Medical & Clinical Research

ISSN: 2577 - 8005 Volume 5 | Issue 11

www.medclinres.org

ISSN: 2577 - 8005

Research Article

Medical & Clinical Research

Successful Reduction of SARS-CoV-2 Viral Load by Photodynamic Therapy (PDT) Verified by QPCR – A Novel Approach in Treating Patients in Early Infection Stages

Hans Michael Weber[*1], Yasaman Zandi Mehran[2], Armin Orthaber[3], Hadi Hosseini Saadat[4], Robert Weber[5] and Matthias Wojcik[6]

[1]*Laser Therapy and Research Center, Lauenfoerde, Germany*

[2]*PhD of Biomedical Engineering, Assistant Professor, IAU, Tehran, Iran*

[3]*Laser Therapy and Research Center, Lauenfoerde, Germany*

[4]*Internal medicine Gandhi Hospital, Tehran, Iran*

[5]*ISLA Research Group, Germany*

[6]*Department of Pharmaceutics and Biopharmaceutics, University of Marburg, Germany*

[*]Corresponding author
Dr. Hans Michael Weber, Laser Therapy and Research Center, Lauenfoerde, Germany, Email: weber@webermedical.com

Submitted: 04 Dec 2020; **Accepted**: 08 Dec 2020; **Published**: 14 Dec 2020

Abstract

Background: *The Covid-19 pandemic is affecting Global Health and the world's economy dramatically since early 2020. After a temporary slowdown in summer 2020 the infection numbers and death rates have been increasing again in recent months leading to various restrictions of social and economic life in many countries. Latest developments of new vaccinations seem to be promising; however large-scale production and worldwide distribution logistics take time while questions such as longevity of immune protection, long-term side-effects etc. are remaining unclear at this point. Furthermore, vaccination is a preventive approach and not a therapy for acutely infected patients. Thus, there are still therapies needed to help people with Covid-19 infections. The objective of this study was to evaluate if Photodynamic Therapy (PDT) with Riboflavin and a specially designed light treatment kit would be able to fill this gap by helping people in early stages of infection. This may lead to a relief for hospitals and intensive care stations.*

Methods: *This study was made up of two groups with 20 patients each with the experiment (verum) group receiving Photodynamic Therapy and daily testing and a control group receiving conventional care plus testing. All patients in both groups had positive Covid-19 test results at the beginning of the study. They were in an early infection stage with mild symptoms like fever, dry cough, headache, hard breathing, fatigue etc. QPCR tests with Ct-viral load were performed on day 1, 2, 3, 4, 5 and 7 in the experiment group and on day 1, 3, 5 and 7 in the control group.*

Results: *All 20 patients in the experiment group showed significant improvement in clinical symptoms and viral load assessment within the 5 days of PDT treatment. 14 out of 20 patients had a negative QPCR test after 5 days of treatment with PDT while the other 6 patients also showed significantly reduced viral load. 20 patients in the control group with conventional care were tested 3 times within 5 days and no significant improvement could be seen, neither clinically nor in viral load assessment.*

Conclusion: *In this primary study the potential of Photodynamic Therapy (PDT) against SARS-CoV-2 could be shown in early infection stages. PDT proved to be successful in improving clinical symptoms, lowering viral loads and in preventing hospitalization and intensive care treatments. The applied treatment is easy to perform at home and it is cost effective. It can be used for prevention after contact with infected people or in case of positive testing but also in early cases with mild to moderate clinical symptoms.*

Background

The principle of Photodynamic Therapy (PDT)

Photodynamic Therapy (PDT) is one of the most interesting and promising approaches for treatment of various cancers and infectious diseases [1, 2]. PDT has a long history and is already approved for several superficial cancers on the skin or on superficial layers in the esophagus, bronchial system, stomach, bile duct, colon and bladder. In contrast to chemotherapy or radiation PDT-treatments normally do not go along with severe side effects. The principle is the stimulation of a light sensitive drug (photosensitizer) which is applied either on the skin as a cream, applied orally or injected into the blood circulation. In cancer treatment the photosensitizer is integrated into tumor tissue by endocytosis and is subsequently irradiated with light of specific wavelength that

matches the absorption spectrum of the photosensitizer. This light activation process induces various chemical processes such as the development of radical oxygen species that ultimately lead to the destruction of tumor tissue or microbes and viruses.

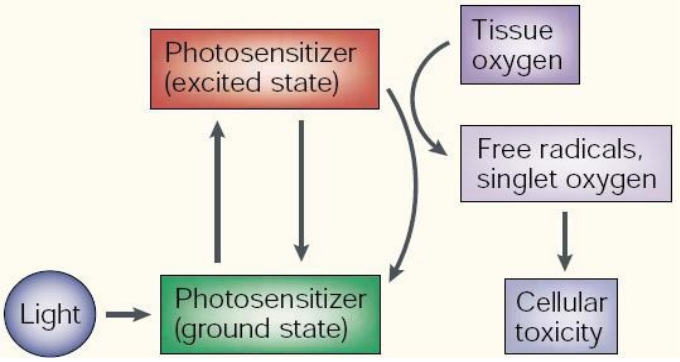


Figure 1: The principle of Photodynamic Therapy

Most of the photosensitizers used in therapeutic applications are derived from natural substances, especially from porphyrin like Haem or Chlorophyll and thus are called Haematoporphyrines or Chlorines [3]. Additionally, there are other natural substances like Curcumin, Hypericin or Riboflavin available which are effective and natural photosensitizers and that can also be stimulated according to their light absorption spectrum [4].

Anti-viral Photodynamic Therapy

It has already been known for a long time that PDT cannot only target and destroy tumor cells but also bacteria, viruses and any other types of microbes. Antimicrobial Photodynamic Therapy (also called aPDT) is a treatment option based on the combination of a photosensitizer that is selectively localized in the target tissue and the application of light of an appropriate wavelength to activate the photosensitizer, resulting in photodamage and cell death. Exposure of the photosensitizer to light of a specific wavelength will lead to light absorption by the photosensitizer, thereby lifting it from a short-lived (nanoseconds) state to an excited singlet electronic state. The singlet-state photosensitizer can then undergo an electronic transition to a much longer-lived (microseconds) triplet state. The longer lifetime allows the triplet photosensitizer to react with ambient (ground state) oxygen by one of two different photochemical pathways, called Type 1 and Type 2 photochemical pathway. Type 1 involves an electron transfer to produce superoxide radicals and then hydroxyl radicals, while Type 2 involves energy transfer to produce excited state singlet oxygen. Superoxide, hydroxyl radicals and excited state singlet oxygen are highly reactive oxygen species (ROS) that can damage nearly all types of biomolecules (proteins, lipids and nucleic acids) and kill cells by inducing irreparable oxidative damages [5]. These processes are called photodamage. Typical examples for those damages are the inhibition of protein synthesis and molecular alterations of DNA strands through DNA-protein cross-linking or strand breaks. These processes alter the transcription of the genetic material during its replication (mutagenic effect) and finally lead to microbial death [6]. The killing of microbial cells via PDT is rapid and only takes a few seconds (while the action of antibiotics or other medications can take hours or days.

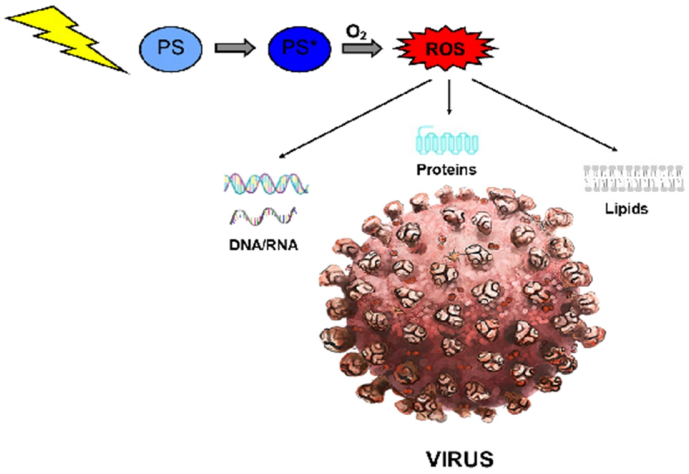


Figure 2: The mechanism of anti-viral Photodynamic Therapy:

Light stimulation boosts the photosensitizer into the excited state (PS*). The reaction with oxygen leads to reactive oxygen species (ROS) which can damage the virus proteins, lipids and nucleic acids.

Riboflavin: A natural photosensitizer with a long history

Riboflavin, also known as Vitamin B2, is a vitamin found in food. It is often used as a dietary supplement to treat Vitamin B2 deficiency and for the prevention of migraine. It can be applied orally or by injection. It is normally very well tolerated and is even safe in pregnancy [7]. Riboflavin is required by the body for cellular respiration. It was discovered in 1920, isolated in 1933 and first synthesized in 1935. It is on the World Health Organization's list of essential medicines. Riboflavin is available as generic medication and over the counter.

In humans there is no evidence for Riboflavin toxicity produced by excessive intakes, partly because it has lower water solubility than other B-vitamins and absorption becomes less efficient as doses increase [8]. Even when 400 mg of Riboflavin per day was given orally to subjects in one study for 3 months to investigate the efficacy in the prevention of migraine headache, no short-term side effects were reported [9]. Although toxic doses can be administered by injection, any excess at nutritional relevant doses is excreted in stool and urine imparting a bright yellow color when present in large quantities. Vitamin B2, along with other B-complex vitamins, is important for metabolism, where it is a co-factor of enzymes involved in the metabolism and catabolism of proteins, fats, carbohydrates, and purines. In addition, Vitamin B2 is required for the generation of energy. Vitamin B2 is also involved in the metabolism of other B-vitamins, in the production of hormones in the adrenal cortex, and in the body's antioxidant defense system, where it is a co-factor of the glutathione reductase enzyme. Thus, Vitamin B2 is of vital importance during all life stages. It is required for heart, brain and cognitive function, bones, joints and muscles, eyes, skin, immune system, liver, and more [10].

Dosage form, formulation, route of administration and pharmacokinetics

Riboflavin can be administered orally or intravenously.

Oral administration of Riboflavin can be regarded as safe because the extent of absorption in the small intestine is limited. Absorption occurs at a specialized segment of the mucosa; drug absorption is limited by duration of drug's contact with this area. Studies have shown that no more than 25 to 30 mg Riboflavin can be absorbed from a single dose [8]. This makes the oral application very safe. Oral absorption is increased when Riboflavin is taken with a meal. So even with extremely high doses of Riboflavin no side effects can be expected because the excess will be excreted by stool. The amount of absorption can be calculated from urine excretion which contributes to 50% of the overall removal of Riboflavin from the plasma. After a single oral dose, biologic half-life is about 66 to 84 minutes in healthy people. Riboflavin is widely marketed as a food supplement and available doses in the formulations vary from 100 – 400 mg. Pure Riboflavin should be replaced by Riboflavin-5-phospate which has much better water solubility and is the active form of Riboflavin. We recommend and used a safe dose of 100 mg Riboflavin-5-phosphate in this study for both systemic and local effects.

Data and literature supporting the proposed use of PDT with Riboflavin for Covid-19 treatment

The potential role of Riboflavin in the photoinactivation of microorganisms and viruses has been known for over half a century [6, 11]. It is still an intensely investigated field, especially in the decontamination of blood products [12-15]. Due to the extensive knowledge of this naturally occurring compound, Riboflavin has been qualified by the US FDA as GRAS (Generally Recognized as Safe). It binds to the nucleic acid bases of virus RNA and upon UV-irradiation, specifically oxidizes the guanine bases in nucleic acids by a single electron transfer reaction. In follow- up reactions, ½ O_2, hydrogen peroxide and hydroxyl radicals are formed. This results in irreversible single strand breaks in nucleic acids with damaging of the pathogens. Riboflavin PDT has been shown to be effective against enveloped as well as several non-enveloped viruses – including HIV, West Nile virus, VSV, IAV, porcine parvovirus, pseudorabies virus, human hepatitis A virus (HAV), encephalomyocarditis virus, Sindbis virus, the MERS coronavirus among others [6]. Riboflavin is the active photosensitizer in the MIRASOL Pathogen Reduction Technology System (Terumo BCT, Lakewood, CO, USA), which is used to treat platelet and plasma products [14]. Moreover, it is also in use for pathogen reduction in whole blood [16]. A new study published in the US in April 2020 showed that Covid-19 virus in plasma products can be eliminated below the limit of detection in a short time with Riboflavin and UV light [17, 18]. At this point we want to emphasize that other substances like Chlorines, Haematoporphyrines, Porphycenes, Curcumin, Methylene Blue or Chlorophyllin can work as photosensitizers as well; however, substances like Haematoporphyrines and Chlorines need to be applied by injection and are very expensive while Curcumin or Methylene Blue could only be applied locally without any systemic effects because of their low resorption in the intestine [6]. Therefore, we decided for Riboflavin as it is cheap, non-toxic, and well absorbed. Another big advantage is that it works with blue and UVA light. Both wavelengths and especially UVA have already an inhibitory effect on viruses and microorganisms and fulfill all safety requirements [18].

Safety requirements for in vivo use and rationale for UVA/blue light usage

The above-mentioned studies on virus elimination in blood products have all been performed in vitro [12-15]. The applied light for Riboflavin stimulation in the in vitro studies was produced in UV lamps that deliver a broad spectrum of UV light including UVC, UVB and UVA light. Range of UVC-light is from 100 – 280 nm, UVB from 280 – 315 and UVA from 315 – 400 nm. The Mirasol system for cleaning blood products from viruses is CE approved but uses UV light with wavelengths between 280 and 400 nm (UVA and UVB together). Other studies showed effective virus inactivation by high energy UVB/UVC light which is also used in sterilization boxes (i.e., for mobile phones) for home-use nowadays. However, such an approach with UVB/UVC light cannot be used in humans as such shorter wavelengths in the UVC and UVB spectrum with high energy are known to be potentially dangerous for cancer development or damaging healthy cells. A possible explanation for the usage of such wavelengths is that all studies have been done in vitro; thus, it was not necessary to worry about potential side effects in humans.

Due to those safety issues, we developed a new and safe protocol based on photodynamic mechanisms with Riboflavin as photosensitizer in combination with UVA/blue light stimulation. By using a photodynamic process, lower light energy with higher wavelengths is necessary to trigger virus inactivation. This approach is safe for in vivo applications as no side-effects should be expected.

According to the absorption spectrum of Riboflavin-5-phosphate maximum absorption and stimulation can be found at 375 and 447 nm (fig.4). In a new study from Rezaie et al. from August 2020 [18] all safety issues of narrow banded UVA light including cell, animal and histological studies were investigated carefully. They showed no relevant risk for clinical application in humans.

Additionally, blue light is absorbed by erythrocytes especially through thin mucous membranes in mouth and throat without pigmentation. Erythrocytes carry oxygen and nitric oxide (NO) in a complex. Irradiation of the erythrocytes with blue light releases free nitric oxide which leads to quick vasodilatation of the blood vessels [19]. This effect can be regarded as an additional benefit for thrombus and embolism protection [20]. The benefit of NO for Covid-19 treatment is currently under investigation in different studies. Thus, the light system in our presented study was equipped with multiple light diodes with a combination of 375 nm narrow banded UVA and 447 nm blue wavelengths for maximum safety and therapeutic efficacy.

Photodynamic process by interaction of Riboflavin with light:

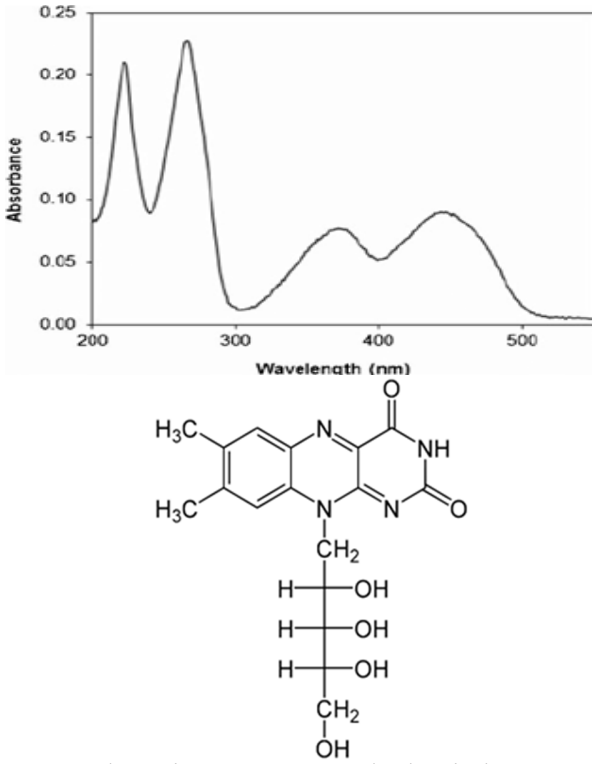


Figure 3: Absorption spectrum and chemical structure of Riboflavin in the literature

Figure 3 shows that Riboflavin absorbs light in the UVC-, UVB-, UVA-and blue range. For treatment in humans, we must neglect the UVB and UVC peaks with 220 and 280 nm because of risk of tissue damage or cancer development.

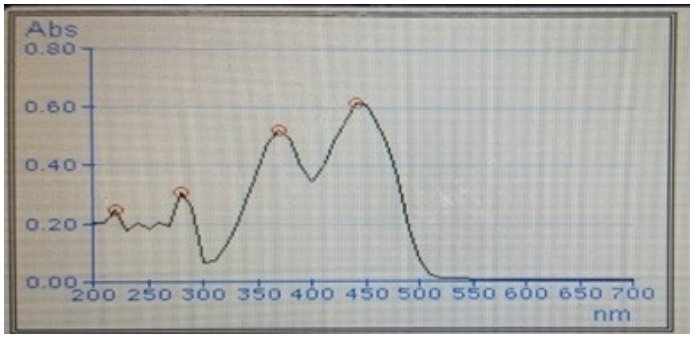


Figure 4: Physiological absorption spectrum of Riboflavin-5-phosphate used in this study

Fig.4 shows a different spectrum of Riboflavin-5-phosphate from Ultra Botanica (USA) with higher peaks in UVA and blue range. This formulation has been used in our presented study.

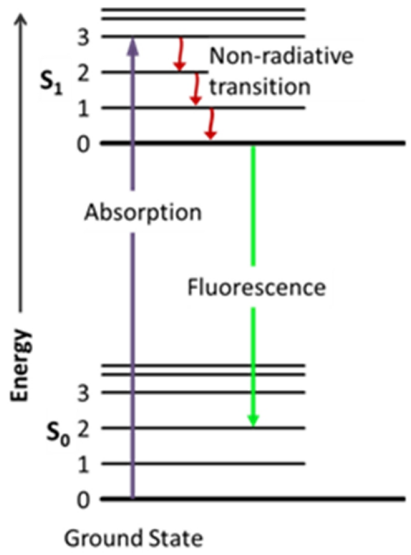


Figure 5: Fluorescence effect after activation

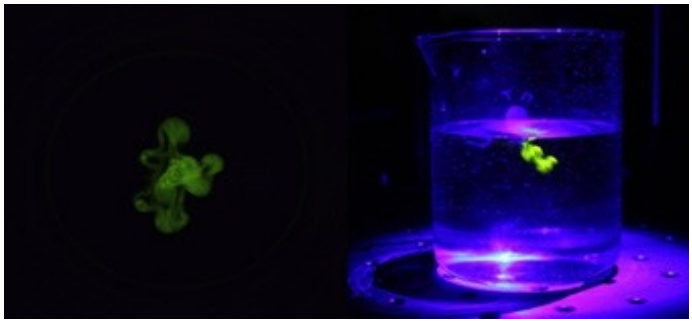


Figure 6: Fluorescence of Riboflavin with blue light

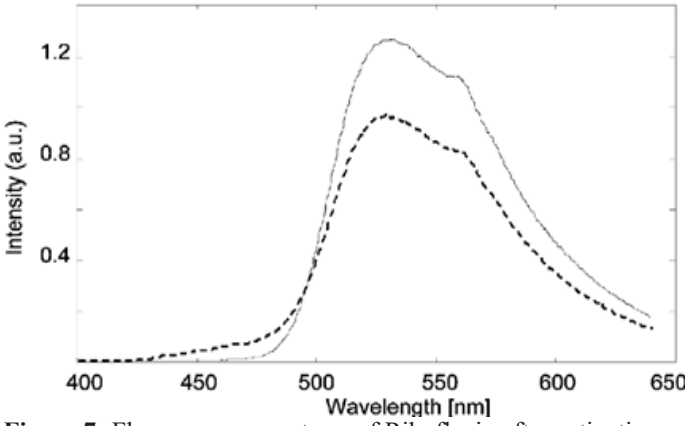
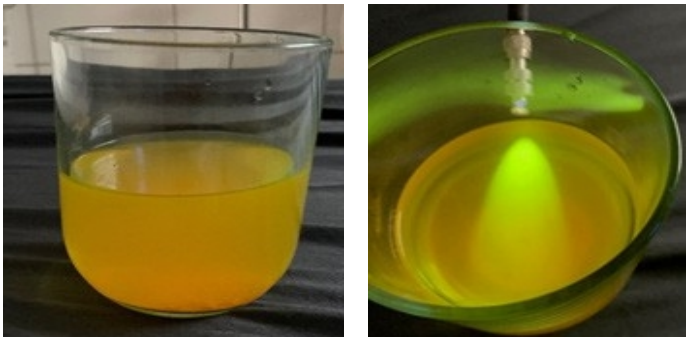


Figure 7: Fluorescence spectrum of Riboflavin after activation with UVA/blue light

After activation of the photosensitizer with light according to its absorption spectrum a different wavelength of light (color) is emitted as fluorescence. This fluorescence effect can be used in diagnostics.

Fig 8 and 9 show the fluorescence effect of Riboflavin after activation with UV and blue light.



a b

c

Figure 8a: Yellow Riboflavin solution in water
Figure 8b: Stimulation with UVA 375 nm with green fluorescence 532 nm
Figure 8c: Stimulation with Blue 447 nm with green fluorescence 532 nm

Figure 8 a,b,c show the fluorescence effect of Riboflavin by irradiation with UVA (375 nm) and blue (447 nm) light which leads to a fluorescence response with green light (532 nm). The same effect can be shown on a human tongue (Figure 9).

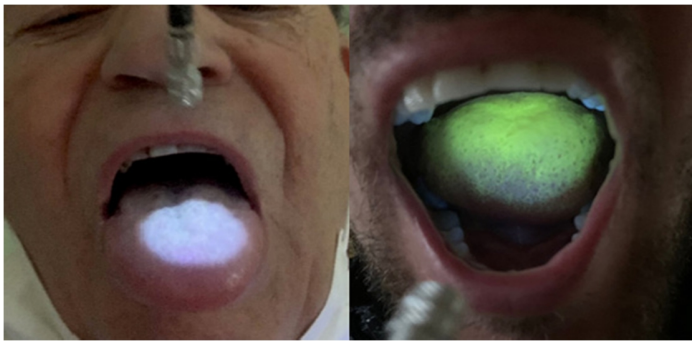


Figure 9: UV 375 nm on the tongue before and after spraying a Riboflavin-5-phosphate solution on the tongue

Materials and methods

Riboflavin-5-phosphate (100 mg capsules) was provided by Ultra Botanica (Oklahoma, USA). All analysis certificates and product specifications were provided by Ultra Botanica. The anti-viral equipment was developed and provided by W Medical Systems GmbH (Germany) and consists of a small aluminum suitcase with

- 60 Riboflavin-5-phosphate 100 mg capsules
- 1 Light treatment device ("Spectra Watch" with 4 red laser diodes and 2 blue, 2 green and 2 yellow LEDs) for systemic treatment of blood via the wrist arteries
- 1 spray bottle with mouth and nose applicator
- 1 nose treatment applicator with 1 blue LED (447 nm) and 1 UVA LED (375 nm)
- 1 mouth treatment applicator with 14 blue LEDs (447 nm) and 14 UVA LEDs (375 nm)

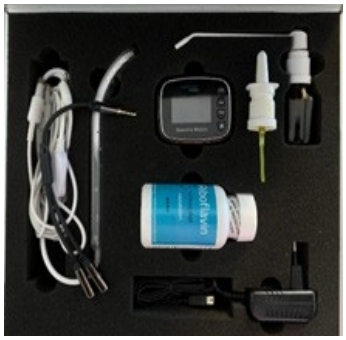


Figure 10: Antiviral equipment box

Figure 11: Riboflavin-5-phosphate

Figure 12: Spray bottle

a b

Figure 13a: Laser/LED watch front side

Figure 13b: Laser/LED watch back side

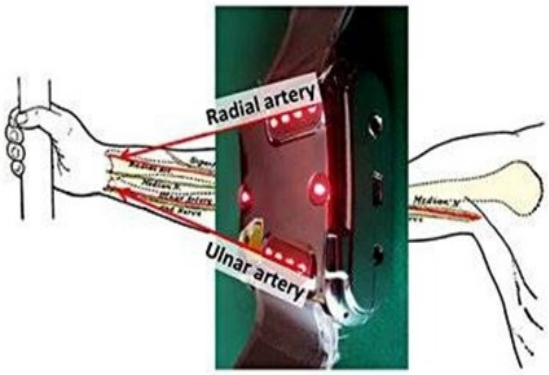
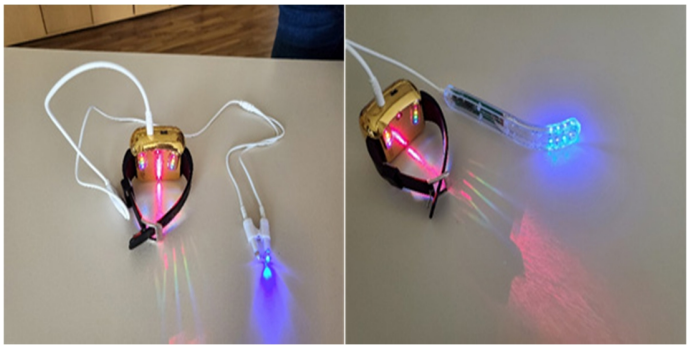


Figure 14: Spectra Laser Watch and systemic irradiation of wrist arteries



a b

Figure 15a: Laser/LED watch with connected nose applicator

Figure 15b: Laser/LED watch with connected mouth applicator

a b

Figure 16a: 20-minute treatment with nose applicator

Figure 16b: 20-minute treatment with mouth applicator

Light treatment kit and rational for use

As we know, the infection route in Covid-19 patients starts in the upper respiratory tract. The light treatment kit used in this study is targeting the nose, mouth, and throat area as well as the patient's circulating blood. Local treatment of the respiratory tract is done by special applicators for nose and mouth/ throat: The nose applicator contains 1 blue LED (447 nm) and 1 UVA LED (375 nm) while the mouth/throat applicator contains 14 blue LEDs (447 nm) and 14 UVA LEDs (375 nm). The diodes in this applicator are built in a way that light radiates upwards, downwards, lateral and to the front. Due to the curved end, it is possible to irradiate deeply into the throat.

The Spectra Laser Watch for systemic treatment is equipped with 4 red laser diodes (658 nm), 2 green LEDs (532 nm), 2 blue LEDs (447 nm) and 2 yellow LEDs (589 nm). The device is fixed on the wrist so that the light can penetrate the arteries (Figure 14) for systemic effects of the different wavelengths and stimulation of the circulating Riboflavin.

Litscher et al. could demonstrate an improved microcirculation after usage of the laser watch with improved oxygen supply [22]. Other effects like improved immune stimulation and anticoagulation, anti-inflammatory effects, elevated nitric oxide and improved oxygen uptake by erythrocytes can be derived from research data on intravenous laser therapy [23, 24]. However, we decided for a non- invasive approach as we were looking for an effective home-use treatment tool.

Most important in this context is the systemic stimulation of the circulating Riboflavin by the blue diodes (447 nm). We know today that Covid-19 infections also affect the inner organs like liver, kidney, heart, and brain, sometimes without or low respiratory symptoms. Hagman et al. could show that the risk of critical disease and mortality correlates with the amount of virus RNA detected in the blood stream [25]. So Photodynamic Therapy of the blood via the wrist arteries might have a preventive effect on disease progression to critical state.

Treatment protocol

1. Take 1 capsule Riboflavin-5-phosphate (100 mg) with a meal for systemic application.

2. Open a second capsule (100 mg) and dissolve it in a glass of 200 ml of water (for local application in nose, mouth, and throat).
3. After 1 hour fix the light treatment device ("Spectra Watch") on the wrist and switch on to 60 minutes (for additional systemic effects).
4. After 15 minutes fill up the spray bottle with the dissolved Riboflavin solution and spray 3 times into both nostrils.
5. Flush the mouth three times with the rest of the solution of the glass with gargling (and drinking is recommended).
6. After 15 minutes, attach nose and mouth applicator to the laser watch and treat each nostril for 10 minutes with blue and UVA light (switch sides after 10 minutes) and inside the mouth and throat for 20 minutes.

Study design

Definition of infection stages for our study rationale

Stage 1: In the first step after infection the Covid-19 virus is bound and detected in cells and tissue of the upper respiratory tract. At this time the QPCR test already shows a positive result but the patient is still asymptomatic.

Stage 2: In this stage (normally after 5-7 days) typical symptoms occur: Sore throat, fever, coughing and loss of smell and taste. The infection is still in the upper respiratory tract but may also start to infect the bronchial system and lung.

Stage 3: In case of progressed disease with lung infection patients usually need to receive oxygen supply; however, without artificial respiration or intubation.

Stage 4: This stage refers to patients in hyperinflammation phase with acute respiratory distress syndrome (ARDS) and other organ failures. Patients are usually intubated and receive artificial respiration.

The treatment presented in this study is recommended in early cases of stage 1 and 2 where the treatment can be done by the patient at home or by supervision of a local physician for prevention of hospitalization, intubation, artificial respiration, and late disease complications.

The focus of the applied treatment protocol is on treating the infected areas like nose, mouth, and throat. Additionally, we achieve a systemic effect by applying light into the wrist arteries with the "laser watch". PDT could also be administered in stage 3 or 4 patients with a different protocol by applying Riboflavin and light directly in the intrabronchial system by special fiberoptic technology. This is under investigation as well and should be discussed further in another publication.

Patient selection and study protocol

Inclusion criteria: Men and women, 10-90 years, stage 1 or 2 according to definition above. Exclusion criteria: Stage 3 or 4 patients, pregnant women, children below 10 years.

For this study 20 stage-2 patients (according to the definition above) were chosen as experiment (verum) group. All of them had positive QPCR test results and Covid-19 related symptoms like fever, sore throat, coughing etc.; none of them had to be supplied with oxygen at that time. 13 women and 7 men with age between 18 and 80 years were enrolled into the study.

On day 1 an extensive clinical evaluation took place including QPCR test and medical questionnaire. Afterwards the first PDT treatment was initiated. On the following 4 days QPCR tests were done every day before the PDT treatment including Ct (cycle threshold) value for viral load evaluation. The patients received a total of 5 PDT treatments within 5 days. The last tests were done on the 5th and 7th day.

20 additional patients under care of the same clinic served as a control group for this study (12 women and 8 men, with age between 22 and 77 years). They were supplied with conventional treatment modalities according to the recommended standards for Covid-19 management; none of those patients received PDT additionally. This group was also evaluated by QPCR and medical questionnaire.

QPCR and Ct values were done by Omnid Hospital laboratory in Teheran, Iran.

Results

Clinical symptoms of patients from the experiment group before PDT treatment are summarized in table 1. 18 out of 20 (90%) patients in the experiment group had fever before PDT treatment. 17 out of 20 (85%) had a dry cough and 9 out of 20 (45%) suffered from headache. 19 out of 20 (95%) reported chest pain/pressure. All 20 (100%) patients suffered from tiredness. 11 out of 20 (55%) had sleep disorder and 18 out of 20 (90%) had lost their sense of taste or smell.

Table 2 shows the post treatment symptoms of the patients from the experiment group (after 5 treatment days): No (0 out of 20) patient had fever anymore. Only 6 (30%) reported a dry cough and 5 (25%) still suffered from headaches. The pain conditions improved in 14 (70%) patients, while only 5 (25%) still reported chest pain/pressure. Tiredness decreased in 8 (40%) patients, with 12 people still feeling fatigue. In 4 (20%) patients sleep quality improved. The loss of taste or smell remained in all 18 (90%) affected persons.

Table 1: Symptoms of 20 patients in the experiment (verum) group before therapy

Experiment group			SYMPTOMS						
Gender	Case number	FEVER	DRY COUGH	HEADACHE	HARD BREATHING, CHEST PAIN OR CHEST PRESSURE	FATIGUE OR TIREDNESS	SLEEP DISORDER	LOSS OF TASTE OR SMELL	How many days before did the first symptoms occur?
F	1	■	■		■	■	■	■	5
M	2	■	■			■		■	7
F	3	■	■		■	■		■	5
F	4	■	■		■	■	■	■	3
F	5	■	■		■	■	■	■	3
F	6	■		■	■	■	■	■	4
M	7	■	■	■	■	■	■	■	7
F	8	■	■		■	■		■	7
M	9	■	■		■	■			7
M	10	■	■		■	■		■	8
M	11	■	■		■	■		■	3
F	12	■	■	■	■	■	■	■	2
M	13				■	■		■	4
F	14	■			■	■			3
F	15	■		■	■	■	■	■	4
F	16	■	■	■	■	■	■	■	5
F	17		■	■	■	■	■	■	2
F	18	■	■	■	■	■	■	■	4
M	19	■	■	■	■	■		■	5
F	20	■	■	■	■	■	■	■	3

Table 2: Symptoms of the experiment (verum) group after 5-day PDT

Experiment group			SYMPTOMS					
Gender	Case number	FEVER	DRY COUGH	HEADACHE	HARD BREATHING, CHEST PAIN OR CHEST PRESSURE	FATIGUE OR TIREDNESS	SLEEP DISORDER	LOSS OF TASTE OR SMELL
F	1		■		■	■	■	■
M	2					■		■
F	3							■
F	4						■	■
F	5		■				■	■
F	6			■				■
M	7		■	■				■
F	8					■		■
M	9					■		
M	10				■	■		■
M	11		■		■	■		■
F	12		■	■		■	■	■
M	13					■		■
F	14							
F	15			■				■
F	16			■	■			■
F	17				■	■	■	■
F	18					■	■	■
M	19					■		■
F	20		■			■	■	■

Table 3.: Test results with Ct values in the experiment (verum) group

Experiment group			QPCR CYCLE THRESHOLD VALUE							
AGE	Gender	Case number	PRE DAY 0	AFTER DAY 1	AFTER DAY 2	AFTER DAY 3	AFTER DAY 4	AFTER DAY 5	DAY 6 NO TREATMENT	DAY 7
49	F	1	+	37.2	38.9	Negative	Negative	Negative		Negative
52	M	2	+	35.2	34.5	36.8	37.8	Negative		Negative
24	F	3	+	28.3	30.3	35.7	35.1	Negative		Negative
18	F	4	+	35.1	39.3	Negative	Negative	Negative		Negative
37	F	5	+	28.3	30.5	32.2	35.2	Negative		Negative
27	F	6	+	29.8	30.2	31.2	33.4	Negative		Negative
40	M	7	+	29.7	29.2	35.4	37.2	Negative		Negative
57	F	8	+	29.2	29.2	34.3	37.8	Negative		Negative
50	M	9	+	22.7	27.3	25.6	30.1	33.7		35.1
31	M	10	+	27.1	30.2	32.2	36.7	Negative		Negative
53	M	11	+	23.9	24.6	27.7	34.4	35.1		34.1
80	F	12	+	34.6	Negative	Negative	38.8	39.1		Negative
39	M	13	+	34.1	35.7	38.8	39.1	Negative		Negative
25	F	14	+	24.1	26.6	29.7	35.7	Negative		Negative
50	F	15	+	26.7	28.8	33.4	37.2	Negative		Negative
67	F	16	+	35.5	34.3	36.7	38.1	Negative		Negative
39	F	17	+	35.4	37.1	38.9	39.2	Negative		Negative
45	F	18	+	31.2	32.9	35.4	35.5	37.2		38.1
39	M	19	+	21.4	25.2	23.4	26.9	32.2		34.2
37	F	20	+	23.6	27.9	25.4	26.2	29.7		30.3

QPCR Cycle Threshold measurements

What does Ct mean? In a real-time PCR assay a positive reaction is detected by accumulation of a fluorescent signal. The Ct (Cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold (i.e., exceeds background level). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (i.e., the lower the Ct level the greater the amount of target nucleic acid in the sample). Real time assays undergo 40 cycles of amplification. Interpretation of the Ct is as follows:

Ct < 29 are strong positive reactions indicative of abundant target nucleic acid in the sample
Ct of 30-37 are positive reactions indicative of moderate amounts of target nucleic acid
Ct > 38 = A marker of probable very low levels of virus in the specimen, near the levels to undetectable
Negative: Indicate that the pathogen may present at a level below the lower limit of this assay

Results

The result of the QPCR (cycle threshold) measurements are summarized in table 3 for the experiment group. All patients in this group had a positive pre-treatment test:

Between day 1 and 3 the average cycle threshold value increased by 3.99 between.

Ct increased further between day 3 and 5 by 4.98.

On day 5, 14 out of 20 patients had a cycle threshold value below detection limits.

Table 4: Symptoms of the control group first day

control group			SYMPTOMS						
Gender	Case number	FEVER	DRY COUGH	HEADACHE	HARD BREATHING, CHEST PAIN OR CHEST PRESSURE	FATIGUE OR TIREDNESS	SLEEP DISORDER	LOSS OF TASTE OR SMELL	How many days before did the first symptoms occur?
F	1		■	■	■	■		■	6
F	2	■	■	■	■	■	■	■	3
F	3	■	■		■	■		■	4
F	4	■	■		■		■	■	5
F	5	■	■	■	■		■	■	3
F	6	■	■		■	■		■	3
M	7		■		■	■	■		5
F	8		■	■	■	■	■	■	3
F	9	■	■	■	■	■			7
M	10	■		■	■			■	9
M	11	■	■	■	■	■	■	■	3
M	12	■	■		■	■	■	■	3
F	13	■	■	■	■	■	■	■	4
F	14	■	■		■	■	■	■	7
F	15	■		■	■	■	■	■	2
F	16	■	■	■	■	■	■	■	5
M	17	■	■	■	■	■	■	■	3
M	18	■	■		■	■		■	6
M	19		■	■	■	■		■	5
M	20		■	■	■	■		■	3

Clinical symptoms of patients from the control group on day 1 are summarized in table 4: 15 out of 20 (75%) patients in the control group had fever on day 1. 18 out of 20 (90%) had a dry cough and 13 out of 20 (45%) suffered from headaches.

All patients reported chest pain/pressure. 17 out of 20 (85%) patients suffered from tiredness. 12 out of 20 (60%) had sleep disorder and 18 out of 20 (90%) had lost their taste or smell.

Table 5: Symptoms of the control group after 5 days without PDT

control group			SYMPTOMS					
Gender	Case number	FEVER	DRY COUGH	HEADACHE	HARD BREATHING CHEST PAIN OR CHEST PRESSURE	FATIGUE OR TIREDNESS	SLEEP DISORDER	LOSS OF TASTE OR SMELL
F	1		■	■	■	■		■
F	2							
F	3					■		■
F	4		■		■		■	■
F	5	■	■	■	■		■	■
F	6	■	■		■	■		■
M	7		■		■	■	■	
F	8							
F	9	■	■	■		■		
M	10	■		■				■
M	11		■	■	■	■	■	■
M	12		■			■	■	■
F	13	■	■	■	■	■	■	■
F	14	■	■		■	■	■	■
F	15	■		■	■	■	■	■
F	16	■	■	■	■	■	■	■
M	17	■	■	■	■	■	■	■
M	18	■	■		■	■		■
M	19		■	■	■	■		■
M	20		■	■	■	■		■

Table 5 shows the clinical symptoms of the patients from the control group after 5 days of conventional care: 2 of the patients had to be hospitalized within the 5 days and left the control group. 10 out of 18 (56%) patients still had fever after 5 days. 15 out of 18 patients (83%) were still suffering from dry cough and 11 out of 18 (61%) from headaches. The pain conditions got better in 4 (22%) patients, with 14 (78%) still reporting chest pain/pressure. Tiredness was still affecting 15 out of 18 (83%) patients. 10 out of 18 patients still had sleep disorder (56%). The loss of taste or smell remained in all affected persons.

Table 6: Test results with Ct values of the control group without PDT

			QPCR CYCLE THRESHOLD VALUE							
AGE	Gender	Case number	PRE DAY 0	AFTER DAY 1	AFTER DAY 2	AFTER DAY 3	AFTER DAY 4	AFTER DAY 5	DAY 6	DAY 7 AFTER PDT
55	F	1	+	21.3		22.1		26.2		28.4
45	F	2	+	18.4	ICU					
57	F	3	+	27.8		28.4		31.9		33.2
55	F	4	+	30.5		31.5		32.6		34.1
77	F	5	+	21.8		21.1		21.7		30.1
73	F	6	+	24.6		24.6		23.2		23.3
75	M	7	+	24.1		25.6		25.1		-
45	F	8	+	15.4	ICU					
23	F	9	+	21.6		20.3		20.8		-
34	M	10	+	26.1		25.5		26.2		-
55	M	11	+	22.7		22.7		21.3		-
54	M	12	+	21.6		18.7		24.8		27.3
22	F	13	+	29.4		30.4		32.1		35.2
35	F	14	+	31.5		32.0		31.1		-
45	F	15	+	33.2		34.1		37.0		-
33	F	16	+	26.7		26.6		28.1		-
45	M	17	+	23.1		22.1		21.9		-
46	M	18	+	30.4		28.8		28.2		-
51	M	19	+	30.9		30.6		30.6		-
27	M	20	+	31.3		30.2		31.2		-

Table 6 shows the results of the QPCR (cycle threshold) measurements of the control group after 5 days of conventional care. All patients in this group had a positive pre-treatment test. 2 of the patients had to be hospitalized within the 5 days and left the control group. The other 18 patients showed no significant improvements: Between day 1 and 3 the average cycle threshold value increased by 0.37, between day 3 and 5 by 0.48. All patients still had a positive QPCR test result after 5 days.

A post verum treatment after 5 days testing was offered to all patients of the control group with 7 people accepting the PDT treatment. Improvement could be shown in all 7 patients.

Discussion

The efficacy of Photodynamic Therapy (PDT) against several viruses including SARS-CoV-2 could be shown in several in-vitro experiments over the last couple of years [6, 12-18]. A newly designed PDT treatment kit had been developed in early 2020 for in vivo application of Photodynamic Therapy against viral infections. Several patients with influenza and infections of the upper respiratory tract were treated in the author's clinic with immediate clinical improvements in summer and fall 2020. Our presented study is the first practical approach in using PDT against SARS-CoV-2. The results indicate high effectiveness in improving clinical symptoms and reducing viral load in patients suffering from early stages of Covid-19.

While no significant improvements with conventional care could be observed in the control group, all 20 patients from the experiment group had a significant reduction of clinical symptoms like fever, dry cough, chest pain etc. Furthermore, viral load assessment by QPCR showed very encouraging results with 70% tested negatively after 5 days and the other 30% showing a significant reduction of viral load to low or moderate levels. PDT also proved to be successful in preventing hospitalization and intensive care treatments.

Additionally, we must emphasize that PDT led to no side-effects at all; this is in accordance with all the in-vitro and safety data for Riboflavin-based Photodynamic Therapy. Another advantage of PDT is the simple and cheap application which can be done even at home by the patient in early disease stages. This would not only relieve the hospitals with their intensive care units but also prevent excessive expenses for health insurances and damage of global economy (due to economic and social lockdowns). Conventional medication shows only limited success in prevention of severe Covid-19 infections. Latest developments of new vaccinations seem to be promising; however, vaccination is a preventive approach and not a therapy for acutely infected patients. Furthermore, there are many questions still to be answered regarding vaccinations (especially new mRNA vaccines); i.e., length of immune protection and long-term side effects.

However, our therapeutic approach is not considered to be in competition with vaccinations. The combination of both preventive vaccinations and therapeutic interventions like Photodynamic Therapy (PDT) for infected people is necessary for successful management of this global pandemic. Additionally, there is potential that Photodynamic Therapy might also lead to a natural vaccination effect. Further investigations to check if IgG or CD8+T cells will be developed by our immune system after PDT are already under progress. Because of the encouraging presented data additional studies with lager patient populations will be initiated shortly.

Conclusion

The presented study shows first evidence for a successful treatment of Covid-19 patients in early stages of disease through Photodynamic Therapy. The applied protocol with a combination of oral Riboflavin, UVA and blue light proved to be effective in reducing viral load of patients and in improving clinical symptoms significantly.

This therapeutic intervention is suitable for early stages of infection in the incubation phase after positive testing but also when first clinical symptoms like fever, sore throat or breathing problems arise. Consequently, it is expected to accelerate recovery and prevent patients from getting into advanced stages.

The procedure can also be used in a preventive manner after contact with infected people or after meetings with many people where the risk of getting infected is potentially high. If used widely this Photodynamic Therapy approach can assist in lowering Covid-19 cases in addition to vaccinations and other treatments. Since not all patients of the world can be vaccinated quickly and the long-term success is still unclear it is an efficient treatment tool that everybody can use at home.

Acknowledgement: We cordially thank Dr. Alireza Najafi, Dr. Masoumeh Shabani, Dr. Sadeghi Tabar, Dr. Kalanaki, Dr. Harati, Dr. Seyed Ali Golestanha, Mr. Nima Kazemi and Dr. Mojgan Mirakhorli for their great contribution to this study.

References

1. Allison RR, Sibata CH (2010) Oncologic photodynamic therapy photosensitizers: A clinical review. Photodiagnosis and Photodynamic Therapy 7: 61-75.
2. Gitika B Kharkwal, Sulbha K Sharma, Ying-Ying Huang, Tianhong Dai, Michael R Hamblin (2011) Photodynamic Therapy for Infections: Clinical Applications. Lasers Surg Med 43: 755-767.
3. Ron R Allison, Gordon H Downie, Rosa Cuenca, Xin-Hua Hu, Carter Jh Childs, et al. (2004) Photosensitizers in clinical PDT. Photodiagnosis and Photodynamic Therapy 1: 27-42.
4. Nittiya Suwannasom, Ijad Kao, Axel Pruss, Radostina Georieva, Hans Bäumler (2020) Riboflavin: The Health benefits of a forgotten natural Vitamin. International Journal of Molecular Sciences 950: 1-22
5. Xiaoqing Hu, Ying-Ying Huang, Yuguang Wang, Xiaoyuan Wang, Michael R Hamblin (2018) Antimicrobial Photodynamic Therapy to Control Clinically Relevant Biofilm. Infections. Front. Microbiol 9.
6. Arno Wiehe, Jessica M O'Brien, Mathias O Senge (2019: Trends and targets in antiviral phototherapy. Photochem. Photobiol Sci 18: 2565-2612.
7. Unna K, Greslin JG (1942) Studies on the toxicity and pharmacology of riboflavin. J Pharmacol Exp Ther 76: 75-78.
8. Janos Zempleni, John R Galloway, Donald B McCormick (1996) Pharmacokinetics of orally and intravenously administered riboflavin in healthy humans. American Journal of Clinical Nutrition 63: 54-66.
9. Boehnke C, Reuter U, Flach U, Schuh-Hofer S, Einhäupl KM, et al. (2004) High-dose riboflavin treatment is efficious in migraine prophylaxis: an open study in a tertiary care centre. European Journal of Neurology 11: 475-477.
10. Hillary J Powers (2003) Riboflavin (vitamin B-2) and health. The American Journal of Clinical Nutrition 77: 1352-1360.
11. M Wainwright (2004) Photoinactivation of viruses. Photochem. Photobiol Sci 3: 406-411.
12. E Ben-Hur, RP Goodrich (2011): Pathogen Reduction in Blood for Transfusion Using Photodynamic Treatments. In:
M. R. Hamblin and G. Jori (Eds.): Photodynamic Inactivation of Microbial Pathogens. Medical and Environmental Applications, RSC Publishing, Cambridge, UK 2011: 235- 263.
13. P Schlenke (2014) Pathogen Inactivation Technologies for Cellular Blood Components: An Update, Transfus. Med Hemother 41: 309-325.
14. Shawn D Keil, Richard Bowen, Susanne Marschner (2016) MERS-CoV in plasma products using a riboflavin-based and ultraviolet light-based photochemical treatment, Transfusion 56: 2948-2952.
15. R Schuyler (2001) Use of riboflavin for photoinactivation of pathogens in blood components, Transfus. Apher Sci 25: 189- 190.
16. Goodrich Raymond (2011) Pathogen Reduction Technology Treatment of Platelets, Plasma and Whole Blood Using Riboflavin and UV Light. Transfusion medicine and Hemotherapy 38: 8-18.
17. Shawn D Keil, Izabela Ragan, Susan Yonemura, Lindsay Hartson, Nicole K Dart, et al. (2020) Inactivation of severe acute respiratory syndrome coronavirus 2 in plasma and platelet products using a riboflavin and ultraviolet light-based photochemical treatment. Vox sanguinis 6: 495-501.
18. Ali Rezaie, Gabriela G S Leite, Gil Y Melmed, Ruchi Mathur, Maria Jesus Villanueva-Millan, et al. (2020) Ultraviolet A light effectively reduces bacteria and viruses including coronavirus. PLoS ONE 15: 1-11.
19. Rainer Mittermayr (2007) Blue Laser Light Increases Perfusion of a Skin Flap Via Release of Nitric Oxide from Hemoglobin. Molecular Medicine 13: 22-29.

20. Joseph Loscalzo (1998) The antithrombotic effect of nitric oxide. Vascular endothelium 294: 99-102.
21. Iqbal Ahmad Q, Fasihullah Faiyaz, HM Vaid (2006) Effect of light intensity and wavelengths on photo degradation reactions of riboflavin in aqueous solution. Journal of Photochemistry and Photobiology B, Biology 82: 21-27.
22. Litscher G, Litscher D (2016) A Laser Watch for Simultaneous Laser Blood Irradiation and Laser Acupuncture at the Wrist. Integr Med Int 3: 75-81.
23. VA Mikhaylov (2015) The use of Intravenous Laser Blood Irradiation (ILBI) at 630–640 nm to prevent vascular diseases and to increase life expectancy. Laser Ther 24: 15-26.
24. Ahmad Amjadi, Hossein Mirmiranpour, Seyed Omid Sobhani, Niloofar Moazami Goudarzi (2020) Intravenous laser wavelength radiation effect on LCAT, PON1, catalase, and FRAP in diabetic rats. Lasers in Medical Science volume 35: 131-138.
25. Karl Hagman, Magnus Hedenstierna, Patrik Gille-Johnson, Berit Hammas, Malin Grabbe, et al. (2019) Severe Acute Respiratory Syndrome Coronavirus 2 RNA in Serum as Predictor of Severe Outcome in Coronavirus Disease 2019: A Retrospective Cohort Study Clinical Infectious Diseases: CIAA 1285: 1-7.