

Efficacy Of Interval Aerobic Training Versus Laser Puncture Therapy On The Severity Of Restless Leg Syndrome In Chronic Obstructive Lung Patients

Ola Mohamed Elgohary^{1*}, Rania Ahmed Diab², Lamiaa S. Tolba³, Mai I. Tolba⁴, Alyaa Abdallah Atallah Ahmed Zaid⁵, Mai M. Abdallah⁶, Engi E. Sarhan^{7,8}, Sami Ahmed Zaher Basha⁹

¹ Lecturer of Physical Therapy Department of Cardiovascular Pulmonary and Geriatrics, Faculty of Physical Therapy, Pharos University, Alexandria, Egypt; ORCID: 0000-0002-7577-0080

² Lecturer of Physical Therapy, Department of Basic Science, Pharos University, Alexandria; ORCID: 0000-0002-7390-5703

³ Lecturer of Physical Therapy, Department of Integumentary Faculty of Physical Therapy Pharos University, Alexandria, Egypt; ORCID: 0000-0002-2571-8305

⁴ Lecturer of Physical Therapy for Women Health, Faculty of Physical Therapy, Horus University-Egypt, New Damietta, Egypt; ORCID: 0009-0006-5291-958X

⁵ Lecturer of Physical Therapy, Department of Physical Therapy for Internal Medicine and Geriatrics, Faculty of Physical Therapy, Horus University-Egypt, New Damietta, Egypt; ORCID: 0009-0008-0465-8746

⁶ Lecturer of Physical Therapy, Department of Orthopedic, Faculty of Physical Therapy, Pharos University, Alexandria, Egypt; ORCID: 0009-0000-5373-7565

⁷ Lecturer of Physical Therapy for Neurology and Its Surgery at Neurology Department Faculty of Physiotherapy at Kafr Elsheikh University, Egypt; ORCID: 0009-0001-8019-3775

⁸ Assistant professor, Department of Physical Therapy, College of Applied Medical Science, Qassim University, Buraydah 51452, Saudi Arabia

⁹ Assistant Professor in Physical Therapy department, Faculty of Allied Medical Sciences, Zarqa University, Jordan; ORCID: 0009-0006-1416-0496

Abstract

Background: Although restless legs syndrome (RLS) is a common neurological condition, little is known about how it manifests and is treated in patients with chronic obstructive pulmonary disease (COPD). The benefits of interval aerobic training and laser puncture therapy on RLS severity, fatigue, sleep quality, hemoglobin levels, and functional ability in individuals with COPD were compared in this randomized controlled experiment.

Methods and materials: Sixty male patients aged 40–60 years, diagnosed with COPD and RLS for at least six months and with a body mass index (BMI) between 25.0–29.9 kg/m², were recruited from the outpatient clinic at Aboker Hospital and Zezini PT Private Center. Participants were randomly allocated into two equal groups (n = 30 each). Group A underwent an interval aerobic training program using a cycle ergometer in addition to standard medical treatment, whereas Group B received laser puncture therapy three times per week for 12 weeks alongside conventional medical care. Outcome measures included the Restless Legs Syndrome Rating Scale (RLSS), hemoglobin concentration, the Fatigue Severity Scale (FSS), the Pittsburgh Sleep Quality Index (PSQI), and the Six-Minute Walk Test (6MWT).

Results: Group A demonstrated significantly greater improvements than those in Group B. Specifically, mean differences were 6.53 ± 0.68 on IRLS, 3.90 ± 0.72 g/dL in hemoglobin, 6.70 ± 0.60 on FSS, 5.50 ± 1.41 on PSQI, and 16.70 ± 7.85 meters on the 6MWT (p < 0.001 for IRLS, Hb, and FSS; p = 0.001 for PSQI; and p = 0.037 for 6MWT)

Conclusion: In patients with COPD who have restless leg syndrome, both interval aerobic training and laser puncture therapy have been shown to improve functional performance, reduce RLS symptoms, and alleviate fatigue and poor sleep. Nonetheless, for individuals who are unable to participate in formal physical training, laser puncture therapy can be an additional choice.

Keywords: Interval aerobic training; laser puncture therapy; restless legs syndrome; fatigue

1. INTRODUCTION

The primary issue with restless legs syndrome, a sensory condition, is moving the legs, with or without unpleasant sensations. Symptoms, which usually worsen at rest, are momentarily alleviated by

movement. RLS is becoming more widely known to patients with chronic obstructive pulmonary disease, and early detection of the illness can significantly improve their quality of life.

The etiology of RLS is multifactorial, with contributing factors including iron-deficiency anemia, hypoxemia, smoking, renal dysfunction, and cardiovascular comorbidities such as hypertension, stroke, and liver disease. Many of these risk factors are highly prevalent in COPD patients, which may explain the higher occurrence of RLS among them (Gupta et al., 2017; Winter et al., 2013). Epidemiological studies indicate that more than 30% of patients with chronic diseases, including COPD, present with RLS—a prevalence considerably higher than in the society. In spite sleep problems is a common complaint in COPD, little research has specifically examined the prevalence and characteristics of RLS in these patients (Cavalcante et al., 2012). Pathophysiological links have also been proposed. Chronic hypoxemia in COPD is associated with systemic inflammation, anemia, and activation of the hypoxia-inducible factor-1 (HIF-1) pathway. Interestingly, HIF-1 activation has been demonstrated in the substantia nigra and brain vasculature of patients with RLS. In genetically susceptible individuals, this mechanism enhances dopamine uptake, contributing to the clinical expression of RLS (Maharjan et al., 2020)

From a therapeutic perspective, traditional continuous moderate-intensity exercise is often limited in COPD patients due to ventilatory constraints, making it insufficient to induce beneficial physiological adaptations. Conversely, interval training has proven more effective in enhancing cardiovascular health, muscular conditioning, and exercise tolerance while requiring less time commitment compared to steady-state exercise (MacDonald et al., 2009). Intermittent exercise training (IET) improves cardiorespiratory fitness, strengthens peripheral muscles, reduces dyspnea, and alleviates leg discomfort—factors that may directly benefit COPD patients with RLS (Alexiou et al., 2021)

In addition, mitochondrial dysfunction in chronic illness leads to impaired ATP synthesis due to increased nitric oxide (NO) production, which interferes with oxygen utilization. Low-level laser therapy (LLLT), applied at appropriate wavelengths, can dissociate NO from mitochondria, restore oxygen availability, and improve cellular energy production. Preliminary evidence has demonstrated improvements in pulmonary function and gas exchange in respiratory disorders following LLLT, but further studies are warranted to confirm its efficacy in COPD patients with RLS ((Karu, 2020; Mилоjević and Kuruc, 2003)

OBJECTIVE:

The objective of this study was to evaluate the effectiveness of interval aerobic training and laser puncture therapy in reducing restless legs syndrome severity, alleviating fatigue, and enhancing chronic obstructive pulmonary disease patients' quality of life.

2- MATERIALS AND METHODS

2.1 Study Design:

This randomized controlled clinical trial was conducted at the outpatient clinics of Aboker Hospital and Zezini Private Physical Therapy Center between March and June 2024. A total of sixty male patients with COPD and concomitant restless legs syndrome (RLS) were recruited. Since smoking-related pulmonary obstruction is more common among men in Egypt, male patients were specifically targeted for inclusion.

2.2 Inclusion Criteria

Eligible participants were male COPD patients aged 40–60 years with a body mass index of 25.5–29.0. All had been diagnosed with COPD for at least two years, presenting with **GOLD 2 (Moderate severity)** $50\% \leq FEV_1 < 80\%$ predicted. Patients had not received any medication for RLS during the six months prior to the study and demonstrated adequate cooperation. RLS was diagnosed according to the International Restless Legs Syndrome Study Group criteria revised in 2014, which include: (1) a strong need to move the legs, frequently accompanied with unpleasant feelings; (2) a worsening of the symptoms when at rest (3) a reduction in the symptoms when walking or stretching; (4) a preponderance of symptoms in the evening and (5) exclusion of symptoms attributable to other medical or behavioral conditions (e.g., arthritis, myalgia, cramps, or positional pain) (da Cunha Moraes et al., 2018; Allen et

al., 2014)

2.3 Exclusion Criteria

Patients were excluded if they had: a history of renal disease, hiatus hernia, severe gastro-esophageal reflux, osteoporosis, severe anemia or congestive heart failure, acute infections or malignant disease, or severe neurological or musculoskeletal abnormalities were not allowed to participate.

2.4 Randomization

Sixty eligible patients were randomly allocated into two same groups (n=30 each) using a computer-generated randomization list:

- **Group A (Interval Aerobic Training):** Patients performed interval cycling ergometer training three times per week, 15–30 minutes per session, for 12 consecutive weeks.
- **Group B (Laser Puncture Therapy):** Patients received low-level laser therapy at acupuncture points (Shenshu [BL23], Xuehai [SP10], Chenshan [BL57], Zusanli [ST36], Sanyinjiao [SP6], and Taixi [KD3]) three times per week for 12 weeks, 5 minutes per point, with each session lasting 15–30 minutes.

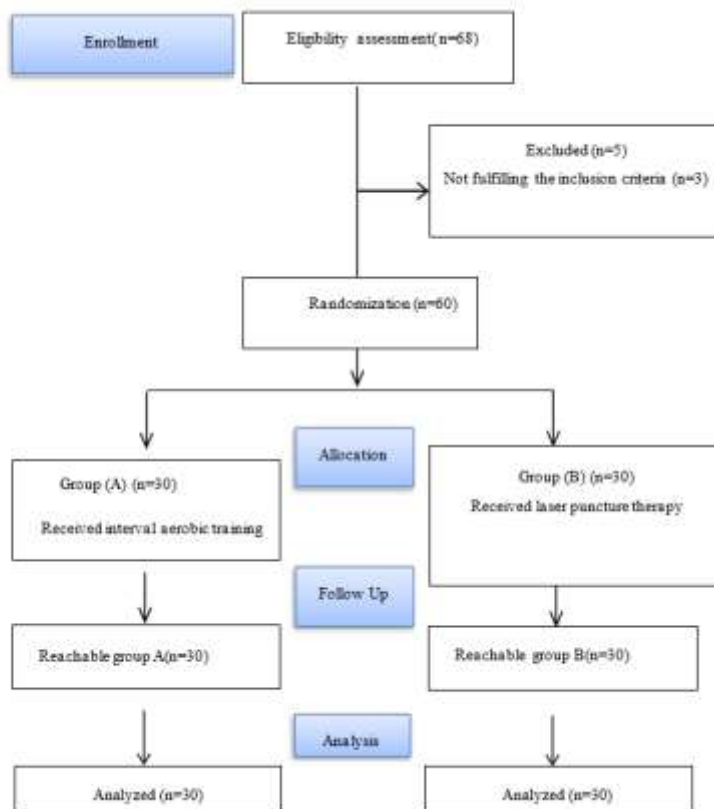


Figure 1. Participants' flow chart for the study

3-Evaluation procedures

A comprehensive battery of validated assessment tools was employed to evaluate both groups before and after the intervention. These tools were carefully selected to capture multiple dimensions relevant to patients with COPD and coexisting RLS, including symptom severity, fatigue, sleep quality, hematological status, and functional exercise capacity.

3.1-Restless Legs Syndrome Rating Scale (IRLS): was used as the primary outcome measure for RLS severity. This self-administered 10-item questionnaire assesses the intensity and frequency of RLS symptoms and their impact on daily activities and sleep. Each item is scored on a Likert scale ranging from 0 (no symptoms) to 4 (very severe), producing a total score between 0 and 40. Scores are categorized as mild (1–10), moderate (11–20), severe (21–30), or very severe (31–40). The average

administration time was approximately 10 minutes. (Allen et al., 2003; Youssef, 2014)

3.2 -Fatigue Severity Scale (FSS): was employed to quantify both physical and cognitive fatigue. This instrument consists of nine statements, each rated from 1 (strongly disagree) to 7 (strongly agree). The mean score represents the overall fatigue burden, with higher scores reflecting more disabling fatigue. A cutoff score below 36 indicates the absence of clinically significant fatigue, whereas scores above this threshold suggest moderate to severe fatigue. (Krupp et al., 1989; Lerdal et al., 2011)

3.3 -Pittsburgh Sleep Quality Index (PSQI): a widely validated 19-item questionnaire. It generates seven component scores (subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction), each rated on a 0–3 scale. The global PSQI score ranges from 0 to 21, with higher scores denoting poorer sleep quality. A score greater than 5 is generally indicative of clinically significant sleep disturbances. ((Jennings et al., 2007; Buysse, 2003)

3.4 Hemoglobin Concentration

Hematological assessment was conducted by measuring hemoglobin levels via venous blood sampling as part of a complete blood count (CBC). Hemoglobin concentration was expressed in grams per deciliter (g/dL). For males, anemia was defined as hemoglobin <13.5 g/dL, while for females the threshold was <12 g/dL. Monitoring hemoglobin was essential due to its strong association with hypoxemia, fatigue, and RLS severity in COPD patients. (Means et al., 2020)

3. 5- Six-Minute Walk Test (6MWT):

the standardized Six-Minute Walk Test, performed along a flat 30-meter indoor corridor. Patients were instructed to walk as far as possible in six minutes, with the total distance covered recorded in meters. monitoring of HR, BP, peripheral o₂ saturation, and dyspnea (via Borg scale) was conducted before and after the test. This submaximal exercise test reflects daily activity tolerance and is sensitive to changes following pulmonary rehabilitation or adjunct therapies and exercise capacity. (Casanova et al.,2007)

4-Treatment Procedures:

Group A: (Aerobic Training Exercise)

Patients in this group underwent an aerobic interval training program using the Pro-Hanson Fitness Exercise Bike with Hand Pulse 100 KG. Each patient was seated on the stationary bicycle, and the protocol was clearly explained prior to initiation. Exercise intensity was continuously monitored using the modified Borg scale (0–10), with values maintained between 4 and 7, corresponding to low-to-moderate exertion levels. In addition, the target training zone was calculated using the Karvonen heart rate reserve (HRR) formula, set at 50–75% of HR_{max}. Each training session lasted 30 minutes, beginning with a 5-minute warm-up, followed by alternating 3-minute intervals of low and moderate intensity with equal duration, and ending with a 5-minute cool-down. The intervention was performed 3times/ week for a total of 12 weeks (SHEREEN et al., 2018)

Group B: (Laser Acupuncture Therapy)

Patients in this group received low-level laser therapy (LLLT) delivered by the Phyaaction CL (Phyaaction, Bilzen, Belgium) GymnaUniphy N.V.-device. The specifications included a frequency of 500 Hz, output power of 5–20 mW, and a wavelength of 905 nm. A laser probe was applied directly to selected acupuncture points for six minutes per site. (Limansky et al., 2011) Both the patient and the physiotherapist wore protective eyewear during the session to safeguard their eyes.

The standardized acupuncture points were as follows:

- **Xuehai (SP10):** Located on the medial thigh, approximately two inches above the patella, directed toward the body midline.
- **Chenshan (BL57):** Found at the lower calf, in the depression formed during toe extension.

- **Zusanli (ST36):** Positioned two inches below the patella, about half an inch medial to the tibial crest.
- **Sanyinjiao (SP6):** situated about two inches above the medial malleolus on the medial side of the lower leg.

Taixi (KD3): located in the hollow that separates the Achilles tendon from the medial malleolus. The laser acupuncture intervention was conducted 3 times /week for 12 weeks, matching the duration of the aerobic training program, to ensure comparable exposure time and treatment consistency.

5-RESULTS:

Statistical analysis: Before starting the study, a power analysis was performed using G*Power version 3.1.9.2 to ensure an adequate sample size. Calculations were based on a two-group *t*-test, with the significance level (α) set at 0.05 and the power set at 80% ($\beta = 0.20$). An expected effect size of 0.778 was derived from the main outcome measures, namely hemoglobin levels and the six-minute walk test. The analysis indicated that a minimum of 44 participants (22 per group) would be required to detect meaningful differences with sufficient statistical confidence

5.1-Data analysis using statistics:

The data were analyzed using IBM SPSS version 20.0 (Armonk, NY: IBM Corp, 2011). Normality of continuous variables was checked using the Shapiro-Wilk test. Results are presented as mean \pm standard deviation, median, and range. Depending on data distribution, comparisons between the two groups were carried out using either the independent *t*-test or the Mann-Whitney test, while changes within each group were assessed using the paired *t*-test. A *p*-value of ≤ 0.05 was considered statistically significant.

At baseline, both groups were well-matched with no significant differences in age, BMI, or any of the clinical outcomes (IRLS, FSS, hemoglobin, 6-minute walk test, and PSQI) (Table 1, Figure 2). After treatment, both groups showed clear improvements; however, Group A demonstrated greater benefits in IRLS, hemoglobin levels, and walking distance, as well as more marked improvements in fatigue and sleep quality. In contrast, post-treatment differences between the two groups were not significant for the Fatigue Severity Scale ($p = 0.347$) or the Pittsburgh Sleep Quality Index ($p = 0.123$) (Table 2, Figure 3). Within-group analysis confirmed that all outcomes improved significantly in both groups ($p < 0.001$), indicating that while both interventions were effective, the effects were more pronounced in Group A. Both groups showed significant improvements after treatment ($p < 0.001$). However, Group A demonstrated greater reductions in RLS severity, fatigue, and sleep disturbance, as well as higher gains in hemoglobin and walking distance, compared with Group B ($p \leq 0.05$). Between-group differences for FSS ($p = 0.347$) and PSQI ($p = 0.123$) were not significant. Overall, the intervention in Group A proved more effective, with consistently superior outcomes across most clinical measures.

Table (1): Comparison between the two studied groups according to demographic data:

	Group (n = 30)	A Group (n = 30)	B	t	P
Age (years)					
Min. - Max.	40.0 - 58.0	40.0 - 58.0		0.308	0.759
Mean \pm SD.	45.53 \pm 4.31	45.20 \pm 4.06			
Median (IQR)	45.0 (42.0 - 49.0)	45.0 (42.0 - 48.0)			
BMI (kg/m²)					

Min. – Max.	25.0 – 30.0	25.0 – 30.0	0.144	0.886
Mean ± SD.	27.37 ± 1.40	27.42 ± 1.30		
Median (IQR)	27.33(26.40 – 28.50)	27.50(26.50 – 28.50)		

SD: Standard deviation; t: Student t-test; p: p value for comparing between the two studied groups

Table (2): Comparison between the two studied groups according to different parameters

		Group A (n = 30)	Group B (n = 30)	t	P
IRLS	Pre				
	Min. – Max.	25.0 – 40.0	25.0 – 40.0	t= 0.197	0.844
	Mean ± SD.	33.37 ± 3.90	33.57 ± 3.95		
	Median (IQR)	32.0 (31.0 – 37.0)	32.50 (31.0 – 37.0)		
	Post				
	Min. – Max.	19.0 – 34.0	20.0 – 36.0	t= 2.227*	0.030*
	Mean ± SD.	26.83 ± 3.79	29.10 ± 4.09		
	Median (IQR)	26.0 (24.0 – 30.0)	28.50 (26.0 – 32.0)		
	t0 (p0)	52.513* (<0.001*)	29.861* (<0.001*)		
Mean Differences	6.53 ± 0.68	4.47 ± 0.82	t=10.622*	<0.001*	
FSS	Pre				
	Min. – Max.	36.0 – 60.0	36.0 – 60.0	t= 0.020	0.984
	Mean ± SD.	47.93 ± 6.40	47.97 ± 6.46		
	Median (IQR)	49.0 (43.0 – 52.0)	49.0 (43.0 – 52.0)		
	Post				
	Min. – Max.	29.0 – 53.0	30.0 – 55.0	t= 0.949	0.347
	Mean ± SD.	41.23 ± 6.37	42.80 ± 6.42		
	Median (IQR)	42.0 (37.0 – 45.0)	43.50 (38.0 – 47.0)		
	t0 (p0)	61.577* (<0.001*)	29.792* (<0.001*)		
Mean Differences	6.70 ± 0.60	5.17 ± 0.95	t=7.489*	<0.001*	
Pittsburgh sleep quality index	Pre				
	Min. – Max.	13.0 – 21.0	13.0 – 21.0	t= 0.272	0.786
	Mean ± SD.	17.30 ± 2.38	17.13 ± 2.36		
	Median (IQR)	17.0 (16.0 – 19.0)	17.0 (15.0 – 19.0)		
	Post				
	Min. – Max.	7.0 – 16.0	9.0 – 17.0	t= 1.563	0.123
	Mean ± SD.	11.80 ± 2.04	12.67 ± 2.25		
	Median (IQR)	11.50 (10.0 – 13.0)	12.50 (11.0 – 14.0)		
	t0 (p0)	21.394* (<0.001*)	33.500* (<0.001*)		
Mean Differences	5.50 ± 1.41	4.47 ± 0.73	t=3.568*	0.001*	
Hemoglobin (g/dl)	Pre				
	Min. – Max.	7.0 – 13.0	7.0 – 13.0	t= 0.000	1.000
	Mean ± SD.	9.53 ± 1.56	9.53 ± 1.56		
	Median (IQR)	9.50 (8.50 – 10.50)	9.50 (8.50 – 10.50)		
	Post				
Min. – Max.	11.0 – 16.50	10.0 – 15.50	t=	0.024*	

	Mean ± SD.	13.43 ± 1.52	12.53 ± 1.49	2.312*	
	Median (IQR)	13.25 (12.50 - 14.0)	12.50 (11.50 - 13.50)		
	t0 (p0)	29.505* (<0.001*)	21.153* (<0.001*)		
	Mean Differences	3.90 ± 0.72	3.0 ± 0.78	t=4.642	<0.001*
6 mint test by meter	Pre				
	Min. - Max.	350.0 - 501.0	350.0 - 501.0	t= 0.079	0.937
	Mean ± SD.	447.6 ± 41.83	448.5 ± 42.92		
	Median (IQR)	454.0 (418.0 - 483.0)	457.0 (418.0 - 486.0)		
	Post				
	Min. - Max.	365.0 - 521.0	365.0 - 520.0	t= 0.114	0.909
	Mean ± SD.	464.3 ± 45.32	463.0 ± 42.80		
	Median (IQR)	472.0 (435.0 - 508.0)	469.5 (428.0 - 499.0)		
	t0 (p0)	11.648* (<0.001*)	13.810* (<0.001*)		
Mean Differences	16.70 ± 7.85	14.53 ± 5.76	U=315.0 0*	0.037*	

SD: Standard deviation; t: Student t-test; U: Mann Whitney test; t0: Paired t-test

p: p value for comparing between the two studied groups

p0: p value for comparing between Pre and Post

*: Statistically significant at p ≤ 0.05

Table (3): Comparison between the two studied groups according to % of improvement

	Group A (n = 30)	Group B (n = 30)	Test of Sig	p
IRLS				
Min. - Max.	↓15.0 - 25.81	↓5.56 - 20.69	t= 8.312*	<0.001*
Mean ± SD.	19.79 ± 2.70	13.52 ± 3.13		
Median (IQR)	19.14 (18.18 - 21.62)	13.33 (11.11 - 15.63)		
FSS				
Min. - Max.	↓11.11 - 19.44	↓2.04 - 16.67	t= 5.423*	<0.001*
Mean ± SD.	14.22 ± 2.30	10.94 ± 2.38		
Median (IQR)	13.84 (12.24 - 16.00)	11.34 (10.00 - 12.00)		
Pittsburgh sleep quality index				
Min. - Max.	↓14.29 - 46.15	↓19.05 - 35.71	t= 3.446*	0.001*
Mean ± SD.	31.80 ± 7.25	26.35 ± 4.73		
Median (IQR)	33.33 (26.32 - 36.84)	25.66 (23.08 - 31.25)		
Hemoglobin (g/dl)				
Min. - Max.	↑18.18 - 41.67	↑14.29 - 38.46	t= 3.265*	0.002*
Mean ± SD.	29.26 ± 5.92	24.13 ± 6.24		
Median (IQR)	29.10 (3.50 - 4.50)	22.73 (2.50 - 3.50)		
6 mint tests by meter				
Min. - Max.	↑3.90 - 5.64	↑2.80 - 4.89	U= 311.000*	0.040*
Mean ± SD.	3.54 ± 1.72	3.16 ± 1.36		
Median (IQR)	3.82 (3.19 - 4.46)	3.29 (2.55 - 3.93)		

SD: Standard deviation

t: Student t-test

U: Mann Whitney test

p: p value for comparing between the two studied groups

*: Statistically significant at $p \leq 0.05$

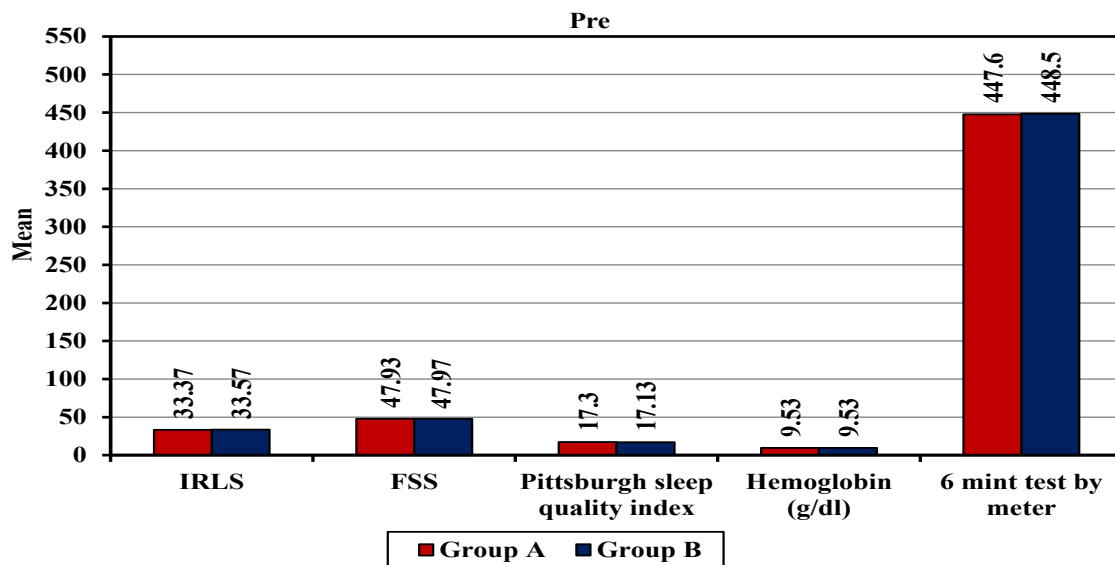


Figure (2): Comparison between the two studied groups according to different parameters in pretreatment

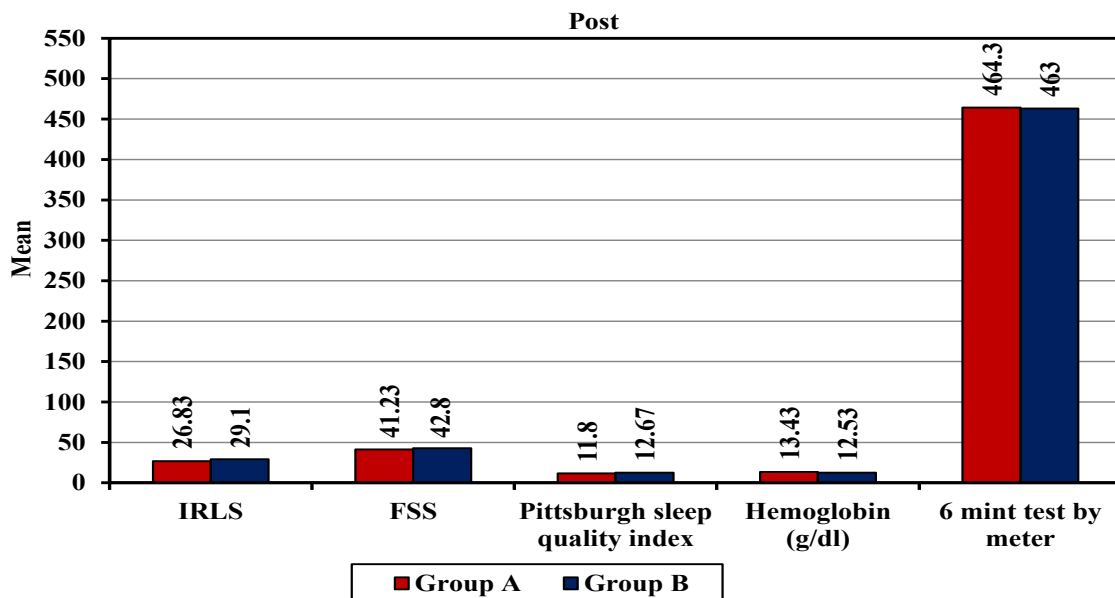


Figure (3): Comparison between the two studied groups according to different parameters in posttreatment.

4. DISCUSSION:

Recent research has indicated a higher prevalence of RLS in between patients with chronic obstructive pulmonary disease with shared risk factors such as chronic hypoxemia, anemia, and nicotine use potentially contributing to this association (Aras et al., 2011). The coexistence of RLS and COPD exacerbates sleep disturbances, increases fatigue, and impairs overall quality of life, thereby adding an additional burden to this vulnerable patient population

As a safe substitute for traditional treatments, laser acupuncture has become a popular non-invasive, non-pharmacological treatment. In order to influence physiological functions like circulation,

neurotransmitter activity, and pain perception, this method stimulates particular acupoints using low-level lasers (Mohammadi et al., 2018). Mechanistically, it has been demonstrated that near-infrared therapy causes the release of nitric oxide (NO), which raises endorphin synthesis, improves neurotransmission, and encourages vasodilation—all of which are directly linked to the reduction of restless symptoms.

The study's findings demonstrated that while all groups benefited from the medication, Group A's gains in haemoglobin levels, IRLS, and sleep quality were more pronounced. Both groups showed improvements in FSS and the 6-minute walk test. The likelihood that Group A's intervention was more effective is increased by the statistical significance of group differences. Low-intensity laser light is used in laser acupuncture, a non-pharmacological treatment, to activate particular body acupoints. This approach is typically thought of as a non-invasive, safe therapeutic approach (Mohammadi et al., 2018).

The beneficial effects of acupuncture in RLS are likely multifactorial. Evidence suggests that acupuncture can modulate dopamine release, enhance peripheral and cerebral microcirculation, and increase endogenous endorphin production (Mitchell et al., 2011; Tang et al., 2024). In COPD patients, chronic hypoxemia may further impair dopaminergic signaling pathways, reinforcing the potential for acupuncture to provide symptomatic relief through both central and peripheral mechanisms (Maharjan et al., 2024)

Additionally, interval aerobic training has emerged as an effective rehabilitation strategy. Compared with continuous aerobic exercise, interval training offers comparable or superior benefits in functional capacity, symptom control, and overall quality of life. It reduces leg discomfort, enhances oxidative capacity, and improves exercise tolerance—findings consistent with previous COPD rehabilitation studies (Kortianou et al., 2010). These improvements may also contribute to RLS symptom relief, potentially through enhanced circulatory function and dopaminergic activity (Song et al., 2022)

Emerging evidence also indicates that near-infrared light therapy may provide temporary relief of RLS symptoms. Its effectiveness appears to depend on both the duration and continuity of treatment, as symptoms frequently reappear upon discontinuation. Although the findings of the present study are promising, the existing literature remains limited. Thus, more extensive, superior randomized controlled trials are required to prove laser acupuncture's effectiveness. and interval training, particularly in COPD patients with comorbid RLS (Huang et al., 2021; Medina & Goldberg, 2024).

One remarkable discovery is the considerable improvement in hemoglobin levels. This is explained in the aerobic training group by the well-established benefits of exercise on promoting the synthesis of red blood cells, which aids in the fight against anemia in chronic illnesses. (Montero et al., 2017). The improvement for the laser therapy group might be because the treatment lowers the breakdown of red blood cell membranes by stabilizing and shielding them from harm. 2. Despite the fact that both therapies produced favorable outcomes, it seems that their modes of action differ. (Lenser, et al., 2020).

5-CONCLUSION:

Both interval aerobic training and laser puncture therapy contributed to enhanced quality of life while effectively decrease the severity of restless legs symptoms. According to the study's findings, laser therapy might offer extra advantages to people who are unable or unwilling to exercise or who might experience adverse effects from restless syndrome while taking medication for copd. Future studies utilizing different sample numbers, study durations, and point laser sites are needed to ascertain the effect of laser therapy on RLS

Ethical approval:

The human use research has been approved by the Pharos University Faculty of Physical Therapy's institutional review board, which also complies with all relevant national regulations, institutional policies, and the Declaration of Helsinki's tenets (approval No.: PUA 03202505253356).

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