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Negative Air Environment-Induced Rehabilitation of Spasticity And Behavior During Physiotherapy in Cerebral Palsy Patients

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Abstract: Introduction: Negative air ions (NAIs) are beneficial in improving memory, anti-depressant effects, productivity, psychological health, and well-being by inducing alkalinity in the body. Cerebral Palsy (CP) is a neurological disorder that impairs movement and elicits behavioral issues like anxiety, anger, agitation, dependency on others, hyperactivity, incompatibility, and stubbornness. Such behaviors may hamper their cooperation or willingness for physiotherapy. **Methodology**: Evidence-based studies to support that NAIs can augment the rehabilitation of CP are scant so, the present study was designed to determine if exposure to NAIs can normalize behaviors and physical outcomes. Ethical approval was taken from IBC, University of Karachi followed by the consent of the participants and the Al Umeed Rehabilitation Association where the intervention study was conducted in 2021. CP-inflicted participants were randomly allocated to the control and intervention groups. Both groups continued their regular twice-a-week physiotherapy sessions, but the intervention group also received 40-minute exposure to 10000 negative ions/cm^3 for 6 weeks. Outcomes were assessed using a researcher-designed physiotherapist reporting questionnaire and Modified Ashworth Scale. **Results**: Analysis using SPSS v.28 demonstrated significant improvement in calmness, cooperation, physical condition, and willingness for physiotherapy along with minimal alleviation of spasticity. The possible role of neuronal pathways, synaptic response, and neurotransmitters such as acetylcholine, dopamine, gamma-aminobutyric acid, and serotonin have been discussed. **Conclusion**: The study concludes that NAIs may alleviate spasticity and normalize behaviors during physiotherapy sessions. We suggest the inclusion of this novel intervention in the multidisciplinary rehabilitation plan after going through the second and third-phase clinical trials.

Keywords: Behavior, Cerebral Palsy, Negative Ionizer, Physiotherapy, Spasticity

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Introduction

A negative air ionizer is an electric device that produces negative air ions (NAIs) and charges the air molecules present in the surrounding. Ionizers are also called Chizhevsky's chandeliers. NAIs are oxygen atoms that contain an extra negatively charged electron. They are invisible, tasteless, and odourless. Superoxide and other activated oxygen species are responsible for the biological effects of NAIs [1].

Several commercial products can generate negative ions including air purifiers, energy stones, antibacterial showers, anion napkins, sanitary pads, special bracelets, Himalayan rock salt, and salt lamps. NAIs are abundant in the environment, especially in the sea, rainfall, waterfalls, green mountains, or forests. NAIs are a great blessing of Allah as they keep the human body alkaline, protecting it against several viruses, cancers, bacteria, infections, and other incurable ailments [2].

NAIs are also known as 'Nature's battery chargers' as they provide sufficient energy and are considered as essential as air or water [3]. Several researchers have reported improvement in different conditions using NAIs likely anti-aging, allergies, asthma, attention span, cognition, depression, emotions, mental energy, mood, reaction time, respiratory issues, and many other physiological functions [4-10]. A concentration of 1,600 to 1,500,000 ions/cm^3 of NAIs for less than an hour to weekly intervals have been used in different experiments [4]. Apart from human studies, a negative air environment provided using salt lamps has been reported to enhance 5-HT metabolism in animal models [11]. Hence, NAIs have potential therapeutic effects on several ailments in animals and humans.

Cerebral palsy (CP) is a neurological disorder that affects movement [12]. The signs and symptoms vary from person to person based on the extent of the brain injury. Worldwide, the ratio of male CP patients is higher and the 1.4:1 male-to-female CP ratio has been reported in Karachi, Pakistan [13]. Primarily, it manifests poor coordination, stiff or weak muscles, and tremors but can also affect abilities to sense, see, hear, or speak. CP patients may have spastic or flaccid bodies with dominant behavioral issues like anxiety, anger, agitation, dependency on others, hyperactivity, incompatibility, stubbornness, and many more [12].

Spasticity manifests in almost 80% of CP-inflicted cases which causes either weakness or stiffness of voluntary movement of joints [12]. Spasticity is normally associated with intermittent or permanent activation of voluntary muscles resulting in weakness or rigidity while moving joints. Spasticity occurs when the nerve pathways that control muscle movement are interrupted or damaged due to brain injury, spinal cord injury, or diseases like amyotrophic lateral sclerosis and multiple sclerosis. It hinders normal functioning by causing rigidity, pain, disturbed sleep patterns, and difficulties in accomplishing the activities of daily life (ADL). Spasticity can aggravate and joints may become immovable because of rigidity if timely interventions are not provided [14]. Several interventions to treat spasticity including anticonvulsant medicines, bimanual training, botulinum toxins, bisphosphonates, context-focused therapies, hip surveillance, fitness training, goal-directed training, home interventions, occupational therapies, and selective dorsal rhizotomy have been reported [15,16]. In earlier studies, we reported the assertive effects of environmental enrichment techniques and Al Fatiha administration on the behavioral and physical outcomes in spastic CP-inflicted patients [17, 18].

Due to the presence of spasticity and behavioral deficits, most CP patients cry, shout, and throw outbursts as observed in our preliminary study at the rehabilitation centre. Behavioral issues may hamper the cooperation or willingness of CP patients for physiotherapy which is difficult for physiotherapists and the patients themselves. It is very challenging for physiotherapists to conduct the sessions without the calm, cooperative, and good communicative behavior of CP patients. Though several researchers have reported the use of NAIs for many ailments in animal or human models, and to the best of our knowledge there are no evidence-based studies to support the use of NAIs for the rehabilitation of CP-inflicted patients. This novel intervention study was an attempt to determine if exposure to NAIs can alleviate spasticity and normalize behaviors in CP-inflicted patients.

Method

Study design & subject selection

It was a first-phase clinical trial for which the sample size can be lower than twenty [19]. The associated pediatric psychiatrist at Al Umeed Rehabilitation Association (AURA) referred some regular participants for the study. The administration of AURA used a concealed method to allocate participants to the control and intervention groups (Figure 1). Twenty-eight CP-inflicted participants were allocated to seven classrooms with four participants in each room and all classrooms were 40 square feet. This study was conducted in the year 2021 from mid of February to the mid of April. Both groups continued their regular (twice-a-week) physiotherapy sessions

while the intervention group also received additional negative ionizer exposure along with regular physiotherapy sessions.

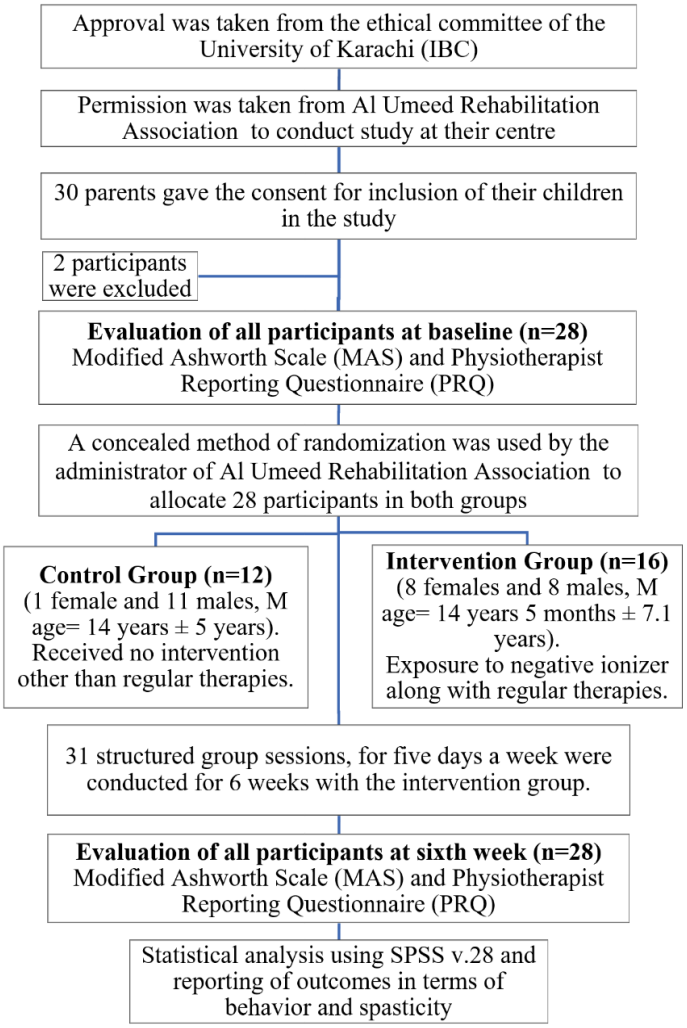

Figure 1: Schematic presentation of the protocol

Ethical consideration

Ethical approval was taken from the 'Institutional Bio-Ethics Committee' of the University of Karachi (Approval IBC-2017).

Characteristic details

Each study participant's medical history and physical characteristics were noted on a formally designed form (Table 1).

Inclusion and exclusion criteria

- Participants whose parents voluntarily permitted were included in the intervention study.
- Participants with no history of any surgery to treat spasticity in the last 6 months were included.
- Participants without any medical conditions that might interfere with the administration of intervention were included.
- Participants with frequent attacks of epilepsy were excluded as epilepsy might aggravate spasticity.

Intervention administration

31 structured group sessions, 5 days a week were conducted for 6 weeks with the intervention group. The intervention group received forty-minute exposure to 10000 negative ions/cm^3 in an enclosed classroom. In our pilot study, the ion concentration monitoring indicated that the ionizer took 10 minutes to disperse 10000 ions/cm^3 in a room of 40 square feet. So, for the present study, 40 minutes session was started 10 minutes after turning on the ionizer. The monitoring of ion concentration was performed twice (once at the beginning and another at end of each session).

Apparatus

The negative ionizer model number 'JHQ- 801' and mini air ion counter 'KT-401' were purchased from the online store of Daraz. JHQ-801 can produce $7\times10E^6/cm^3$ ions and has a built-in fan that strongly disperses negatively charged ions in the air. The test range of the ion counter is between 1x10000 - 1999x10000 ions/cm^3. The mechanism of action of the negative air ionizer is illustrated in Figure 2.

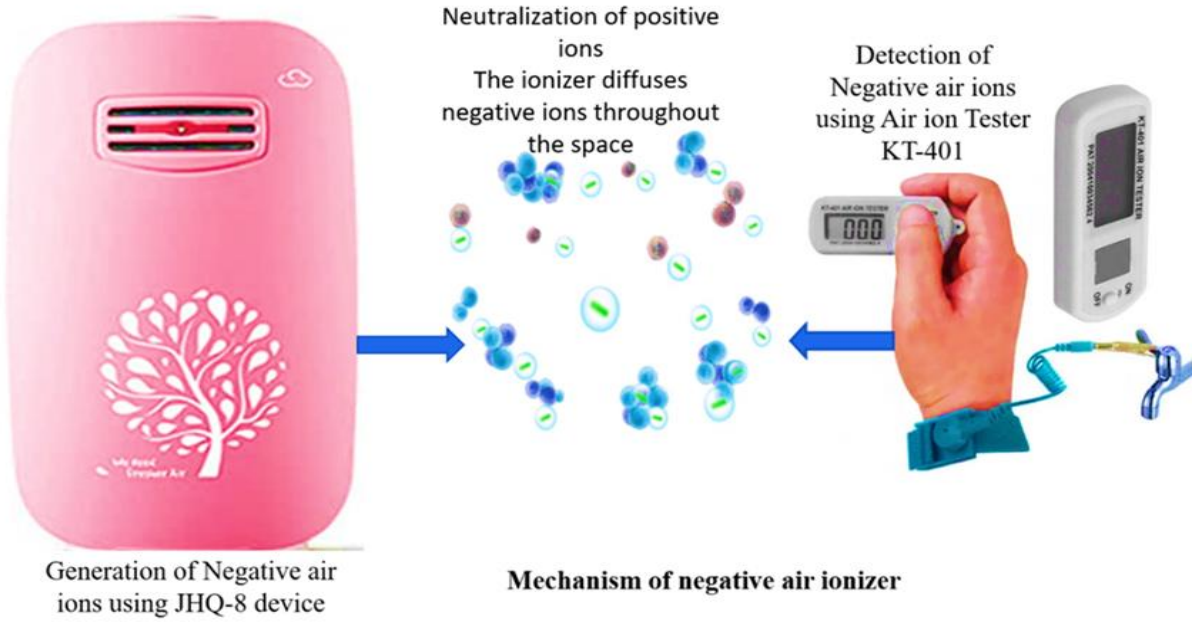

Figure 2: Mechanism of action of negative air ionizer and detector.

Measurement scales

Two scales namely the researcher-designed 'Physiotherapist reporting questionnaire (PRQ)' and 'Modified Ashworth Scale (MAS)' were employed for this study. The PRQ encompassed ten variables and was designed on a seven-point Likert Scale (far below "1", moderately below "2, slightly below "3", met expectations "4", slightly above "5", moderately above "6", and far above "7"). The physiotherapy staff at AURA also helped in designing the PRQ. Spasticity was assessed by using the MAS [20]. The muscle tone is graded ranging from normal tone (0) to severe spasticity (4). The upper extremity joints included evaluation of the elbow, shoulder, and wrist, whereas the lower extremity joints included the evaluation of abduction, adduction, ankle, hip, and knee. Clinically a reduction of ≥1 in the MAS scoring in at least 1 of the upper/lower limbs is considered a "MAS change".

All respective physiotherapists assessed participants for PRQ and MAS scales at baseline (Time 1), and in the sixth week (Time 2). It was a single-blind design for physiotherapists as they were kept unaware of the assigned groups for all participants. All marked forms were rechecked by senior management, the director, and the deputy director of therapy at AURA.

Statistical analysis
SPSS version 28 was used to perform Wilcoxon signed rank test and descriptive statistics were calculated to determine pre-to-post PRQ and MAS changes.

Results
The study participants in the intervention group encompassed all levels of the Gross Motor Function Classification System (GMFCS) (I, n=2; II, n=2; III, n=4; IV, n=7; V, n=1). Dominant physical characteristics of participants in the intervention group were 62.5% spastic, 56.2% diplegic, 50% moderately severe, 81.2% wheelchair-bound, and 43.7% GMFCS level IV (Table 1). Whereas the control group encompassed 4 GMFCS levels (I, n=3; II, n=1; III, n=2; IV, n=6). Dominant physical characteristics of participants in the control group were 58.3% spastic, 33.3% diplegic, 33.3% dystonia, 66.6% mildly severe, 41.6% wheelchair-bound, 41.6% independent, and 50% GMFCS level IV (Table 1).

Table 1: Physical characteristics of the participants

Categories	Sub-categories	Control Group	Intervention Group
Gross Motor Function Classification System (GMFCS)	Level I	3 (25)	2 (12.5)
	Level II	1 (8.33)	2 (12.5)
	Level III	2 (16.6)	4 (25)
	Level IV	6 (50)	7 (43.7)
	Level V	0	1 (6.25)
Muscle tone	Spastic	7 (58.3)	10 (62.5)
	Hypotonic	5 (41.6)	6 (37.5)
Mobility	Wheelchair-bound	5 (41.6)	13 (81.2)
	Independent	5 (41.6)	1 (6.25)
	Walker	2 (16.6)	2 (12.5)
Severity	Mild	8 (66.6)	4 (25)
	Moderate	2 (16.6)	8 (50)
	Severe	2 (16.6)	4 (25)
Topographical Distribution	Diplegia	4 (33.3)	9 (56.2)
	Athetoid	3 (25)	3 (18.7)
	Hemiplegia	1 (8.33)	2 (12.5)
	Dystonia	4 (33.3)	0
	Monoplegia	0	1 (6.25)
	Triplegia	0	1 (6.25)

Values are frequencies and percentages.

PRQ
In the intervention group, participant's mean calmness during physiotherapy improved from 3.43 to 3.93 (p<0.05), and mean cooperation during physiotherapy improved from 4.5 to 5 (p<0.05), mean improvement in physical condition from 4.12 to 4.75 (p<0.05) and mean willingness for physiotherapy also improved from 4 to 4.81 (p<0.05). Whereas in the control group none of the median post-test ranks were statistically higher than the pre-test ranks (Table 2).

Table 2: Pre-to-Post comparison of physiotherapist response to PRQ

Variables	Group	Baseline		Sixth week		Wilcoxon signed rank	
		Mean	SD	Mean	SD	z value	*p*-value
Calmness during	Control	4.083	1.975	3.916	2.020	-.378	0.705

physiotherapy	Intervention	3.437	1.504	3.937*	1.768	-1.994	0.046
Communication with therapist	Control	4.75	1.602	5	1.651	-1.089	0.276
	Intervention	3.937	1.436	4.312	1.302	-1.857	0.063
Cooperation during physiotherapy	Control	4.666	1.922	4.583	2.020	-1.000	0.317
	Intervention	4.5	1.366	5*	1.211	-2.530	0.011
Crying during physiotherapy	Control	1.583	1.240	1.5	1	-.447	0.655
	Intervention	2.187	1.721	2.062	1.730	-.816	0.414
Fine motor skills	Control	4.25	1.422	4	1.651	-1.342	0.180
	Intervention	3.375	1.746	3.5	1.712	-1.000	0.317
Decrease in spasticity	Control	2.583	1.621	2.75	1.815	-.447	0.655
	Intervention	4.125	1.821	4.187	1.833	-.144	0.885
Gross motor skills	Control	4.333	1.969	4.416	1.975	-.447	0.655
	Intervention	3.875	1.892	3.937	1.806	-.378	0.705
Improvement in the physical condition	Control	4.083	1.505	4.333	1.302	-1.342	0.180
	Intervention	4.125	1.707	4.75*	1.341	-2.232	0.026
Involuntary movement	Control	3	1.954	3.25	1.912	-1.732	0.083
	Intervention	3	2.250	3.125	2.247	-1.000	0.317
Willingness for physiotherapy	Control	4.583	1.621	4.583	1.564	.000	1
	Intervention	4	1.632	4.812*	1.470	-2.401	0.016

Values are means and SD is evaluated using Wilcoxon signed-rank test for the control and intervention groups. Significant differences at $^{*}p<0.05$.

MAS

Figure 3 shows the frequency of spastic and normal joints in both extremities of both groups at baseline and sixth week. Initially, the overall spasticity of any grade from 1-4 on the MAS scale was categorized as spastic joints and normal joints were the ones that were graded 0 as per the MAS scale by the physiotherapists. This helped in the pre-to-post comparison of differences among both groups. There were comparable differences i.e., ≤1 among both groups in pre-to-post comparison of normal and spastic joints in both extremities.

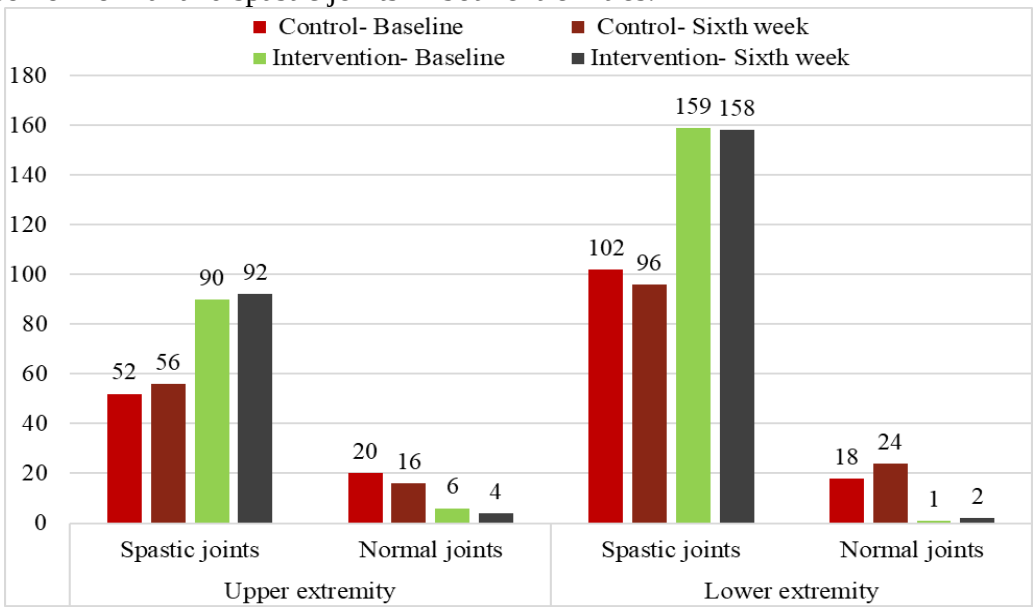

Figure 3: Frequency of normal and spastic joints in both groups at baseline and sixth week.

For the MAS scale, the participants were evaluated in grades 0 to 4 to report MAS changes by categorizing them as stable, deteriorated, or alleviation in spasticity. If the pre-to-post MAS

grading for a joint was the same it was categorized as 'stable', for an increased muscle tone it was categorized as 'deteriorated' and for a decreased muscle tone joint was categorized in the category of 'alleviation of spasticity'. Table 3 shows the descriptive pre-to-post MAS changes for both groups. Table 4 shows that the stability and deterioration of joints in both extremities of the intervention group were higher (81.6%, and 16.8%) as compared to the control group (77.6%, and 11.9%) respectively. Moreover, the alleviation of spasticity in both extremities of the intervention group was comparably lower (3.51%) than the control group (10.4%).

Table 3: Descriptive pre-to-post MAS changes in joints

Extremity	Joint	Groups	Stable	Deteriorated	Alleviation of spasticity
Upper	Right Elbow	Control	11 (91.6)	1 (8.33)	0
		Intervention	14 (87.5)	2 (12.5)	0
	Left Elbow	Control	11 (91.6)	0	1 (8.33)
		Intervention	14 (87.5)	2 (12.5)	0
	Right Shoulder	Control	9 (75)	3 (25)	0
		Intervention	13 (81.5)	3 (18.7)	0
	Left Shoulder	Control	8 (66.6)	4 (33.3)	0
		Intervention	12 (75)	4 (25)	0
	Right Wrist	Control	12 (100)	0	0
		Intervention	12 (75)	3 (18.7)	1 (6.25)
	Left Wrist	Control	12 (100)	0	0
		Intervention	12 (75)	4 (25)	0
Lower	Right Ankle	Control	8 (66.6)	2 (16.6)	2 (16.6)
		Intervention	14 (87.5)	1 (6.25)	1 (6.25)
	Left Ankle	Control	8 (66.6)	2 (16.6)	2 (16.6)
		Intervention	15 (93.7)	1 (6.25)	0
	Right Abduction	Control	8 (66.6)	1 (8.33)	3 (25)
		Intervention	13 (81.5)	2 (12.5)	1 (6.25)
	Left Abduction	Control	9 (75)	0	3 (25)
		Intervention	14 (87.5)	2 (12.5)	0
	Right Adduction	Control	7 (58.3)	3 (25)	2 (16.6)
		Intervention	12 (75)	2 (12.5)	2 (12.5)
	Left Adduction	Control	7 (58.3)	2 (16.6)	3 (25)
		Intervention	13 (81.5)	2 (12.5)	1 (6.25)
	Right Hip	Control	9 (75)	2 (16.6)	1 (8.33)
		Intervention	12 (75)	4 (25)	0
	Left Hip	Control	9 (75)	2 (16.6)	1 (8.33)
		Intervention	13 (81.2)	3 (18.7)	0
	Right Knee	Control	11 (91.6)	0	1 (8.33)
		Intervention	12 (75)	2 (12.5)	2 (12.5)
	Left Knee	Control	10 (83.3)	1 (8.33)	1 (8.33)
		Intervention	14 (87.5)	1 (6.25)	1 (6.25)

Values are frequencies and percentages.

Table 4: Pre-to-Post MAS changes concerning both extremities

Group	Extremity	Stable n (%)	Deteriorated n (%)	Alleviation of

				spasticity n (%)
Control	Upper	63 (87.5)	8 (11.1)	1 (1.38)
	Lower	86 (71.6)	15 (12.5)	19 (15.8)
Intervention	Upper	77 (80.2)	18 (18.7)	1 (1.04)
	Lower	132 (82.5)	20 (12.5)	8 (5)

Values are frequencies and percentages.

Discussion

PRQ evaluation showed that intervention significantly exhibited improvement in calmness during physiotherapy, cooperation during physiotherapy, the physical condition of the participants, and willingness for physiotherapy (Table 2). Sometimes, it is a very challenging task for physiotherapists to convince CP-inflicted patients for physiotherapy sessions as such patients may have disabilities in vision, hearing, speaking or other functions too along with abnormal behaviors [12]. Since NAIs have several potential therapeutic effects [2-10] so, we anticipate that NAIs may have normalized the agitative, anti-social, hyperactive, incompatible, and stubborn behaviors of participants as reported in PRQ.

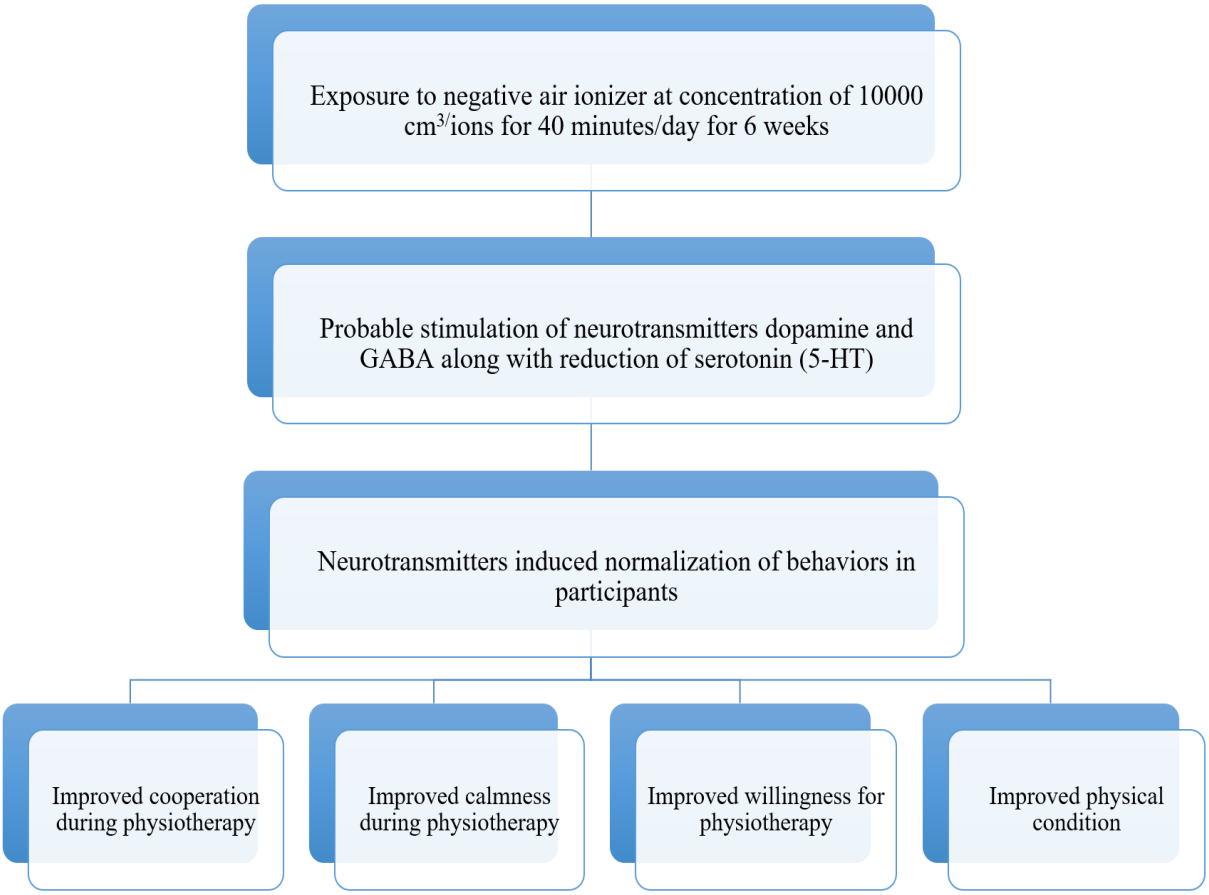

Figure 4: A suggested mechanism for improvement in PRQ evaluation

Gamma-aminobutyric acid (GABA) is known as an inhibitory neurotransmitter that reduces the activity in the CNS by blocking certain signals from the brain to produce a calming effect. GABA lowers heart rate and blood pressure which helps the brain to relax and fall asleep [21]. Dopamine is known as the brain's reward system and regulates voluntary movement [22]. Serotonin (5-HT) plays important role in controlling the central nervous system (CNS) and the peripheral nervous system (PNS). In CNS, serotonin acts as neurotransmitter to control appetite, autonomic neural activity, body temperature, mood, sleep, and stress response. In PNS, serotonin acts as a peripheral

hormone to regulate motor and secretory functions. Moreover, it has a fundamental role to regulate cell growth, heart rate, homeostasis, vascular tone, immunity, inflammatory response, and respiratory drive [23]. NAIs reduce serotonin levels in the blood or brain [24]. Current research also helped us to anticipate that there may be a possible NAIs-induced stimulation of neurotransmitters dopamine, and GABA along with a reduction of serotonin which exhibited improvement in behaviors (Figure 4). However, we did not measure dopamine, GABA, or serotonin in the current study. Measurement of serotonin and dopamine in CP animal models could help us to assert our findings.

MAS scale findings showed that the overall ratio of stability and deterioration of joints were relatively higher but the alleviation of spasticity in the intervention group than in the control group (Table 4). The physical characteristics data showed that were more serious cases of CP in the intervention group at baseline in terms of spasticity, topographical distribution, severity, mode of transition, and GMFCS levels (Table 1). The minimal alleviation of spasticity was observed in the intervention group which might be due to the partial reinstation of the damaged nerve pathways which facilitated the alleviation of spasticity. A CP patient may have a different pattern of spasticity on both sides of the limbs [14]. So, if each case of CP is different from one other, the intervention-induced physical outcomes of participants cannot be similar for all inducted participants as depicted in our findings.

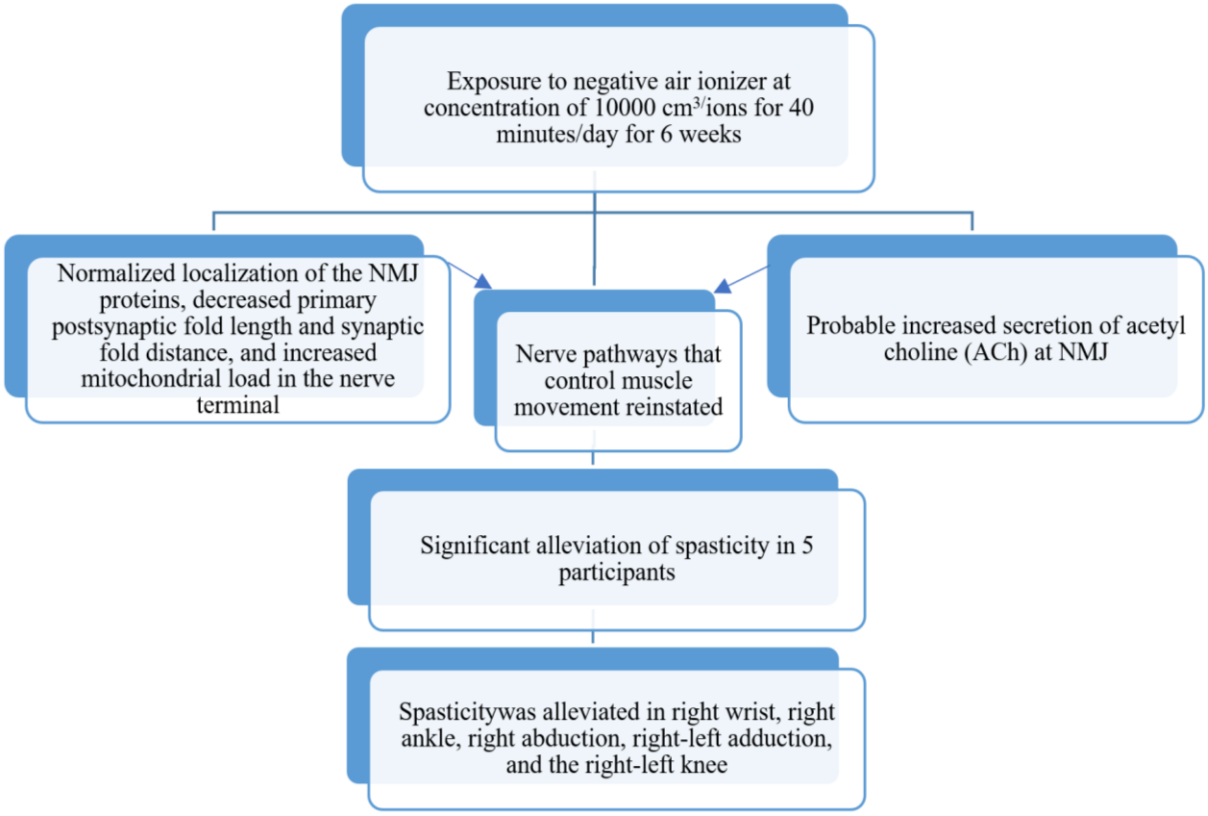

Figure 5: A suggested mechanism for the alleviation of spasticity using NAIs in CP

Acetylcholine (ACh) is a neurotransmitter that plays a key role in muscle movement, learning, and memory. It is released from cholinergic interneurons and works at the neuromuscular junction (NMJ). The NMJ is the junction where motor nerves communicate with muscle fibers and it requires signaling among motor neurons, muscle fibers, and perisynaptic neurolemmocytes/Schwann cells. CP patients may have altered NMJ protein localization, greater postsynaptic fold length and synaptic fold distance, and reduced mitochondrial load in the nerve terminal. Understanding NMJ deficits and odd neuromotor functions in a CP patient would be a

diagnostic pathway for understanding abnormal movement [25]. We predict that NAIs in the current study may have reinstated neuronal pathways, increased secretion of ACh, normalized localization of the NMJ proteins, decreased primary postsynaptic fold length and synaptic fold distance, and increased mitochondrial load at the nerve endings in the intervention group (Figure 5).

At the cellular level, the superoxide and other activated oxygen species in the NAIs prevent the oxidation of free radicals which revitalize the cells to provide immunity and resistance [1, 24]. These neutralized cells may produce reliable effects on cardiovascular, digestive, immune, respiratory, and mental health [2-10]. Since there are no studies on this topic so, the exact mechanism of action of NAIs at the cellular and molecular level behind the normalization of behaviors or spasticity is yet to be elucidated.

Though the current findings were not very remarkable as per our expectation, we envisage that a longer duration of exposure to NAIs may be more beneficial in ameliorating behavioral and physical outcomes in CP-inflicted patients. The limitations of the study are that it was a first-phase clinical trial and the absence of any pre-established scale to assess behaviors of CP-inflicted patients during physiotherapy. Based on our findings, we suggest the inclusion of this novel intervention in the multidisciplinary rehabilitation plan after going through the second and third-phase clinical trials.

Conclusion

The study concludes that NAIs may alleviate spasticity and normalize behaviors during physiotherapy sessions. However, we recommend further research with a longer duration and bigger sample size to validate our reported findings.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals were used in this study. The study on humans was conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

None.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

1. Goldstein NI, Goldstein RN, Merzlyak MN. Negative air ions as a source of superoxide. Int J Biometeorol 1992; 36(2): 118-22.
2. Jiang SY, Ma A, Ramachandran S. Negative air ions and their effects on human health and air quality improvement. Int J Mol Sci 2018; 19(10): 2966.
3. Perez V, Alexander DD, Bailey WH. Air ions and mood outcomes: a review and meta-analysis. BMC Psychiatry 2013; 13(1): 1-20.
4. Alexander DD, Bailey WH, Perez V, Mitchell ME, Su S. Air ions and respiratory function outcomes: a comprehensive review. J Negat Results Biomed 2013; 12(1): 1-6.
5. Hagiwara G, Mankyu H, Tsunokawa T, Matsumoto M, Funamori H. Effectiveness of positive and negative ions for elite Japanese swimmers' physical training: Subjective and biological emotional evaluations. Appl Sci 2020; 10(12): 4198.
6. Tom G, Poole MF, Galla J, Berrier J. The influence of negative air ions on human performance and mood. Hum Factors. 1981; 23(5): 633-6.
7. Wallner P, Kundi M, Panny M, Tappler P, Hutter HP. Exposure to air ions in indoor environments: Experimental study with healthy adults. Int J Environ Res Public Health 2015; 12(11): 14301-11.
8. Hawkins LH, Barker T. Air ions and human performance. Ergonomics 1978; 21(4): 273-8.
9. Inbar O, Rotstein A, Dlin R, Dotan R, Sulman FG. The effects of negative air ions on various physiological functions during work in a hot environment. Int J Biometeorol 1982; 26(2): 153-63.
10. Ryushi T, Kita I, Sakurai T, Yasumatsu M, Isokawa M, Aihara Y, *et al*. The effect of exposure to negative air ions on the recovery of physiological responses after moderate endurance exercise. Int J Biometeorol 1998; 41(3): 132-36.
11. Naz H, Haleem DJ. Exposure to illuminated salt lamp increases 5-HT metabolism: A serotonergic perspective to its beneficial effects. Pak J Biochem Mol Biol 2010; 43(2): 105-108.
12. Vitrikas K, Dalton H, Breish D. Cerebral Palsy: An Overview. Am Fam Physician 2020; 101(4): 213-20.
13. Rafique A, Naz H. A Survey-based report on the occurrence of Cerebral Palsy in Urban areas of Karachi. J Pak Med Assoc 2020; 70(8): 1442-4.
14. Barnes MP. Management of spasticity. Age Ageing 1998; 27(2): 239-45.
15. Novak I, Mcintyre S, Morgan C, Campbell L, Dark L, Morton N, *et al*. A systematic review of interventions for children with cerebral palsy: state of the evidence. Dev Med Child Neurol 2013; 55(10): 885-910.
16. Fan J, Milosevic R, Wang S. Selective peripheral neurotomy (SPN) as a treatment strategy for spasticity. Brain Sci Adv 2020; 6(1): 30-41.
17. Rehman A, Naz H, Rafique A. Effectiveness of environmental enrichment techniques on spastic diplegia and behavioral modulation of three cerebral palsy Pakistani children. Int J Sci Eng Res 2019; 10(12): 228-32.
18. Rafique A, Naz H, Farah D. Perception Towards Quranic Intervention and Chapter Al Fatiha Induced Amelioration of Cognitive, Behavioral, and Physical Skills in a Child with Cerebral Palsy: Possible Involvement of Brain Stimulation. Br J Med Health Sci 2022; 4(8): 1297-306.
19. Storer, B.E. Design and analysis of phase I clinical trials. Biometrics 1989; 45(3): 925-937.
20. Bohannon Rw, Smith M. Interrater reliability of a modified Ashworth scale of muscle spasticity. Phys Ther. 1987; 67(2): 206-7.
21. Roberts E. γ-aminobutyric acid and nervous system function—A perspective. Biochem Pharmacol 1974; 23(19): 2637-49.
22. Teleanu RI, Niculescu AG, Roza E, Vladâcenco O, Grumezescu AM, Teleanu DM. Neurotransmitters—Key factors in neurological and neurodegenerative disorders of the central nervous system. Int J Mol Sci 2022; 23(11): 5954

23. Kanova M, Kohout P. Serotonin—Its synthesis and roles in the healthy and the critically ill. Int J Mol Sci 2021; 22(9): 4837.
24. Krueger AP, Reed EJ. Biological impact of small air ions. Science. 1976; 193(4259): 1209-13.
25. Nishimune H, Shigemoto K. Practical anatomy of the neuromuscular junction in health and disease. Neurol Clin 2018; 36(2): 231-40.