Clinical and videocapillaroscopic features and serum concentration of nitric oxide in patients with Raynaud's phenomenon after Multiwave Locked System laser therapy

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ABSTRACT

Purpose: The aim of the study was the assessment of the influence of MLS laser therapy on morphological changes in nailfold videocapillaroscopy (NVC), clinical features, and the serum NO level in patients with primary and secondary Raynaud's phenomenon (RP).

Materials and methods: The analysis was performed on a group of 78 patients with RP and 30 healthy volunteers, who underwent NVC examination. NO concentration was assayed using the Griess method in blood serum before and after 3-weeks of laser biostimulation. MLS was performed with a Laser-M6 ASA Company device, for 3 weeks with weekend breaks, using the following parameters: a frequency of 1500 Hz, a dose of 25 J/cm2, and a time of 2.5 minutes on one hand.

Results: After 3 weeks of MLS laser therapy, the beneficial clinical effects manifested by a decrease of duration and number of RP attacks and degree of pain score on the visual analogue scale (VAS) in patients with primary and secondary RP. Clinical improvement after MLS laser therapy was reflected in the assessment of microcirculation disorders in NVC examination. Moreover, the tendency of normalization of NO concentration in the serum of patients with primary and secondary RP may suggest a favorable effect of laser biostimulation on the regulation of processes taking part in microcirculation disorders.

Conclusions: The results showed that NVC is a useful diagnostic tool in the evaluation of dynamic microvascular involvement in RP patients. MLS laser therapy has a beneficial effect in patients with primary and secondary Raynaud's phenomenon

Keywords: Raynaud's phenomenon, Multiwave Locked System laser therapy, videocapillaroscopy, nitric oxide

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INTRODUCTION

Raynaud's phenomenon (RP) refers to paroxysmal pallor or cyanosis, mainly of the digits of the hands or feet owing to cold-induced vasoconstriction of the digital arteries, precapillary arterioles, and cutaneous arteriovenous shunts [1, 2].Raynaud's phenomenon reflects an exaggeration of normal central and local vasomotor responses to cold or emotion. RP has been classified as primary, occurring as an isolated condition, or secondary, associated to an underlying disease, mainly a connective tissue disease [3].

The pathophysiology of RP is still not completely understood. The following can be observed in the pathogenesis of RP: dysregulation of autonomic and sensitive nerve fibers, functional and structural vessel changes, intravascular alterations [3, 4].

Functional alterations of blood vessel tone can be mediated by endothelium-dependent and/or endothelium-independent factors.

Nitric oxide (NO) plays a critical role in the control of vascular tone. NO is generated constitutively by the endothelium as a messenger molecule which acts as an antithrombotic and cytoprotective agent. NO can also cause tissue damage [5].

The impairment of constitutive NO synthesis and release as a consequence of vascular endothelium injury leads to the dysregulation of vascular tone control and progressive disorganization of vascular architecture [6,7].

The role of NO in rheumatic diseases may be protective and anti-inflammatory or cytotoxic depending on the amount of NO produced and the targets available for action [8].

Clinically, RP can manifest as an isolated, acute or subacute disorder presenting with cold, pain and numbness of the fingers, secondary to ischemia that can lead to ulcerations and/or gangrene [1]. Therefore, early diagnosis of microcirculatory abnormalities and distinction between primary and secondary RP is crucial for better treatment strategy and prognosis of the disease.

Nailfold videocapillaroscopy (NVC) has been proposed as a first-line investigation in the early differential diagnosis between primary and secondary RP [9,10]. Moreover, nailfold capillaroscopy is very helpful as a screening method for detecting patients with RP at high risk for the development of connective tissue disease [11-13].

The first line RP therapy consists of lifestyle changes. A drug-based treatment may lead to adverse events. Surgical treatment is reserved for complicated conditions not responding to pharmacological therapy [14]. Thus, establishing a new approach to improve clinical treatment of RP seems to be necessary.

Multiwave Locked System (MLS) therapy, characterized by a synchronized generation of continuous and pulsed laser light, is a new technique used to increase the effect of laser irradiation [15].

It has been postulated that the analgesic effect of the pulsed emission as well as the anti-edema and anti-inflammatory effects of the continuous irradiation potentiate each other, increasing the intensity of the therapeutic effect on both pain and inflammation [14-16].

The aim of this study was to determine the influence of two-wave MLS laser therapy on the clinical features, microvascular changes, and NO concentration in patients with primary and secondary Raynaud's phenomenon. Additionally, we aimed to evaluate the potential correlation between the concentration of circulating modulator released as a consequence of endothelium injury, such as NO, and microvascular abnormalities detected by NVC.

MATERIALS AND METHODS

Patients

We enrolled 78 patients with RP (75 women and 3 men, mean age 42.6 years) recruited from the Rehabilitation Outpatient Clinic in the Department of Rehabilitation at the Medical University of Bialystok.

All patients were evaluated by extensive clinical and laboratory studies. All RP patients were classified into two groups, 38 patients with primary RP and 40 patients with secondary RP. The patients with primary RP (36 women and 2 men; age range 19-77 years) were diagnosed according to the criteria of LeRoy and Medsger and exhibited exclusively Raynaud's phenomenon without any clinical or laboratory signs of the presence of a systemic autoimmune disease [17].

The individuals with primary RP showed an absence of an NVC scleroderma pattern and negative findings of antinuclear autoantibody assays.

The group of secondary RP (40 patients; 39 women and 1 man; age range 26-66 years) included 28 patients with systemic sclerosis (SSc), six cases with systemic lupus erythematosus (SLE), and six patients with undifferentiated connective tissues disease (UCTD), according to international criteria [18-20].

Exclusion criteria included previous contact with vasodilators or immunosuppressive therapy with cyclosporine or cyclophosphamide. Control samples were obtained from 30 healthy subjects, who had no evidence of RP and matched for sex and age.

The Ethical Committee of the Medical University of Bialystok approved the study, and all participants gave their written informed consent before entry into the examination enrolment (agreement No R-1-002/338/2012).

Clinical and laboratory analysis

Clinical, capillaroscopy, and laboratory examinations were performed on the days of blood sample collection, i.e. one week before the start of the treatment and one week after the last session (one month after the first session) of MLS laser therapy.

The patients were instructed to record any Raynaud's attacks in diaries one week before treatment commencement, during the 3 weeks of MLS therapy, and one week after the end of exposure sessions. The number of attacks, mean duration of Raynaud's attack and severity of pain assessed by means of a 10-cm visual analogue scale (VAS) had to be recorded at bed time.

Clinical, capillaroscopy, and laboratory data were recorded at the time of blood serum collection. The serum samples were stored at -80°C. NO concentration was measured by Griess reaction. Assays were performed following the manufacturer's instructions.

Nailfold videocapillaroscopy (NVC)

Nailfold videocapillaroscopy (NVC) examination was performed in all patients and healthy subjects using a stereomicroscope SZ 4045 (Olympus, Germany) by the same experienced investigator. Cold illumination was provided by a fiber optic light source and filter. Each patient was acclimatized for 20 minutes at room temperature (20-24°C) prior to the examination.

The nailfolds of all fingers except the thumbs and fingers affected by recent local trauma were examined in each patient. To obtain the best visibility of the microvascular network, a drop of immersion oil was placed on the nailfold bed. Only capillaries in the distal row of the nailfold were analyzed. The intensity of the morphological changes was evaluated on the basis of a semiquantitative method that included the following parameters: loop density; capillary length variability (shorter or longer capillaries); percent of loops with architectural derangement, such as tortuous, meandering, enlarged, ramified or bushy capillaries; irregular distribution of the capillary array; and the presence of extravasations in the perivascular tissue. The intensity of capillaroscopic changes was expressed according to a previous classification [21] and ranged from 0 to 3, where: a score of 0 indicated no changes, 1 – mild (25-50% morphologically changed capillaries without perivascular changes), 2 – moderate (50% - 75% morphologically changed loops with diminished loop density, increased visibility of the sub-papillary venular plexus, and without extravasations), and 3 – severe changes (more than 75% morphologically changed loops with the extended architectural derangement of the microvascular network, heterogeneous features of angiogenesis, extravasations into perivascular tissue and extensive visible venous plexus). The mean score for each subject was obtained from analysis of all the fingers.

The diagnosis of RP was based on episodic, thriphase changes of skin color associated with exposure to cold or a stress agent. The data were verified by the induction of symptoms in response to putting hands under a cold water stream for 5 minutes. If there was no response, the hands were put into 4°C water bath with ice for 5 minutes.

MLS laser therapy

Patients with RP received MLS laser (ASA Company) irradiation with the following parameters: intensity 50%, frequency 1500 Hz, energy 129.3 J, procedure time 2.5 minutes on a single hand. Laser energy was administered on the dorsal parts of hands.

Statistical analysis

The significance of differences between the control group and particular patient groups were tested using the Mann-Whitney rank sum test. The Wilcoxon signed rank test was used to evaluate differences before and after MLS laser therapy. The probability of differences in frequency distributions was determined by the chi-square test or Fischer's exact test. The data were correlated by Spearman's rank-order correlation. A *P* value lower than 0.05 was considered statistically significant.

RESULTS

Clinical effects of MLS laser therapy

Table 1 shows the clinical differences between patients with primary and secondary Raynaud's phenomenon prior to MLS laser treatment. In the whole study group, the number of RP attacks during 1 week ranged from 1-76 (mean 19.9), in patients with primary RP from 1-76 (mean 14.2), in secondary RP from 1-75 (25.3), respectively.

The mean duration of a single RP attack was 25.8 min (5-120).

The mean duration of an RP attack in patients with primary RP was longer than in the case of secondary RP (29.6 min vs. 22.1 min.) (Table1).

Pain intensity was assessed by the Visual Analogue Scale (VAS). The mean value in the whole group was 33.5 (0-96), including 27.9 and 38.9 in patients with primary and secondary RP, respectively.

The 3-week MLS laser therapy showed a reduction in the number of RP attacks per week, duration of a single RP attack, and pain assessed by VAS (Table 2).

Table 1. Clinical differences in patients with primary and secondary Raynaud's phenomenon (RP) before MLS laser treatment

Characteristics	RP together (n=78)	Primary RP (n=38)	Secondary RP (n=40)	P value
Sex (M/F)	3/75	2/36	1/39	NS
Age (years) (mean, range)	42.7 (19-77)	36.5 (19-77)	48.9 (26-66)	NS
Disease duration (years, range)	10.9 (1-40)	9.5 (2-40)	12.5 (1-30)	NS
Number of RP attacks per week (mean, range)	19.9 (1-76)	14.2 (1-76)	25.3 (1-75)	0.02
Duration of single RP attack (minutes) (mean, range)	25.8 (5-120)	29.6 (10-120)	22.1 (5-60)	NS
VAS (mm) (mean, range)	33.5 (0-96)	27.9 (0-96)	38.9 (0-87)	NS

F – female; M – male; N - not significant; VAS – Visual Analogue Scale

Table 2. Clinical characteristics of patients with primary and secondary Raynaud's phenomenon (RP) before and after MLS laser treatment

		Primary RP	P value	Secondary RP	P value
Number of RP attacks per week	Before MLS laser therapy	14.2 ± 16.4	<0.001	25.3 ± 21.9	<0.001
	After MLS laser therapy	9.3 ± 9.6		19.7 ± 18.4	
Duration of single RP attack	Before MLS laser therapy	29.6 ± 27.1	<0.001	22.1 ± 18.0	<0.001
	After MLS laser therapy	16.7 ± 13.5		13.5 ± 11.5	
VAS (mm)	Before MLS therapy	27.9 ± 30.4	<0.001	38.9 ± 28.6	<0.001
	After MLS laser therapy	20.6 ± 21.8		29.9 ± 24.3	

Data are presented as mean ± standard deviation; MLS - Multiwave Locked System; VAS – Visual Analogue Scale

Capillaroscopic effects of MLS laser therapy

Mild changes (score 1) in NVC were present in 18 (23.1%) patients with Raynaud's phenomenon, while moderate and advanced abnormalities were observed in 17 (21.8%) and 23 (29.5%) patients, respectively (Table 3). After 3 weeks of MLS laser therapy an increase in the number of patients (from 20 to 27) without microcirculation abnormalities was noticed. Moreover, a decrease in the number of patients (from 23 to 17) with advanced changes in NVC (score 3) was observed. Mild (score 1) changes in NVC were observed in 16 out of 38 (42.1%) patients with primary RP, while moderate capillaroscopy changes (score 2) were found in 2 patients (5.3%).

After MLS laser therapy, an increase in the number of patients with a score of 0 was observed (from 52.6% to 71%). Simultaneously, a decrease in the number of patients with a score of 1 was noticed (from 42.1% to 23.7%).

The presence of a score of 3 in NVC was found neither before nor after MLS laser therapy.

In the group of patients with secondary RP, a reduction of the score of 3 was noticed (from 57.5% to 42.5%) after MLS laser therapy. Moreover, we observed an increased number of patients with a score of 1 (from 5% to 20%) while the number of patients with a score of 2 was invariable.

Prior to treatment commencement, a positive correlation between microcirculation score abnormalities and the number of RP attacks ($r=0.24$, $p<0.05$), and VAS ($r=0.27$, $p<0.02$) was found.

After MLS laser therapy, only a positive correlation between NVC score and pain assessed by VAS was noticed ($r=0.23$, $p<0.05$) in all RP patients.

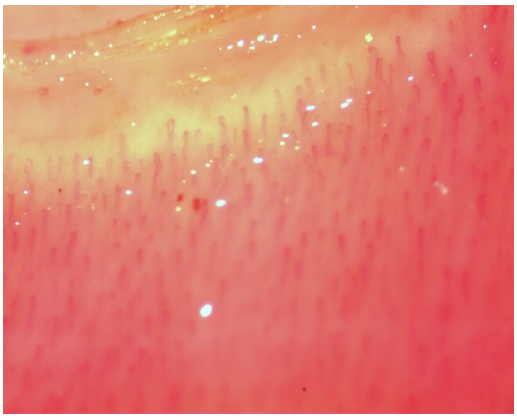

Photo 1a (by J.Kita)

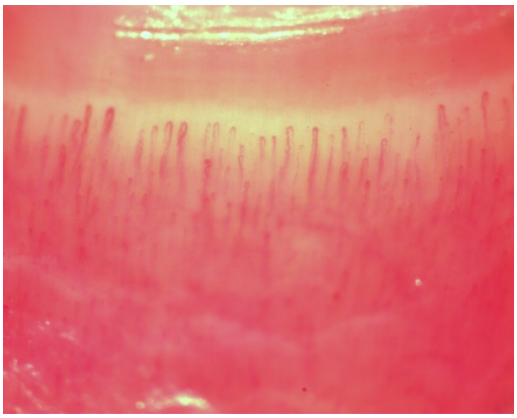

Photo 1b (by J.Kita)

Photo 1. Intensity of capillaroscopic changes in patients with primary RP before (photo 1a) and after (photo1b) MLS laser stimulation

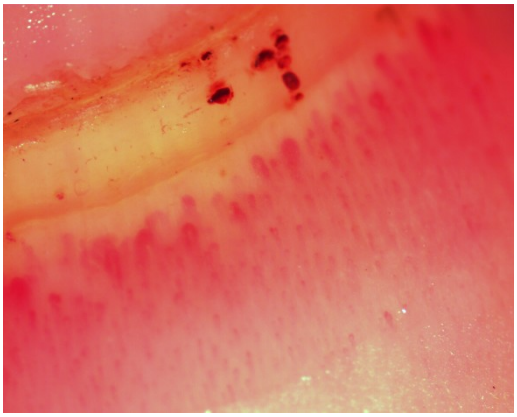

Photo 2a (by J.Kita)

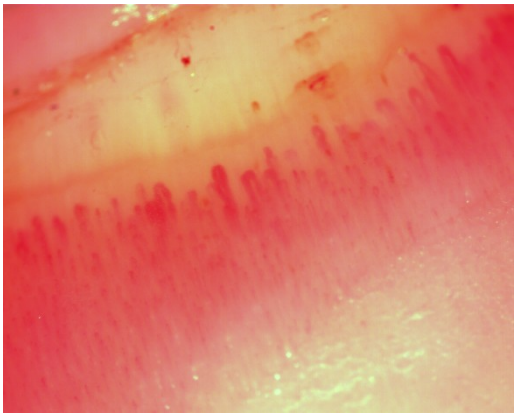

Photo 2b (by J.Kita)

Photo 2. Intensity of capillaroscopic changes in patients with secondary RP before (photo 2a) and after (photo 2b) MLS laser therapy

MLS laser therapy decreases serum NO concentration

NO concentration was elevated in both groups with RP compared with the control group, before and after treatment (P<0.001).

Interestingly, patients with secondary RP had significantly increased NO values compared with patients with primary RP, before (P<0.001) and after (P<0.05) MLS laser therapy.

In patients with secondary RP, a decrease in NO concentration was observed after treatment (P<0.001) (Table 3).

Before laser therapy, the lowest NO values were noticed in patients with scores 0 and 1 (P<0.05). However, after the treatment a significant decrease in NO serum level was observed in patients with scores 2 (P<0.001) and 3 (P<0.05) (Table 4). In patients with primary RP, a positive correlation between attack duration and pain (VAS) was observed (r = 0.65, P<0.001). In patients with secondary RP, a negative correlation between the number of RP attacks and NO concentration in serum was detected (r = -0.32, P<0.05).

Table 3. Capillaroscopic characteristics and serum Nitric Oxide (NO) concentration in patients with primary and secondary Raynaud's phenomenon (RP) before and after MLS laser therapy

Capillaroscopy score	Primary RP (n=38)	Secondary RP (n=40)	P value
Score 0 (n/%)	20/52.6	0	0.001
Score 1 (n/%)	16 /42.1	2/5.0	0.01
Score 2 (n/%)	2.0/5.3	15/37.5	0.01
Score 3 (n/%)	0	23/57.5	0.001
NO concentration (µM)			
Before MLS therapy (mean ± SD)	35.7 ± 9.5	44.1 ± 8.8	0.001
After MLS therapy (mean ± SD)	33.8 ± 7.1	37.0 ± 5.8	0.05

Table 4. Correlation between serum nitric oxide (NO) concentration with morphological changes in nailfold videocapillaroscopy (NVC) in RP patients before and after MLS therapy

Score	Serum NO concentration (μM)		
	before MLS laser therapy	after MLS laser therapy	*P**
0	33.7 ± 7.7	33.6 ± 6.3	ns
1	36.5 ± 10.8	36.8 ± 7.8	ns
2	47.9 ± 9.0	35.8 ± 5.6	$P < 0.001$
3	42.2 ± 7.2	36.5 ± 6.9	$P < 0.05$

Data are presented as mean ± standard deviation; * Wilcoxon signed rank test; MLS - Multiwave Locked System; ns – not significant

DISCUSSION

Many different mechanisms have now been implicated in the pathophysiology of Raynaud's phenomenon, i.e. increased vasospasm and reduced vasodilatation, structural abnormalities of large and small vessels, and coagulopathy. The agents playing a major role in these mechanisms include: endothelin-1, nitride oxide (balance between them), the α_2-adrenoreceptors, free radicals, and platelet activation/aggregation. Progress in the pathophysiology of the RP has led to new approaches to treatment.

The necessity of immediate assessment and treatment in severe forms of the disease with digital ulcers is emphasized. A large number of medications is used in the treatment of RP, yet no gold standard exists. Mild forms of primary RP can be controlled by non-pharmacologic approaches [21,22].

The developement of diagnostic techniques of microcirculation abnormalities, such as NVC, allowed for early diagnosis of connective tissue disease. According to recent studies, the presence of abnormal skin capillaries in autoimmune disease reflects changes in systemic microcirculation (i.e. coronary microcirculation) [22,23]. Dysfunctions of microcirculation in the course of RP are associated with homeostasis disturbances between vasoconstrictor (endothelin, angiotensin II) and vasodilator factors (NO, prostacyclin). Previous pharmacologic treatment is associated with adverse events, which limits the possibilities of therapy [24,25].

Low level laser therapy stimulates microcirculation, reduces pain and the inflammatory process, and reduces the frequency and intensity of attacks in patients with primary and secondary RP [26-29]. Regarding the clinical efficacy of MLS laser therapy, only a few reports may be considered as evidence in the treatment of patients with primary and secondary RP [14, 26].

In the present study, the clinical improvement manifested by a decrease in the number of RP attacks, duration of a single attack, and pain intensity was observed in both groups of patients after three weeks of MLS laser therapy.

In randomized placebo-controlled trials after 3 weeks of infrared thermography, the authors observed a decrease in the number of attacks, the duration of a single attack, and intensity [27]. The beneficial effect of MLS treatment was observed in patients with knee osteoarthritis [28]. MLS laser therapy allowed modulating the inflammatory response in an experimental model of collagen-induced rheumatoid arthritis, and the therapy is effective in both the early and the late progression stage of collagen-induced rheumatoid arthritis [29].

In our study, mild changes in NVC were detected in 16 out of 38 patients (42.1%) with primary RP and 2 out of 40 patients (5%) with secondary RP. Moderate abnormalities (score 2) were present in 2 out of 38 patients (5.3%) with primary RP and in 15 out of 40 patients (37.5%) with secondary RP. Advanced changes (score 3) were observed in 23/40 (57.5%) patients with secondary RP. In the group of 40 patients with secondary RP, systemic sclerosis was diagnosed in 28 subjects.

The above results confirm the findings of other authors and indicate the presence of significant differences between capillary density and width in patients with primary and secondary RP in the course of systemic sclerosis [30, 31]. According to previous studies, the presence of NVC abnormalities was observed between 19-37% of patients with primary RP, and in 32-88% of patients with secondary RP. In the present study, the increased percentage of microcirculation abnormalities (47% primary RP, 95% secondary RP patients) could result from the use of NVC in comparison with traditional capillaroscopic examination. The NVC method allows for picture recording, archiving, and repeated analysis.

From a clinical point of view, a noticeable aspect is the correlation between the number of attacks and pain intensity with the advancement of microcirculation abnormalities in NVC. The above findings confirm the association between the degree of microvascular changes and disease progression.

As regards the available literature, only one study describing the influence of laser biostimulation on microcirculation disorders in NVC can be found [30]. This study showed significant improvement of microcirculation abnormalities with a decrease in the number of patients (primary and secondary RP) with advanced changes in NVC after MLS laser therapy.

The role of agents controlling angiogenesis (e.g. VEGF, Ang-2, NO) in the pathogenesis of RP has not been completely described thus far [30].

Recent studies suggest that NO has an important function as a regulator of angiogenesis. In addition, previous findings underline the pleiotropic activity of NO in the inflammatory process [8]. Diminished NO concentration protects endothelial cells, while elevated NO level induces apoptosis [32]. Increased values of NO were detected in patients with SSc as well [32]. The results obtained by Dooley et al. showed that plasma NO was elevated in primary RP and limited scleroderma, while in diffuse scleroderma the levels were normal [32].

In the case of in vitro studies, NO inhibits the secretion of Ang-2 from endothelial cells. In patients with sepsis, a negative correlation between Ang-2 and NO bioavailability was observed. The authors suggest that the diminished NO level is responsible for the impairment of the internal organs [33].

In the present study, an increased NO concentration was observed in both groups of patients with RP, especially in the case of secondary RP. A significant negative correlation between Ang-2 and NO was also noticed in patients with primary RP (our unpublished data). Similar results were obtained by Davis et al. [34]. The present study showed that patients with advanced abnormalities in NVC have increased levels of Ang-2 and NO. The elevation of Ang-2 with enhanced changes in NVC might be caused by the impairment of NO bioavailability and thus the disturbance of NO regulation may explain the dysfunction of microcirculation.

According to the results of our study, a significant decrease in serum NO concentration after MLS treatment was observed in both groups of patients. This can be the consequence of the influence of MLS biostimulation on inflammatory process mechanisms.

Recent demonstrations of the inactivation of $O^{-}2$ by NO suggest that NO acts as a useful endogenous free radical scavenger. NO can be regarded as a scavenger of the superoxide anion and it is suggested that NO may provide a chemical barrier to cytotoxic free radicals.

Also, NO may have a considerable protective effect on cellular viability and can act as an antioxidant, which both protects cells from oxidant-induced damage and prevents endothelial apoptosis.

Moreover, NO is considered to have a biphasic effect in physiological and pathological conditions, being both beneficial and detrimental depending on the concentration and local environment.

In this study, we investigated the influence of MLS laser therapy on the clinical features, microvascular changes in nailfold video-capillaroscopy, and an indicator of endothelial function in patients with primary and secondary Raynaud's phenomenon.

Our results indicate that in patients with Raynaud's phenomenon NVC is a non-invasive and useful tool for monitoring the development and progression of the disorder. The 3 weeks of MLS laser therapy showed a decrease in the duration and number of RP attacks as well as the degree of pain score on the VAS scale in patients with primary and secondary RP.

The evaluation of a biochemical marker of endothelium dysfunction (NO) shows a tendency of normalization of NO concentration in the serum of primary and secondary RP patients after MLS laser therapy. This observation may suggest the beneficial effect of MLS laser therapy on the regulation process involved in microvascular disorders.

CONCLUSIONS

Our study suggests that MLS laser therapy leads to beneficial clinical effects in patients with primary and secondary RP. NVC is a non-invasive, useful tool for monitoring the development of the disorder and may reflect microvascular abnormalities associated with clinical improvement after MLS laser therapy in patients with primary and secondary RP. Moreover, NO serum level may be a useful marker of microvascular disturbances in patients with primary and secondary RP treated with MLS therapy.

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Conflicts of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for his work that could have affected its outcome.

Authors' contributions

AKM conceived the idea for the study. AKM and AH contributed to the design of the research. JK was involved in data collection. AH

and JK analyzed the data. All authors edited and approved the final version of the manuscript.

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