NEGATIVE AIR IONS INDUCED AMELIORATION OF BIOCHEMICAL PARAMETERS IN CEREBRAL PALSY PATIENTS

Ammara Rafique
College of Physical Therapy & Rehabilitation, Indus University of Health Sciences, Karachi , Pakistan

Correspondence:
Ammara Rafique,
College of Physical Therapy & Rehabilitation, Indus University of Health Sciences, Karachi , Pakistan

Email:ammara-rafique92@gmail.com

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ABSTRACT
Cerebral palsy (CP) is an umbrella term for a group of neurological abnormalities that impair a person's ability to stand, balance, walk, and maintain posture. It is caused by injury or anomalies in the developing brain, which tend to happen before or soon after birth or in early childhood. The study aimed to explore the influence of negative air ions (NAIs) on biochemical parameters in CP-inflicted patients and was conducted from February 1st to March 30th, 2021. Thirty-one structured sessions of exposure to NAIs were conducted for six weeks after randomly allocating CP-inflicted participants into control (n=12; Median age: 14±5 years) and intervention groups (n=16; M age: Median age=14.5±7.1 years). Biochemical parameters included blood urea nitrogen, calcium, creatinine, electrolytes (bicarbonate, chloride, potassium, and sodium), liver function tests (alkaline phosphatase, alanine aminotransferase, aspartate transaminase, bilirubin total, direct bilirubin, and γ-glutamyl transpeptidase), protein tests (albumin, globulin, A/G ratio, and total proteins), and random blood sugar. For all tests, a trained phlebotomist collected blood samples from the premises of the rehabilitation center at baseline and at sixth week. The biochemical parameters were normal in both groups at baseline and sustained normality in assessment at the sixth week. The NAI intervention showed no negative effects, indicating that it can be a progressive, non-pharmacological, cost-effective, and a potential method to improve the quality of life of CP-inflicted patients.

Key Words: Cerebral Palsy, Biochemical parameters, Intervention, Negative Air Ions

INTRODUCTION

Cerebral palsy (CP) is an umbrella term for a group of neurological abnormalities that impair a person's ability to stand, balance, walk, and maintain posture. It is caused by injury or anomalies in the developing brain, which tend to happen before or soon after birth or in early childhood. CP may alter normal blood parameters, cognitive ability, hearing, metabolism, muscle coordination, motor movement, oral health, speech, vision, and many other functions (1,2). Some of the key etiological factors include birth asphyxia, consanguinity, genetic abnormalities, head injuries, home labor, hypoxic-ischemic encephalopathy, intrauterine growth restriction, infections, low gestational weight, microcephaly, multiple pregnancies, smaller gestational age, and many more (1,3). The majority of identified risk factors for CP are associated with the male gender and the prenatal developmental period (3,4). Nearly 17 million people are affected by CP all over the world (1) but the epidemiology and the etiology of CP are yet to be determined in Pakistan (5,6).

Certain abnormal biomarkers including amino acids, ammonia, calcium, cholesterol ethers, creatine phosphokinase, electrolytes, iron, phosphate, protein, urea, uric acid, and growth status have been reported in CP-inflicted patients (7-11).

Interventions are efforts done to enhance cognitive, motor, sensory, or social components of one's surroundings when there are clear cognitive or motor deficiencies. Although CP is a lifelong impairment, several interventions can assist in modifying its impact on the body and activities of daily life. Healthcare providers have employed a variety of interventions to deal with behavioral and emotional issues, bowel problems, controlling pain, drooling, dysphagia, epilepsy, hearing and vision problems, insomnia, intellectual disability, movement issues, problems with communication, and spinal and hip abnormalities in CP-inflicted patients (12). Enriched

environment techniques can modulate behavior, spasticity, and cognitive outcomes in CP-inflicted patients (13).

The negative air ionizer/generator generates negative air ions (NAIs) by charging air molecules in the surrounding environment with high voltage. NAIs' biological function is reliant on superoxide and activated oxygen. Ion deficiency is the primary cause of many ailments, and inhaling NAIs is a successful treatment. Studies have demonstrated NAIs induced improvement in aging, allergies, attention, asthma, depression, cancer, cognition, depression, memory, mental energy, metabolism, mood, performance, reaction time, respiratory issues, sleep, spasticity, stress, and numerous physiological functions (14-18). Exposure to negative ionizers can be used as part of environmental enrichment strategies to benefit individuals inflicted with CP. Some studies suggest potential positive effects on humans and animals, while others find no significant impact or even report negative outcomes. Despite several findings, no studies have demonstrated the use of NAIs in the rehabilitation of individuals with CP. As a result, health practitioners remain dubious about employing negative air ionizers to treat CP. It was also an attempt to replenish the scarcity of data on NAI intervention-induced alterations in biochemical parameters of CP-inflicted patients. Thus this study was designed including CP-inflicted patients.

METHODS

The study was conducted from February 1st to March 30th, 2021, after ethical permission from the Institutional Bioethics Committee (Ref: IBC-2017) and the Al-Umeed Rehabilitation Association (AURA), where the intervention study was carried out. The pediatric psychiatrists at AURA identified patients with CP and these patient's participated in this study. Parental consent was taken for the inclusion of their children in the study. A total of 30 parents gave consent for the inclusion of their children in the study. Two participants were later excluded, and the remaining twenty-eight were divided into seven classes, each class with four participants. Each classroom measured roughly forty square feet. The administration of AURA employed a concealed randomization method to allocate all inducted participants to one of two groups: (a) Control (n=12; median age: 14±5 years) or (b) intervention groups (n=16; median age=14.5±7.1 years).

Due to a limited number of consents, participants were chosen regardless of age, gender, muscle tone, mode of transition, topographic presentation, or other additional impairments. Participants with any medical condition, a history of surgery or medical procedure, or blood transfusions in the previous six months were excluded. Participants who were denied permission by the institute were also barred from participating.

The control group was chosen to ensure that the comparisons and results were valid, acceptable, and generalizable. The intervention group participated in 31 scheduled sessions of negative ionizer exposure for 5 days/week for six weeks. Participants in the intervention group attended all of the intervention sessions regularly. 'Negative ionizer JHQ- 801' and 'KT-401 mini air ion counter' were purchased from the Daraz online shop for intervention administration and ion concentration monitoring, respectively. It was ensured that a concentration of 10000 NAIs/cm3 was present in whole session. Throughout the intervention, all study participants in each group continued to receive regular speech, occupational, physical, and medical care. All participants were already receiving several interventions at the center and the negative ionizer was added to the interventions offered to the intervention group.

Biochemical parameters included blood urea nitrogen, calcium, creatinine, electrolytes (bicarbonate, chloride, potassium, and sodium), liver function tests (alkaline phosphatase, alanine aminotransferase, aspartate transaminase, bilirubin total, direct bilirubin, and γ-glutamyl transpeptidase), protein A/G ratio (albumin, globulin, A/G ratio, and total proteins), and random blood sugar. For all blood tests, a trained phlebotomist collected blood samples from the premises of the rehabilitation center at baseline and sixth week. The samples were transported carefully to perform tests at 'The Laboratory' situated in Karachi, Pakistan.

Serum was collected from whole blood using the 'Labofuge 400R Centrifuge'. The 'Roche/Hitachi Cobas 6000 c 501' was used to measure blood urea nitrogen, calcium, creatinine, liver function tests, protein, and random

blood sugar levels in serum using a photometric method. The 'Nova biomedical 4+ electrolyte analyzer' was used to do electrolyte tests using the direct ion-selective electrode technique.

Statistical analysis

Statistical Package for Social Sciences (SPSS version 28.0) was used to analyze the collected data. To examine the demographical characteristics of the inducted CP-inflicted patients, descriptive statistics was used for calculating the frequencies and percentages. The demographic characteristics data encompassed gender, gross motor functional classification system (GMFCS) levels, muscle tone, topographical presentation, mode of transition, and additional impairments. For the biochemical parameters, the mean, standard deviation, standard error, and 95% confidence were determined. The data was parametric so, paired t-test was applied to measure the within-group mean differences for each parameter at baseline and the sixth week.

RESULTS

Demographic characteristics

Out of 28 participants, 9 (32.2%) were males and 19 (67.8%) were females. Table 1 shows the characteristics of inducted participants. In the intervention group, dominant cases presented GMFCS level IV in 43.7%, spastic muscle tone in 62.5%, wheelchair-bound mobility in 81.2%, and diplegic presentation in 56.2%, whereas, in control group, dominant cases presented GMFCS level IV in 50%, spastic muscle tone in 58.9%, wheelchair-bound mobility in 58.3%, and triplegic presentation was seen in 58.3%.

Biochemical parameters

In the control group, paired t-tests showed significantly increased blood urea nitrogen ($p<0.01$), chloride ($p<0.001$), creatinine ($p<0.01$), direct bilirubin ($p<0.01$), and sodium ($p<0.001$) (Tables 2, 3 and 4). In the intervention group, paired t-tests showed significantly increased chloride ($p<0.01$), creatinine ($p<0.01$), globulin ($p<0.05$), sodium ($p<0.001$), and total protein ($p<0.05$) (Tables 2, 3 and 4) along with a significant decrease in the A/G ratio test ($p<0.05$) (Table 2).

Table 1: Characteristics of the study participants (n=28)

Characteristics	Control group n(%)	Intervention group n(%)
Gender		
Male	1(8.3)	8(50)
Female	11(91.6)	8(50)
Gross Motor Functional Classification System (Levels)		
I	3(25)	2(12.5)
II	1(8.33)	2(12.5)
III	2(16.6)	4(25)
IV	6(50)	7(43.7)
V	0	1(6.25)
Muscle tone		
Spastic	7(58.3)	10(62.5)
Hypotonic	5(41.6)	6(37.5)
Topographical presentation		
Monoplegic	0	1(6.25)

Hemiplegic	1(8.33)	2(12.5)
Diplegic	4(33.3)	9(56.2)
Triplegic	7(58.3)	4(25)
Quadriplegic	0	0
Mode of transition		
Wheelchair	7(58.3)	13(81.2)
Independent	3(25)	1(6.25)
Walker	2(16.6)	2(12.5)
Additional impairments		
Epilepsy	2(16.6)	1(6.25)
Poor attention	4(33.3)	2(12.5)
Speech impairment	3(25)	2(12.5)

Table 2: Summary of blood parameters: Intervention versus control group

Group	Evaluation	Mean±SD	Std. Error	95% Confidence Interval for Mean		*p*-value
				Lower Bound	Upper Bound	
Total proteins (Normal: 6.6-8.7 G%)						
Control	Baseline	7.6±0.3	0.11	7.35	7.84	0.09
	Sixth week	7.8±0.4	0.12	7.53	8.08	
Intervention	Baseline	7.5±0.4	0.10	7.32	7.78	0.01
	Sixth week	7.8*±0.3	0.09	7.63	8.06	
Albumin (Normal: 3.2-4.5 G%)						
Control	Baseline	4.6±0.3	0.09	4.48	4.89	0.07
	Sixth week	4.8±0.3	0.10	4.58	5.02	
Intervention	Baseline	4.5±0.2	0.07	4.41	4.71	0.19
	Sixth week	4.6±0.2	0.06	4.51	4.81	
Globulin (Normal: 1.9-2.8 G%)						
Control	Baseline	2.9±0.2	0.07	2.74	3.06	0.30
	Sixth week	3.0±0.2	0.05	2.87	3.12	
Intervention	Baseline	2.9±0.4	0.11	2.73	3.23	0.01
	Sixth week	3.1*±0.4	0.10	2.96	3.40	
Albumin/Globulin ratio (Normal: 1.1-2.2)						
Control	Baseline	1.6±0.2	0.05	1.49	1.75	0.75
	Sixth week	1.6±0.1	0.04	1.51	1.70	
Intervention	Baseline	1.5±0.2	0.07	1.41	1.72	0.04
	Sixth week	1.4*±0.2	0.05	1.37	1.59	
Random sugar (Normal: Upto 180 mg%)						
Control	Baseline	83.9±11.9	3.44	76.3	91.4	0.46
	Sixth week	87.5±15.4	4.47	77.6	97.3	

Intervention	Baseline	90.0±18.2	4.56	80.3	99.7	0.25
	Sixth week	94.1±18.5	4.63	84.3	104.0	
Blood urea nitrogen (Normal: 7-21 mg%)						
Control	Baseline	10.4±3.23	0.93	8.36	12.4	0.004
	Sixth week	11.9**±3.72	1.07	9.54	14.2	
Intervention	Baseline	11.1±3.05	0.76	9.55	12.8	0.88
	Sixth week	11.0±2.11	0.52	9.93	12.1	
Creatinine (Normal: 0.6-1.3 mg%)						
Control	Baseline	0.55±0.17	0.04	0.44	0.66	0.003
	Sixth week	0.65**±0.17	0.04	0.54	0.76	
Intervention	Baseline	0.48±0.14	0.03	0.40	0.55	0.006
	Sixth week	0.53**±0.16	0.04	0.44	0.61	
Calcium (Normal: 8.1-10.4 mg%)						
Control	Baseline	9.83±0.30	0.08	9.63	10.0	0.79
	Sixth week	9.85±0.35	0.10	9.63	10.0	
Intervention	Baseline	9.70±0.41	0.10	9.48	9.92	0.10
	Sixth week	9.82±0.39	0.09	9.61	10.0	

Values are significant at $^{*}p$ <0.05 and $^{**}p$<0.01.

Table 3: Summary of Liver Function Test: Intervntion versus control groups

Group	**Evaluation**	**Mean±SD**	**Std. Error**	**95% Confidence Interval for Mean**		***p*-value**
				Lower Bound	**Upper Bound**	
Bilirubin total (Normal: 0.1-1.2 mg%)						
Control	Baseline	0.38±0.19	0.05	0.25	0.50	0.20
	Sixth week	0.45±0.18	0.05	0.33	0.57	
Intervention	Baseline	0.37±0.31	0.07	0.20	0.53	0.98
	Sixth week	0.37±0.36	0.09	0.17	0.57	
Direct bilirubin (Normal: 0.1-0.4 mg%)						
Control	Baseline	0.14±0.05	0.01	0.10	0.17	0.001
	Sixth week	0.19**±0.05	0.01	0.15	0.22	
Intervention	Baseline	0.13±0.09	0.02	0.08	0.18	0.12
	Sixth week	0.15±0.09	0.02	0.09	0.20	
Aspartate transaminase (Normal: Upto 46 U/L)						
Control	Baseline	25.9±11.1	3.22	18.8	33.0	0.43
	Sixth week	23.5±5.85	1.68	19.7	27.2	
Intervention	Baseline	23.5±7.41	1.85	19.5	27.4	0.68
	Sixth week	23.2±6.77	1.69	19.6	26.8	
Alanine aminotransferase (Normal: Upto 49 U/L)						
Control	Baseline	22.9±18.3	5.30	11.2	34.5	0.27

	Sixth week	17.0±6.06	1.74	13.1	20.8	
Intervention	Baseline	17.3± 6.8	1.71	13.7	21.0	0.05
	Sixth week	14.8± 5.01	1.25	12.2	17.5	
Gamma-glutamyl transferase (Normal: 7-32 U/L)						
Control	Baseline	12.4±4.64	1.33	9.46	15.3	0.15
	Sixth week	13.1±4.70	1.35	10.1	16.1	
Intervention	Baseline	12.1±4.19	1.04	9.95	14.4	0.56
	Sixth week	11.8±3.03	0.75	10.2	13.4	
Alkaline phosphatase (Normal: 35-105 U/L)						
Control	Baseline	178.4±90.5	26.1	120.9	235.9	0.85
	Sixth week	176.1±78.1	22.5	126.5	225.8	
Intervention	Baseline	167.1±75.5	18.8	126.8	207.3	0.33
	Sixth week	179.5±100.5	25.1	125.9	233.0	

Values significant at $^{**}p<0.01$.

Table 4: Summary of serum Electrolytes: Intervention versus control groups

Group	**Evaluation**	**Mean±SD**	**Std. Error**	**95% Confidence Interval for Mean**		***p-value***
				Lower Bound	**Upper Bound**	
Sodium (Normal: 136-149 M Eq/L)						
Control	Baseline	139.1±0.90	0.26	138.5	139.6	<0.001
	Sixth week	144.5***±2.24	0.64	143.1	145.9	
Intervention	Baseline	139.5±2.09	0.52	138.4	140.6	<0.001
	Sixth week	144.0***±2.07	0.51	142.9	145.1	
Potassium (Normal: 3.8-5.2 M Eq/L)						
Control	Baseline	4.59±0.39	0.11	4.34	4.84	0.19
	Sixth week	4.39±0.33	0.09	4.18	4.60	
Intervention	Baseline	4.47±0.38	0.09	4.26	4.67	0.25
	Sixth week	4.35±0.42	0.10	4.12	4.57	
Chloride (Normal: 98-107 M Eq/L)						
Control	Baseline	102.9±1.00	0.29	102.3	103.6	0.0002
	Sixth week	105.0***±1.55	0.44	104.0	106.0	
Intervention	Baseline	102.6±2.04	0.51	101.5	103.7	0.006
	Sixth week	104.6**±2.64	0.66	103.2	106.0	
Bicarbonate (Normal: 25-29 M Eq/L)						
Control	Baseline	28.4±1.08	0.31	27.7	29.1	1
	Sixth week	28.4±1.67	0.48	27.3	29.4	
Intervention	Baseline	29.3±1.62	0.40	28.4	30.1	0.06
	Sixth week	28.4±1.45	0.36	27.6	29.2	

Values significant at $^{**}p<0.01$, and $^{***}p<0.001$.

DISCUSSION

In the present study, the participants in the intervention group were exposed to 10000 ions/cm3 for 40 minutes which significantly improved chloride, creatinine, globulin, total proteins, and sodium levels within the normal range.

Electrolytes play a significant role in maintaining blood clotting, blood pressure, chemical reactions, muscle contraction, and water equilibrium for the normal functioning of the human body. The intervention group participants of this study presented an increase in the concentration of electrolytes (i.e. chloride and sodium) and total proteins within the normal range. These changes are consistent with previous study (10) that suggested these changes could be associated with the hypohydration status (19).

NAIs can increase the affinity of hemoglobin for oxygen, raising the partial pressure of oxygen and decreasing the partial pressure of carbon dioxide in the blood. In this way, it reduces the rate of respiration, which improves the metabolism of water-soluble vitamin C and B-complex. Vitamin B12 is required for metabolism, red blood cell formation, and the maintenance of proper central nervous system function. Vitamin B7 is necessary for carbohydrate, lipid, and protein metabolism, and a lack of it can cause anemia, melancholy, cardiac problems, and muscular pains. Vitamin C is an antioxidant that helps with iron absorption, tissue health, bone and collagen formation, wound healing, immunity, and blood vessel strength. Vitamin C deficiency can result in scurvy, poor tissue growth, tooth loss, and impaired wound healing (20). Deficiency in water-soluble vitamins has significant clinical consequences or even death. NAIs activate various biological systems. As a result, NAI-induced improvements in water-soluble vitamins may have contributed to considerably higher levels of globulin and total proteins in the intervention group within the normal range.

The participants in the present study were exposed to a concentration of 10000 ions/cm3 and a concentration over 1000 ions/cm3 has been found to increase immune functioning (21). For a healthy immune system, there must be an equilibrium between the negative and positive ions in the body. NAIs can increase the cell membrane permeability to enable nutrient absorption and waste removal from the cells by re-activating the Na-K pump. NAIs enhance oxygen (O2) absorption across RBCs by increasing the oxygen-carrying capacity of the cells (14). An insufficient immune system has been reported to trigger several diseases including autoimmune diseases, cancers, and inflammatory diseases. NAIs can boost the immune system by strengthening the immune cells and making them healthier and more energetic to boost their ability to fight foreign antigens (13). Therefore, it is anticipated that the exposure to NAIs may have contributed to the strengthening of the immune system.

Serotonin (5-HT) is a neurotransmitter that controls several endocrine, metabolic, and neurovascular functions including addiction, aggression, appetite, anxiety, autism, bladder, brain homeostasis, breathing, circadian rhythms, depression, fear, gut motility, immunity, intestinal absorption, memory, mood, neurogenesis, pain, sleep, thermoregulation, and vascular tone (22). Though serotonin levels were not monitored in the participants, it is anticipated that NAIs may have normalized the 5-HT levels in the intervention group.

Hence, it is anticipated that there may be possible involvement of multiple factors including the enhancement of water-soluble vitamin metabolism, immune system strengthening, restoration of acid-base disturbances in the brain and other systems, and serotonin reduction that contributed to normalizing the blood parameters. NAIs may have improved the digestive, cardiovascular, circulatory, endocrine, immunological, integumentary, lymphatic, neurological, respiratory, and urinary systems in the participants. It is also possible that the provided NAI intervention may have ameliorated undiagnosed biochemical issues that contributed to the improvement of chloride, creatinine, globulin, total proteins, and sodium within the normal range.

The present study has a small sample size due to the limited number of consents obtained from the parents of CP patients at the rehabilitation center. The recruited participants had normal blood parameters at the time of induction, which might be attributed to the therapies provided at the rehabilitation center. Several

biochemical parameters were improved in the present study, it would be interesting to follow the intervention-induced changes in naive CP patients. This study is an endeavor to pool the existing and insufficient data on the blood parameters of CP-inflicted patients.

CONCLUSION

The NAI intervention showed no negative effects, indicating that it can be a progressive, non-pharmacological, cost-effective, and a potential method to improve the quality of life of CP-inflicted patients.

Conflict of interest:
Authors declare no conflict of interest
Funding source:
The study did not receive any external funding
Ethical Approval:
The study was approved by the ethics review committee of the University of Karachi. Verbal and written consent was taken from the parents of all inducted CP-inflicted participants.
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