094$), dan jenis darah ($p = 0.099$). Dapatan ini menunjukkan bahawa kedudukan sentroid dan pucak, puncak fluks dan jumlah fluks berkorelasi tinggi dengan ciri manusia dan boleh menjadi penunjuk penting bagi analisis darah. Tambahan, analisis fluks terkeliling menunjukkan prospek yang baik dalam penyelidikan darah. Ini membawa kepada kaedah yang jitu bagi menjelaskan penyakit berkaitan dengan darah.

Kata kunci: penyinaran laser He-Ne, erythrocytes, hemoglobin, isi padu sel min, hemoglobin sel min.

INTRODUCTION

There are widespread applications of low intensity laser radiation in various areas of the medical field (Lundeberg & Malen 1991; Kipshidze et al. 1994), however, the mechanisms of its effect on human blood components still have not been sufficiently studied and remain a topic for discussion. The most important interest is in photoreactions initiated by intravenous laser irradiation of blood, which is acknowledged to be the most effective laser biostimulation method. He-Ne laser radiation has been found to have a lot of important applications which led to the expanding biomedical use of laser technology, particularly in surgery (Mi et al. 2006). This experimental medicine practice requires detailed information on the mechanisms of their biological effects (Wasik et al. 2007; Halevy et al. 1997) and the increasing understanding of the wavelength selective interaction and associated effects of laser radiation acting on biologic tissue. Despite the fact that the response of blood to the action of a low intensity laser radiation gives important information on the mechanism of interaction of laser radiation with a living organism (Zalesskaya & Sambor 2005; Korolevich et al. 1992), only a small number of works have been devoted to such investigation in living organisms. Also, there is still a lack of information concerning response of blood parameters, such as RBC with low laser light radiation. A wide research exists on the use of low intensity laser radiation in different experimental biological models. The most used laser of low level laser theraby studies is He-Ne

laser emitting light at a wavelength of 632.8 nm (Sikurova et al. 2011; Hend et al. 2010; Wasik et al. 2007; Mi et al. 2006; Yanhong et al. 2007; Gulsoy et al. 2006; Brill et al. 2000). Although some studies have been reported on the effect of low power laser radiation on human blood parameters, especially for the parameter of RBC (Hend et al. 2009; Zalesskaya et al. 2006; Zalesskaya & Sambor 2005; Mi et al. 2004; Siposan & Lukacs 2000), more research need to be done to understand the respond of this parameter with low level laser radiation. The goals of the present study are to compare the blood parameters of HGB, MCV and MCH before and after irradiation of He-Ne laser and to study correlations between HGB, MCV and MCH with human blood characteristics such as age, gender, ethnicity, and blood types, before and after laser radiation.

MATERIALS AND METHODS

Fresh human blood samples were collected from the Wellness Centre, Universiti Sains Malaysia USM, pulau Pinang, Malaysia. Permission was granted from the director of the USM Wellness Centre and also the head of the diagnostic laboratory before the data is collected. A total of 107 blood samples from 53 males and 54 females were used in this study. Blood samples were obtained from patients range from 10 to 80 years old and were labeled with the coding systems according to age, gender, ethnic, blood group, and disease. Blood parameters such as HGB, MCV, and MCH before and after laser exposure in each blood sample were analyzed by using Hematology analyzer, Cell-Dyn 1700.

A 632.8 nm red light laser beam from a 0.95 mW He-Ne laser was used as a power source and Encircled Flux Analysis System (EFAS) model 8350 fPhoton Inc. as a flux detector. The laser beam was delivered for one second to the irradiation spot of a drop of blood mounted on the slide. Readings of beam parameters were acquired after the irradiation. The procedure was repeated for 10 times and the mean of the results computed. The blood sample was irradiated at room temperature (23 ± 2°C) and the whole experimental set-up was performed in the dark enclosure.

Statistical analysis of the acquired data was performed using the statistical package for social science (SPSS). Paired nonparametric tests were used to evaluate the difference between the irradiated samples and non-irradiated controlled samples. An independent non-parametric test was used to evaluate and compare Hemoglobin concentration (HGB), Mean cellular volume of RBC (MCV), mean cellular hemoglobin (MCH) and beam parameters according to age, gender, ethnic, and blood group of patients.

RESULTS

Blood parameters check of the laser exposed and controlled samples show significant difference in HGB, MCV, and MCH (Figure 1). The mean values of these parameters for 107 blood samples before and after laser irradiation are shown in Table 1.

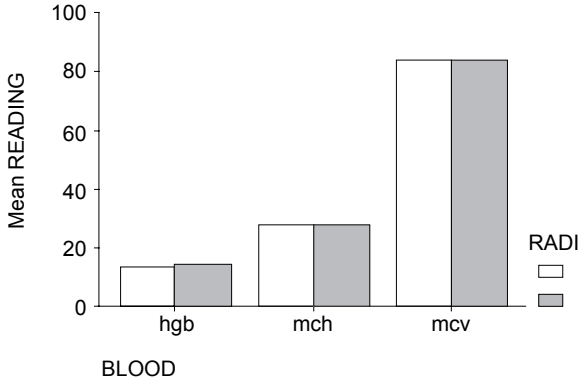

FIGURE 1. Comparisons of blood parameters before and after laser irradiation

TABLE 1. Blood parameters (mean ± standard error mean) for 107 irradiated and 107 controlled blood samples from different donors

Blood parameters	Controlled	Irradiation	Difference	p-value
HGB (g/dl)	13.479 ± 0.1406	14.188 ± 0.1687	0.709 ± 0.1019	0.000
MCV (fl)	83.372 ± 0.6304	83.575 ± 0.6361	0.203 ± 0.0563	0.000
MCH (pg)	28.053 ± 0.2497	28.223 ± 0.2487	0.170 ± 0.0472	0.000

Data are represented as mean ± standard error mean from 107 experiments.

Table 2 shows, a significant difference in mean centroid position horizontal and mean centroid position vertical, the mean centroid position vertical is greater than the mean centroid position horizontal (p-value = 0.000). There is no significant difference in mean peak position horizontal and mean peak position vertical (p-value = 0.261). The mean of flux peak is 139.28 counts. The mean of total flux is 3.55 μW.

TABLE 2. Beam statistics parameters (mean ± standard error mean) for 107 irradiated samples

Beam Statistics Parameters	Mean ± Std. Error Mean	p-value
Flux Peak (cnt)	139.28	-
Total Flux (μW)	3.55	-
Centroid Position Horizontal (μm)	39.67 ± 1.82	0.000
Centroid Position Vertical (μm)	57.64 ± 0.78	
Peak Position Horizontal (μm)	43.91 ± 2.83	0.261
Peak Position Vertical (μm)	48.05 ± 1.70	

Data are mean ± standard error mean from 107 experiments

Table 3 shows, no significant difference in the increase of mean HGB for age range of 10 to 24 years, 25 to 40 years, and more than 40 years old (male and female) for both samples, before and after irradiation. However, there is significant difference in the increase of mean HGB for Malay, Chinese, Indian, and others before and after irradiation. But, no significant difference in the increase of mean HGB for blood groups such as A, B, AB, and O before and after irradiation (Table 3). However, no significant difference in the increase of mean MCV and MCH for age range of 10 to 24 years old, age range of 25 to 40 years old, and age range of 41 years old and above before and after irradiation, and no significant difference in the increase of mean MCV and MCH (male and female), for Malay, Chinese, Indian, and others before and after irradiation. Also, no significant difference in the increase of mean MCV and MCH for blood groups such as A, B, AB, and O before and after irradiation (Table 4, 5).

TABLE 3. Comparison of difference HGB according to human age, gender, ethnic and blood group

Characteristic	Category	Mean ± Std Error Mean	p-value
Age	10-24 years old	0.838 ± 0.294	0.259
	25-40 years old	0.548 ± 0.221	
	>40 years old	0.738 ± 0.126	
Gender	Female	0.804 ± 0.144	0.587
	Male	0.613 ± 0.145	
Ethnic	Malay	0.823 ± 0.116	0.068
	Chinese	0.700 ± 0.314	
	Indian	0.060 ± 0.329	
	Others	0.300 ± 0.425	
Blood Group	A	0.794 ± 0.186	0.178
	B	0.509 ± 0.225	
	AB	0.150 ± 0.430	
	O	0.817 ± 0.154	

Data are mean ± Std error mean from 107 experiments

TABLE 4. Comparison of difference MCV according to human age, gender, ethnic and blood group

Characteristic	Category	Mean ± Std Error Mean	p-value
Age	10-24 years old	-0.162 ± 0.161	0.222
	25-40 years old	-0.361± 0.121	
	>40 years old	-0.159 ± 0.069	
Gender	Female	-0.233 ± 0.080	0.371
	Male	-0.172 ± 0.080	
Ethnic	Malay	-0.170 ± 0.065	0.184
	Chinese	-0.036 ± 0.174	
	Indian	-0.460 ± 0.183	
	Others	-0.517 ± 0.236	
Blood Group	A	-0.159 ± 0.103	0.345
	B	-0.336 ± 0.125	
	AB	-0.367 ± 0.239	
	O	-0.149 ± 0.085	

Data are mean ± Std error mean from 107 experiments

TABLE 5. Comparison of difference MCH according to human age, gender, ethnic and blood group

Characteristic	Category	Mean ± Std Error Mean	p-value
Age	10-24 years old	0.077 ± 0.136	0.609
	25-40 years old	0.200 ± 0.102	
	>40 years old	0.177 ± 0.058	
Gender	Female	0.113 ± 0.066	0.197
	Male	0.228 ± 0.067	
Ethnic	Malay	0.170 ± 0.055	0.869
	Chinese	0.100 ± 0.149	
	Indian	0.260 ± 0.156	
	Others	0.150 ± 0.201	
Blood Group	A	0.225 ± 0.087	0.501
	B	0.164 ± 0.104	
	AB	-0.083 ± 0.200	
	O	0.168 ± 0.071	

Data are mean ± Std error mean from 107 experiments

A significant difference in mean of flux peak after irradiation for age group (p-value = 0.067), between gender (p-value = 0.044), in addition for different ethnicity (p-value = 0.094), and among different blood type (p-value = 0.099) (Table 6).

DISCUSSIONS

In this study, the measurements using a hematology analyser cell-DYn 1700 showed significant differences between control and irradiated blood samples regarding the following parameters: red blood cell level which includes

TABLE 6. Comparison of Flux Peak according to human age, gender, ethnic and blood group

Characteristic	Category	Mean ± Std Error Mean	p-value
Age	10-24 years old	126.0923 ± 50.2848	0.067
	25-40 years old	149.6478 ± 42.8042	
	>40 years old	138.3423 ± 40.6693	
Gender	Female	130.9648 ± 40.4506	0.044
	Male	147.7604 ± 43.1811	
Ethnic	Malay	135.0175 ± 37.4678	0.094
	Chinese	134.2364 ± 56.7069	
	Indian	166.2500 ± 49.9323	
	Others	160.4833 ± 52.9577	
Blood Group	A	143.4781 ± 36.1083	0.099
	B	139.6682 ± 35.0255	
	AB	90.1167 ± 50.5559	
	O	142.5255 ± 45.8499	

Data are mean ± Std error mean from 107 experiments

hemoglobin concentration (HGB) (p-value = 0.000), mean cellular volume of red blood cell (MCV) (p-value = 0.000), and mean cellular hemoglobin (MCH) (p-value = 0.000) as in Table 1. Also the results revealed that an increase of the red blood cells including its components (HGB, MCV, MCH) after irradiation. Similar case found in other previous works that used same laser wave length and different power (Zalesskaya et al. 2006; Zalesskaya & Sambor 2005; Mi et al. 2004; Siposan & Lukacs 2000). This increases maybe due to the red cells form aggregates, which break up when the blood flows (Zhilin et al. 2009).

The studies of blood parameters by flux variations exhibit no significant patterns for peak position horizontal and vertical, while there is a significant difference in mean of the centroid position horizontal and vertical (Table 2). Also found that the mean centroid position vertical was greater than the mean centroid position horizontal (p-value = 0.000), this is maybe due to the exposure area of the blood sample.

The significant differences in the mean of hemoglobin concentration were noticed especially in the cases of the patients' ethnicity. Malay patients show the highest increase in mean of the hemoglobin concentration after irradiation (Table 3). However no significant differences were noticed in the cases of MCV, MCH according to the patients' characteristics, age, gender, ethinic, and blood groups (Table 4 & 5), but we need further research to study the Changes of the blood parameters with patients' characteristics according to the medical history and the body mass index.

There is a significant difference in mean of flux peak after irradiation for all tested ages. Patients aged group from 25 to 40 years old show the highest increase in mean of flux peak. The mean increase of flux peak after irradiation is higher for male than female, Indian patients show the highest increase in mean of flux peak, and patients with blood group A show the highest increase in mean of flux peak (Table 6).

CONCLUSION

The findings from this study show that low level He-Ne laser irradiation on various types of blood samples caused important change to human blood parameters including, hemoglobin concentration (HGB), Mean cellular volume of RBC (MCV), and mean cellular hemoglobin (MCH). The beam statistics parameters such as centroid position, peak position, flux peak and total flux, can become significant parameters in the analysis of blood.

ACKNOWLEDGEMENT

The authors acknowledge the research grant provided by the Universiti Sains Malaysia (USM), Penang, Malaysia that has resulted in this article. We would also like to thank the staff of the Diagnostic laboratory of USM Wellness Centre for their assistance and support of this research

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Received: January 2011
Accepted for publication: July 2011

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