Egypt. J. Chem. **Vol. 65**, No. 11 pp. 297 - 304 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Effectiveness of Low Power He-Ne Laser Irradiation in Improving the Hemoglobin Conformational Structure of Rheumatoid Arthritis Patients: a Biophysical Perspective

Samir AA Aziz[a], Fatma A. A. Ibrahim[a], Sherif Moussa[a*], Atef Attia[a], Aliaa Elgendy[b], Noha Abd El-Latif[a], Azhar M. Elwan[a]

[a]*Department of Biochemistry, National Research Centre,* 12622 *Dokki, Giza, Egypt*
[b]*Department of Complementary Medicine, National Research Centre, ,* 12622 *Dokki, Giza, Egypt*

Abstract

Cold laser has considerable potential curative outcomes. This study desires to assess the relative efficacy of using soft laser as an additive therapy in rheumatoid arthritis by assaying the absorption spectra, studying the thermodynamic parameters of hemoglobin (Hb), plasma malonyldialdehyde (MDA), as well as erythrocyte osmotic fragility. Experiments were performed on 70 subjects with age group 50 ± 5 years. They were divided into three groups. The first group (G1) represents 20 normal adults as a control group. The second group (G2) represents 25 patients treated with non-steroid anti-inflammatory drugs (NSAIDs). The third group (G3) represents 25 patients treated with NSAIDs and undergo to cold laser generated from the mid-infrared laser medical device. Patients are exposed to laser sessions for four weeks every day. The study revealed a significant improvement in the heme-heme interaction, exhibiting the stabilization of oxyhemoglobin induced by dual treatment in the third group. It has enhanced the dynamic motion of Hb as a folding process, which increases oxygen utilization. The results also showed a significant reduction in plasma malondialdehyde (MDA) besides lowering the average elevated osmotic fragility and a decrease in the high rate of hemolysis. In conclusion, by using a cold laser as a complementary therapy, there is a significant enhancement in the connection between hemoglobin synthesis and function.

Keywords: Rheumatoid arthritis; soft laser; non-steroidal anti-inflammatory drugs; osmotic fragility; absorption spectrum of haemoglobin; thermodynamic parameters.

1. Introduction

Rheumatoid arthritis (RA) is a universal chronic inflammatory disease; it causes gradual joint damage and also leads to disability[1]. Environmental and genetic factors are closely related[2]. The incidence of RA is 0.5% to 1% in industrialized countries; the percentage is higher among women and the elderly [3].

For now, although the outlook for patients is favorable, many of them still do not respond to treatments [4]. Rheumatoid arthritis has been caused by a substantial burden on humans and society[5].Thus, it is necessary to discover new and effective therapeutic modalities for RA.

Erythrocytes play a critical role in the human body because of their abundant ability to transport oxygen and carbon dioxide to various tissues. The most essential component in erythrocytes is Hb, which is the primary oxygen-transport protein in humans. Anemia is common in patients with systemic rheumatoid arthritis[6]. Insufficient studies have been performed to determine whether it can alter the structure of Hb at the molecular level in erythrocytes of rheumatoid arthritis patients.

Low level laser therapy (LLLT) is a treatment that generates monochromatic light. This is done through non-thermal or photochemical reactions in cells, known as photobiology or bio-stimulation, photo-bio modulation. Laser propagation in tissues is an issue of increasing importance in the many diagnostic and therapeutic applications in today's photo-medicine. To get any effect by the low-power

*Corresponding author e-mail: sherifmoussa96@gmail.com.; (Sherif Moussa).
Receive Date: 08 February 2022; Revise Date: 02 March 2022; Accept Date: 06 March 2022.
DOI: 10.21608/EJCHEM.2022.120787.5417.

laser, electronic bands of molecular photoreceptors or chromophores must absorb photons. The chromophore in the blood is hemoglobin, which is the main absorber of visible light in the blood [7]. Exposure of blood to low power laser leads to changes in the infra-red (IR) and visible absorption spectra of blood and erythrocytes[8].

This study aspires to shed light on the effect of soft laser being added to the schedule of anti-inflammatory drugs applied in rheumatoid therapy on improving heme stability in RA patients.

2. Experimental

This study was performed on 70 subjects with range 50 ± 5 years old. Attending El-Kasr El-einy Hospital, Cairo University and exposed to Cold LASER or low light laser treatment (LLLT) in complementary medicine clinic, National Research Centre, Cairo, Egypt. It was approved by Research Ethics Committee of NRC.

These subjects were categorized into three groups. The first group (G1) represented 20 normal adults (7 males and 13 females) with no rheumatoid arthritis history. The second group (G2) consisted of25 patients (10males, 15 females) treated with non-steroid anti- inflammatory drugs. The third groups (G3) consisted of25 patients (9 males and 16females) who were treated with NSAIDs and subjected to cold laser.

All Egyptian patients had a diagnosis of arthritis based on 2012 American College of Rheumatology / European League against Rheumatism RA classification criteria[9].

Exclusion Criteria: Patients with co-morbidities that overlap with another connective tissue disease such as systematic lupus erythromatosus (SLE), systematic sclerosis, ischematic heart disease, alcoholics and smokers were eliminated from the study.

All patients underwent complete history taking, careful clinical examination, and RA disease activity was estimated by Disease Activity Score 28 (DAS 28); by assessing tender's number and swollen joints, the visual analogue scale (VAS) of pain as well as the erythrocyte sedimentation rate (ESR). Patients with a DAS 28 score higher than 5.1 are having high disease activity, those with DAS28 more than 3.2 but less than or equal 5.1 are in moderate disease activity. If the patient has score is ≤ 3.2 he has low disease activity, while the patient is considered in remission if his DAS 28 is less than 2.6 [10].

Health assessment questionnaire (HAQ) was used for assessing the functional ability of the patients [11]. It was one of the first measures of functional status (disability) and has become the dominant tool in many areas of disease including arthritis. It is widely used worldwide and outcome measurement has become mandatory for clinical trials in RA.

Blood samples from patients and controls (heparinized and not) were drown and centrifuged at 3000 rpm 4ºC for 15 minutes. Serum and plasma samples were stored at -70ºC till the analysis. Blood samples collected before and after LLLT for experimental tests.

LLLT protocol: Photobiostimulation was done using a He-Ne Laser with the following parameters: Continuous radiation mode, wave length 905 nm, Output power 100 mW of energy density 8J/cm^2. The output divergence after lens is 60 mRad, coaxially connected with 2 infra-red diodes at 904 nm wavelength, with all of the following specifications:

1-Infra- red laser emitters: Peak output power = 5 x 10 w. Average output power = 5 x (0.3 + 5) mw. Pulse width 180 nsec. Pulse frequency min. 200 Hz - Max. 10000 Hz. Output beam divergence 70 m Rad. Number of diodes = 5

2- I.R. Handles: Peak power = 10 W. Average output power = 3 mW (min.). Pulse duration 180 nsec. Pulse frequency 4000 Hz. Output beam divergence 70 mRad.

RA patients susceptible to LLLT on three days a week for four weeks (i.e. 12 sessions per patient).

2.1. Hemoglobin Extraction:

Hemolysates of washed, packed erythrocytes were prepared by a modification of the method of Trivelli et al., (1971)[12].

2.2. Absorption spectrum of hemoglobin

The concentration of hemoglobin, under these conditions was found to be within the range ($2.9 \times 10^{-5} – 3.4 \times 10^{-5}$ M). At this extreme- proper dilution, under air-saturated conditions, deoxyhemoglobin fraction is too low to be detected spectrophotometrically, and therefore it was neglected.

The absorption spectra of HbO_2 were measured in the wavelength range 200 to 700 nm at 25 ± 1°C using a Cary UV/VIS dual-beam spectrophotometer (model 100 UV-VIS), made by Agilent Technologies, Australia.

2.3. Thermodynamic parameters of hemoglobin

In this experiment, the absorbance of hemoglobin solution of concentration (3.4×10^{-5} M, at the spin or spin heme-heme interaction band (A_{578}) were measured at various temperature in the range 25-40°C, 5°C intervals, using temperature controlled spectrophotometer. The spin state constant (K), at each temperature, was calculated by using the following equation:

$$K = \frac{A_{578}}{1 - A_{578}} \qquad (1)$$

2.4. Measurement of malonyldialdehyde (MDA)

The kit used to determine MDA was purchased from the Bio diagnostic Company in Cairo, Egypt. MDA concentrations were measured spectrophotometrically using the method Satoh [13].

2.5. Erythrocyte osmotic fragility of Red blood cells

Erythrocyte osmotic fragility of red blood cells were measured according to Mazeron et al., (2000) [14].

2.6. Statistical Analysis

Data were presented as the mean ± standard error (SE) values. One-way analysis of variance (ANOVA) was carried out, and the statistical comparisons between groups with post hoc and the least significant difference (LSD) tests were performed using statistical programs (Statistical Package for the Social Sciences, version 14 [SPSS Inc., Chicago, IL]. P<0.05 was considered statistically significant.

3. Results

Table 1: Absorbance of different hemoglobin bands of RA patients subjected to various treatments compared to the control group (G1).

Group	Globin-heme interaction (λ = 340 nm)	Soret band (λ = 415 nm)	λ = 540 nm	Heme-Heme interaction (λ = 578 nm)	Ratio A_{578}/A_{540}
G_1	0.7243±0.0025	1.1741±0.0188	0.632±0.0019	0.621±0.00054	0.9825
G_2	0.5892±0.0028 ^{a}	1.1463±0.038^{a}	0.541±0.0022 ^{a}	0.476±0.00038 ^{a}	0.8798
G_3	0.7011±0.0022^{a,b}	1.1688±0.211^{a,b}	0.619±0.00073^{a,b}	0.598±0.00051^{a,b}	0.9660

Values were set as mean ± SE, asignificantly different from controls, P < 0.0005, bsignificantly different from the RA group before Laser irradiation P < 0.0005.

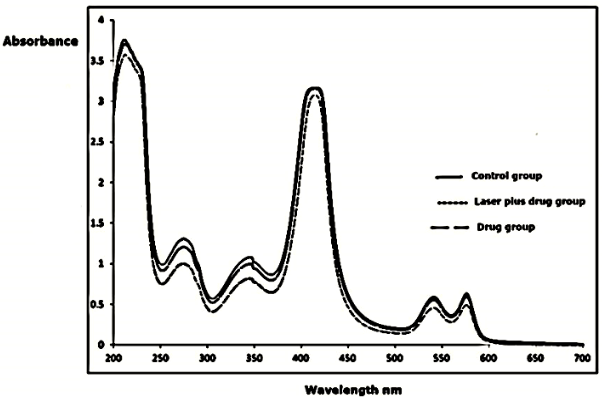

Figure 1: Hemoglobin absorption spectra of RA patients exposed to different treatments compared to the control group (G1).

Absorption spectra and different absorbance bands of hemoglobin for RA patients undergoing different treatments compared to the control group are shown in Table (1) and Fig. (1). The results pointed to a significant decrease in the absorbance band at 340 nm (globin-heme interaction) for patients (G2) associated with a significant increase in patients (G3) compared to the control group (G1).

A significant increase in the HbSoret band was detected at 415 nm from patients (G3) compared to (G2). A significant decrease in the absorbance band at 540 nm was revealed for patients treated with different treatments compared to the control group, while there is a significant increase in this band for patients (G3) compared to group (G2). A highly significant increase in the absorbance band at 578 nm (heme-heme interaction) was identified for patients (G3) compared to (G2). The absorbance ratio of A578/A540 was less than one for all patients who underwent different treatments. There was a significant decrease in the group (G2), with a significant increase in the group (G3) relative to the control group (G1).

Table 2: Absorbance at 275 nm as an indicator of globin motion for RA patients subjected to various treatments to the control group (G1).

Group	Absorbance at 275 nm
G1	1.386 ± 0.0127
G2	1.102 ± 0.0482 ^{a}
G3	1.278 ± 0.0374^{a,b}

Values were set as mean ± SE, ^{a}significantly different from controls, P < 0.0005, ^{b}significantly different from the RA group before Laser irradiation P < 0.0005.

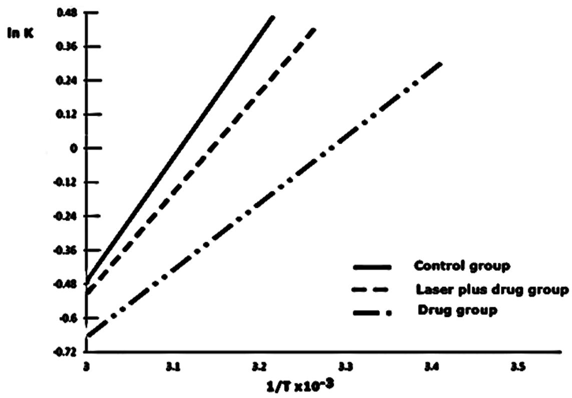

Figure 2: Relationship between the natural logarithm of spin state constant (K) of iron heme and 1/T.

The values of (ln K) were plotted as a function of (1/T) as shown in Fig. (2). Spin state constant was determined by Arrhenius equation.From the slope of the lines, the enthalpy (ΔH) was calculated through the use of equation:

$$\Delta H = -R\left[\frac{\partial \ln K}{\partial \frac{1}{T}}\right] \qquad \textbf{(2)}$$

It is clear that the slope of this relation is remarkable shifted towards control group regarding patients subjected to soft laser and drug (G3), compared to patients of (G2). Spin state constant (K) of heme iron was increased significantly in patients subjected to soft laser (G3) compared to (G2).

Table 3: Spin state constant and thermodynamic parameters of hemoglobin for RA patients subjected to different treatments compared to the control group.

Group	Spin state constant K	ΔF (J/mole)	ΔH (J/mole)	ΔS (J/mole K)
G_1	0.75 ± 0.00042	7.63 $\times 10^2 \pm$ 0.761	-8.64 × $10^3 \pm$ 0.173	-28.86 ± 0.00865
G_2	0.685± 0.00084 [a]	8.05 × $10^2 \pm$ 0.3044 [a]	-7.09 × $10^3 \pm$ 0.272 [a]	-27.38 ± 0.08753 [a]
G_3	0.728 ± 0.00045[a,b]	7.24 × $10^2 \pm$ 0.211[a,b]	-7.76 × $10^3 \pm$ 0.1756[a,b]	- 28.21 ± 0.0374[a,b]

Values were set as mean ± SE, [a]significantly different from controls, P<0.0005, [b]significantly different from the RA group before Laser irradiation P<0.0005

An opposite behavior was observed in free energy (ΔF) in (G3) regarding to (G2), an appreciable decreaseof enthalpy (ΔH) and entropy (ΔS) was detected concerning (G3) as compared to (G2) as shown in table 3.

Table 4: Plasma MDA levels of RA patients exposed to different treatments compared to the control group (G1).

Groups	Plasma MDA nmol/ml
Control (non -rheumatoid patients) (G_1)	3.123±0.213
Rheumatoid patients treated with drugs (G_2)	9.327±0.398[a]
Rheumatoid patients treated with Cold laser plus drugs (G_3)	4.678±0.254[a,b]

Values were set as mean ± SE, [a]significantly different from controls, P < 0.0005, [b]significantly different from the RA group before Laser irradiation P < 0.0005.

In this study, a highly significant decrement in plasma MDA was observed in RA patients from G3, as compared to G2 (Table 4).

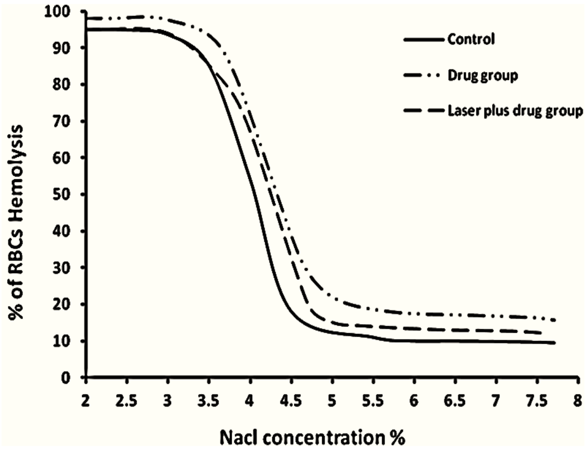

Figure 3: Osmotic fragility curves of RA patients undergoing different treatments compared to the control group (G1).

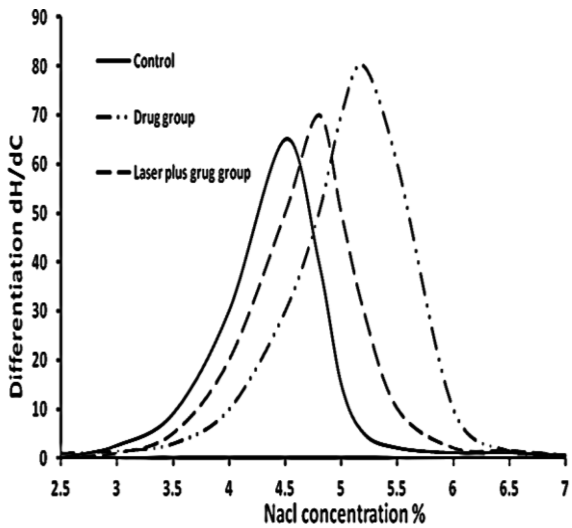

Figure 4: Gaussian curves (hemolysis rate versus NaCl concentration) for RA patients exposed to different treatments compared to the control group (G1).

Table 5: H_{50} center, width(S), and height (P) of Gaussian peaks ofRA patients receiving different treatments compared to the control group (G1).

Group	Average osmotic fragility H_{50} (g/l)	Dispersion of hemolysis S(g/l)	Area (A) mm^2	Height (P) %
Control (non - rheumatoid patients) (G_1)	4.52±0.003	0.99±0.005	84.9 ± 0.542	65.21
Rheumatoid patients treated with drugs (G_2)	5.52±0.001[a]	0.86 ±0.004 [a]	84.1 ± 0.515 [a]	80.11
Rheumatoid patients treated with Cold laser plus drugs (G_3)	4.81±0.003[a,b]	0.94 ±0.005[a,b]	84.6 ± 0.538 [a,b]	70.13

Values were set as mean ± SE, [a]significantly different from controls, $P < 0.0005$, [b]significantly different from the RA group before Laser irradiation $P < 0.0005$

The shift of patients' erythrocyte osmotic fragility from the group (G2) curve to the right of the control is clear; represents a rise in the mean osmotic fragility (H_{50}). Simultaneously, the dispersion of hemolysis (S) showed a steep decrease in Table 5. The results gained from the G2 curve displayed a rise in the maximum rate of hemolysis (P) with a concomitant shift of the center of peaks (C) towards higher values of NaCl concentration (Table 5). Group (G3) Patients treated with soft laser proved a diminishing effect of free radicals on the structural integrity of RBCs. (Fig.3, Fig.4, and Table 5).

4. Discussion

One of the most familiar types of inflammatory diseases is rheumatoid arthritis. It can lead to pain, stiffness, swelling, and damage to function[15]. Ultimately, RA can lead to cumulative disability and early death [16, 17], but until causative factors are discovered that may allow for more precise differentiation and use of more specific forms of treatment, group therapy should continue based on a set of non-specific measures aimed at relieving symptoms, preventing disfigurement, and improving overall health.

Many of the drugs used to treat the rheumatoid disease can intervene with several patterns of the inflammatory response. In the age of new complementary medicines like a soft laser, clinical remission of RA complications remains the dominant treatment target to improve patients' health by eliminating the burden of inflammation[18, 19].

The absorption spectrum of the hemoglobin molecule can confer some intimation about the conformational changes. The lower Soret band absorbance (at 415 nm) associated with lower absorption (at 340 nm) for RA patients (G2) compared to the control group shows some troubles in the electronic states of the heme-prosthetic group in these patients. The significant increase in Soret band absorbance at 415 nm and 340 nm in patients (G3) confirms the stability of hemoglobin as a folding process because of some hemoproteins under different stress conditions which increases the binding of the protein to the new prosthetic groups (change in the globin-heme interaction)[20].

The decrement in the absorbance of the heme-heme interaction band (at 578 nm) consecutively with the decrease at the A578/A540 ratio in (G2) relative to the control group (G1) indicates a destabilization of oxyhemoglobin as a macromolecule in RA patients. These changes are reversed after soft laser treatment exhibiting the improvement in the heme-heme interaction accompanied by an increase in A578/A540 ratio which signifies the reduction of Met-Hb to HbO2, this effect reflects the function of hemoglobin through the drop in its affinity to oxygen and consequently elevates the delivery of oxygen to the tissues, clarifying the stability of oxyhemoglobin induced by dual treatment[21].

One can conclude that the chromophores in the cell are more susceptible to light absorption; endogenous porphyrins that appear in the cells can absorb this light. Porphyrins have a high absorption band at 360 nm region, and four further absorbance bands at 502, 540, 560, and 630 nm with a decrease in intensity[22].

It became clear that proteins are dynamic systems that twist and breathe, and their movements are essential to their functions[23, 24]. Thus, the decrease in the absorbance of globin at 275nm for RA (G2) patients shows an allusion for this abnormal motility that reflects its deviation from the normal functions. The increase in this motility can be attributed to the different degrees of globin unfolding due to changes in the microenvironment and the aggregation state of hemoglobin molecules. The increase in globin absorbance at 275 nm for patients (G3) compared to

(G2) shows the conformational changes, which determine the consequences of the laser; directly on Hb molecules, or indirectly, by affecting several enzyme systems correlated with Hb functions in erythrocytes. There is an assumption that cell components can be redirected by the linear polarization of the laser, and this leads to a reactivation of the metabolic process.

Another clue comes from thermodynamic parameters that enhance the dynamic motion of Hb as a folding process, which increases Hb stability and oxygen utilization [25].

Free energy ΔF represents any reaction in which direction and how far the reaction will go to reach equilibrium. When it occurs in the standard state, ΔF of Hb advances towards equilibrium, its value is negative[26]. Thus, laser irradiation led to drive to equilibrium, as ΔF was lowered in (G3) patients compared to (G2) patients, which signifies the low affinity of Hb to oxygen.

The enthalpy ΔH (heat content) of the molecule shows that the reacting system releases or absorbs heat from its surrounding at constant temperature and pressure. When the reacting system loses heat, the sign of ΔH is negative. This decrement of enthalpy in the group (G3) that was subjected to laser radiation compared to group (G2) means that the system loses heat to the ambient, indicating the folding of Hb as a globular protein and consequently enhances the mechanism of oxygen utilization[27].

Entropy (molecular disorders) increases during oxidation (conversion to MetHb). The notable increase in entropy in the (G2) group compared to the control group is resulting from the oxidation potential, which appeared as a hybrid of Hb and Met-Hb. The significant decrease in entropy as a result of laser irradiation in the (G3) group is due to the stability of Hb probably through the elevation of hydrogen bonds [28].

Another key finding of our study is that erythrocyte osmotic fragility is an indirect process for evaluating oxidative stress as a good reflector for studying the health status of individuals with pathological problems[29]. High MDA, a signal of lipid peroxidation, can disrupt the red blood cells' biochemical and physiological functions. Alteration of lipid peroxidation in the erythrocyte membrane leads to increased fragility of red blood cells in group G2. This indicates a higher maximum rate of hemolysis (peak height), and a decrement in the hemolysis dispersal (below the normal dispersion represents an unexpected rupture of red blood cells). The center of the Gaussian peak (H50) also shifted toward a higher concentration of NaCl. It has been stated that changes in erythrocyte membrane lipid composition have a slight influence on mechanical behavior, whilst changes in membrane skeletal proteins perform a vital role [30].

Most of the free radicals observed in cases of rheumatoid arthritis are oxygen derivatives, especially the superoxide anion and hydroxyl radical. The formation of radicals occurs in the body through various procedures that include both endogenous and environmental influences. As a single electron is added to oxygen, superoxide anion is produced, as well as, many mechanisms can form the superoxide in vivo[31]. Therefore, the alteration caused by osmotic fragility reverses the detrimental effects on the cytoskeleton[32].

The bio-stimulating effect of the laser counteracts the phenomenon of physiological destruction (aging of RBC), which leads to an increase in the vitality of cells in the circulatory system. This effect coincides with the results of [33] which revealed that low energy He-Ne laser generates a preventative effect on erythrocyte membranes, reducing hypotonic, hemolysis and allowing the cell membrane to stabilize. Thus, they show a decrement in osmotic fragility (increased resistance of cells to hypotonic solutions) [34].

5. Conclusions

Surprisingly, laser irradiation recovers the conformational structure of hemoglobin and performs its function as a macromolecule, thus reducing arthritis swelling, alleviating oxidative stress, inflammation as well as enhancing the photo - biological response and energy metabolic status.

6. Conflicts of interest

The authors declare that they have no conflict of interest.

7. Formatting of funding sources

None

8. Acknowledgments

The authors thank and appreciate the National Research Centre of Egypt for the technical supporting of this work.

9. References

[1] Stahl EA, Raychaudhuri S, Remmers EF, Xie G, Eyre S, Thomson BP, et al. Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. Nat Genet 2010;42:508–14. https://doi.org/10.1038/NG.582.

[2] Kim K, Bang S-Y, Lee H-S, Cho S-K, Choi C-B, Sung Y-K, et al. High-density genotyping of

immune loci in Koreans and Europeans identifies eight new rheumatoid arthritis risk loci. Ann Rheum Dis 2015;74:e13. https://doi.org/10.1136/annrheumdis-2013-204749.

[3] Smolen JS, Aletaha D. Rheumatoid arthritis therapy reappraisal: Strategies, opportunities and challenges. Nat Rev Rheumatol 2015;11:276–89. https://doi.org/10.1038/NRRHEUM.2015.8.

[4] Smolen JS, Breedveld FC, Burmester GR, Bykerk V, Dougados M, Emery P, et al. Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international task force. Ann Rheum Dis 2016;75:3–15. https://doi.org/10.1136/annrheumdis-2015-207524.

[5] Cross M, Smith E, Hoy D, Carmona L, Wolfe F, Vos T, et al. The global burden of rheumatoid arthritis: estimates from the Global Burden of Disease 2010 study. Ann Rheum Dis 2014;73:1316–22. https://doi.org/10.1136/annrheumdis-2013-204627.

[6] Swaak A. Anemia of chronic disease in patients with rheumatoid arthritis: Aspects of prevalence, outcome, diagnosis, and the effect of treatment on disease activity. J Rheumatol 2006;33:1467–8.

[7] Sikurova L, Balis P, Zvarik M. Penetration of laser light through red blood cell ghosts. J Photochem Photobiol B Biol 2011;103:230–3. https://doi.org/https://doi.org/10.1016/j.jphotobiol.2011.03.015.

[8] Sallam SM, Abo Salem LI, Abdalfattah B, Rezk, Ibrahim M. Influence of The He-Ne (632.8 nm) laser Radiation on a Human Whole Blood Stored 2020;21:57–64.

[9] Anderson J, Caplan L, Yazdany J, Robbins ML, Neogi T, Michaud K, et al. Rheumatoid arthritis disease activity measures: American College of Rheumatology recommendations for use in clinical practice. Arthritis Care Res (Hoboken) 2012;64:640–7. https://doi.org/10.1002/acr.21649.

[10] Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44–8. https://doi.org/10.1002/art.1780380107.

[11] Aletaha D, Smolen JS. Remission in rheumatoid arthritis: missing objectives by using inadequate DAS28 targets. Nat Rev Rheumatol 2019;15:633–4. https://doi.org/10.1038/s41584-019-0279-6.

[12] Trivelli LA, Ranney HM, Lai H-T. Hemoglobin Components in Patients with Diabetes Mellitus. N Engl J Med 1971;284:353–7. https://doi.org/10.1056/NEJM197102182840703.

[13] Kei S. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta 1978;90:37–43. https://doi.org/10.1016/0009-8981(78)90081-5.

[14] Mazeron P, Didelon J, Muller S, Stoltz JF. A theoretical approach of the measurement of osmotic fragility of erythrocytes by optical transmission. Photochem Photobiol 2000;72:172–8. https://doi.org/10.1562/0031-8655(2000)072<0172:ataotm>2.0.co;2.

[15] Deeks JJ, Higgins JPT, Altman DG, Group CSM. Analysing data and undertaking meta- analyses. Cochrane Handb Syst Rev Interv 2019:241–84.

[16] Sanderson T, Morris M, Calnan M, Richards P, Hewlett S. What outcomes from pharmacologic treatments are important to people with rheumatoid arthritis? Creating the basis of a patient core set. Arthritis Care Res (Hoboken) 2010;62:640–6. https://doi.org/10.1002/acr.20034.

[17] Venkatesh MP, Kumar TP, Pai DR. Targeted drug delivery of Methotrexate in situ gels for the treatment of Rheumatoid Arthritis. Saudi Pharm J SPJ 2020;28:1548–57. https://doi.org/10.1016/j.jsps.2020.10.003.

[18] Glinatsi D, Heiberg MS, Rudin A, Nordström D, Haavardsholm EA, Gudbjornsson B, et al. Head-to-head comparison of aggressive conventional therapy and three biological treatments and comparison of two de-escalation strategies in patients who respond to treatment: study protocol for a multicenter, randomized, open-label, blinded-assessor, pha. Trials 2017;18:161. https://doi.org/10.1186/s13063-017-1891-x.

[19] Kolonics F, Szeifert V, Timár CI, Ligeti E, Lőrincz ÁM. The Functional Heterogeneity of Neutrophil-Derived Extracellular Vesicles Reflects the Status of the Parent Cell. Cells 2020;9:2718. https://doi.org/10.3390/cells9122718.

[20] Walski T, Drohomirecka A, Bujok J, Czerski A, Wąż G, Trochanowska-Pauk N, et al. Low-Level Light Therapy Protects Red Blood Cells Against Oxidative Stress and Hemolysis During Extracorporeal Circulation. Front Physiol 2018;9:647. https://doi.org/10.3389/fphys.2018.00647.

[21] Vanin AA, Verhagen E, Barboza SD, Costa LOP, Leal-Junior ECP. Photobiomodulation therapy for the improvement of muscular performance and reduction of muscular fatigue associated with exercise in healthy people: a systematic review and meta-analysis. Lasers Med Sci 2018;33:181–214. https://doi.org/10.1007/s10103-017-2368-6.

[22] Elwell CE, Gagnon NL, Neisen BD, Dhar D, Spaeth AD, Yee GM, et al. Copper–Oxygen Complexes Revisited: Structures, Spectroscopy, and Reactivity. Chem Rev 2017;117:2059–107. https://doi.org/10.1021/acs.chemrev.6b00636.

[23] Kleckner IR, Foster MP. An introduction to NMR-based approaches for measuring protein dynamics. Biochim Biophys Acta - Proteins Proteomics 2011;1814:942–68. https://doi.org/10.1016/j.bbapap.2010.10.012.

[24] Zhang K, Fan Z, Yao B, Zhan T, Ding Y, Zhu S, et al. Entropy-driven ectrochemiluminescence ultra-sensitive detection strategy of NF-κB p50 as the regulator of cytokine storm 2020;176:112942.

[25] Lanferdini FJ, Krüger RL, Baroni BM, Lazzari C, Figueiredo P, Reischak-Oliveira A, et al. Low-level laser therapy improves the VO2 kinetics in competitive cyclists. Lasers Med Sci

2018;33:453–60. https://doi.org/10.1007/s10103-017-2347-y.
[26] Muller MP, Jiang T, Sun C, Lihan M, Pant S, Mahinthichaichan P, et al. Characterization of Lipid–Protein Interactions and Lipid-Mediated Modulation of Membrane Protein Function through Molecular Simulation. Chem Rev 2019;119:6086–161. https://doi.org/10.1021/acs.chemrev.8b00608.
[27] Khan I, Khan K. Uncoupling of Carbonic Anhydrase from Na-H exchanger-1 in Experimental Colitis: A Possible Mechanistic Link with Na-H Exchanger. Biomolecules 2019;9:E700. https://doi.org/10.3390/biom9110700.
[28] DeMizio DJ, Geraldino-Pardilla LB. Autoimmunity and Inflammation Link to Cardiovascular Disease Risk in Rheumatoid Arthritis. Rheumatol Ther 2020;7:19–33. https://doi.org/10.1007/s40744-019-00189-0.
[29] Chihuailaf RH, Contreras PA, Wittwer FG. Pathogenesis of oxidative stress: Consequences and evaluation in animal health 2002;33:265–85.
[30] Andolfo I, Russo R, Gambale A, Iolascon A. New insights on hereditary erythrocyte membrane defects. Haematologica 2016;101:1284–94. https://doi.org/10.3324/haematol.2016.142463.
[31] Casares D, Escribá P V., Rosselló CA. Membrane Lipid Composition: Effect on Membrane and Organelle Structure, Function and Compartmentalization and Therapeutic Avenues. Int J Mol Sci 2019;20:2167. https://doi.org/10.3390/ijms20092167.
[32] Huisjes R, Bogdanova A, van Solinge WW, Schiffelers RM, Kaestner L, van Wijk R. Squeezing for Life – Properties of Red Blood Cell Deformability. Front Physiol 2018;9:656. https://doi.org/10.3389/fphys.2018.00656.
[33] Iijima K, Shimoyama N, Shimoyama M, Mizuguchi T. Red and Green Low-powered He-Ne Lasers Protect Human Erythrocytes from Hypotonic Hemolysis. J Clin Laser Med Surg 1991;9:385–9. https://doi.org/10.1089/clm.1991.9.385.
[34] Mostafa YM, Amin SN, Abdalwahab S, Elsherbini AAM. Effects of non-coherent and coherent light on complete blood picture and osmotic fragility of human blood. J Blood Disord Transf 2013;4:134.