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ORIGINAL RESEARCH

Effects of transcutaneous auricular vagus nerve stimulation on inflammation, cardiac autonomic modulation, and clinical evolution of patients with COVID-19: protocol for a clinical, controlled, randomized, and blind trial

Efeito da estimulação elétrica transcutânea do nervo vago na inflamação, modulação autonômica cardíaca e evolução clínica dos pacientes com COVID-19: estudo de protocolo para um ensaio clínico, controlado, randomizado e cego

Efecto de la estimulación eléctrica transcutánea del nervio vago sobre la inflamación, la modulación autonómica cardiac y la evolución clínica de pacientes con COVID-19: estudio de protocolo para un ensayo clínico, controlado, aleatorizado y ciego

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ABSTRACT | This study aims to evaluate the effect of transcutaneous auricular vagus nerve stimulation (taVNS) on inflammation, cardiac autonomic modulation, and clinical evolution of patients with COVID-19. This is a clinical, sham-controlled, randomized, and blind trial, in which 52 hospitalized individuals diagnosed with COVID-19 will participate. They will be randomized into: experimental group (usual medical treatment associated with active taVNS) and control group (usual medical treatment associated with sham taVNS). The taVNS will be performed by a neuromuscular electric stimulator (Dualpex model 071 of Quark Medical Products), with the stimulation electrode positioned on the left tragus, with alternating current, at a 30Hz frequency with 50% variation. Intensity will be adjusted to the patient's sensory threshold, with 90-minutes-long stimulation sessions, happening twice per day for seven consecutive days, totaling 14 sessions. Interleukin-6 (IL-6) and interleukin-10 (IL-10), cortisol and C-reactive protein (CRP), blood pressure, heart rate variability (HRV) by low frequency (LF), high frequency (HF) and low and high frequency ratio (LF/HF) parameters will be evaluated before and after the intervention, as well as patients' clinical evolution—including anxiety and depression levels—whose data will be obtained through medical records and questionnaires. A follow-up will also be performed seven and 14 days after the end of the interventions to verify the clinical evolution, including anxiety and depression levels. Memory and attention levels will be evaluated for six months.

Keywords | COVID-19; Vagus Nerve; Vagus Nerve Stimulation; Inflammation.

RESUMO | O objetivo deste estudo é avaliar o efeito da estimulação elétrica transcutânea do nervo vago (EETNV)

This study was conducted at the Universidade Nove de Julho (Uninove), Vergueiro campus, and at the Professor Lydia Storópoli Hospital of Uninove – São Paulo (SP), Brazil.
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na inflamação, modulação autonômica cardíaca e evolução clínica dos pacientes com COVID-19. Trata-se de um ensaio clínico, controlado por *sham*, randomizado e cego, no qual participarão 52 indivíduos hospitalizados com diagnóstico de COVID-19, que serão randomizados em dois grupos de tratamento: grupo experimental (tratamento médico usual associado à EETNV ativa) ou grupo-controle (tratamento médico usual associado à EETNV *sham*). A EETNV será realizada por meio de um estimulador elétrico neuromuscular (modelo Dualpex 071 da Quark Medical Products), com o eletrodo de estimulação posicionado sobre o tragus esquerdo, com corrente alternada, frequência de 30Hz e variação de 50%, intensidade ajustada para o limiar sensorial do paciente, com duração de 90 minutos cada sessão de estimulação, duas vezes ao dia, durante sete dias consecutivos, totalizando 14 sessões. Serão avaliados pré e pós-intervenção o nível de interleucina-6 (IL-6) e interleucina-10 (IL-10), cortisol e proteína C reativa (PCR), pressão arterial, variabilidade da frequência cardíaca pelos parâmetros de baixa frequência (BF), alta frequência (AF) e pela razão de baixa e alta frequência (BF/AF), além de evolução clínica dos pacientes, incluindo o nível de ansiedade e depressão, cujos dados serão obtidos por meio de prontuários e questionários. Será realizado também um acompanhamento 7 e 14 dias após o término das intervenções para verificar a evolução clínica, incluindo nível de ansiedade e depressão, e durante seis meses serão avaliadas memória e atenção.

Descritores | COVID-19; Nervo Vago; Estimulação do Nervo Vago; Inflamação.

RESUMEN | El objetivo de este estudio es evaluar el efecto de la estimulación eléctrica transcutánea del nervio vago (EETNV) sobre la inflamación, la modulación autonómica cardíaca y la evolución clínica de pacientes con COVID-19. Se trata de un ensayo clínico, controlado por simulado, aleatorizado y ciego, en el que participarán 52 individuos hospitalizados diagnosticados de COVID-19, que serán aleatorizados en dos grupos de tratamiento: grupo experimental (tratamiento médico habitual asociado a la EETNV activa) o grupo control (tratamiento médico habitual asociado a la EETNV simulada). La EETNV se realizará mediante un estimulador eléctrico neuromuscular (modelo Dualpex 071 de Quark Medical Products), con el electrodo de estimulación colocado en el trago izquierdo, con corriente alterna, frecuencia de 30Hz y 50% de variación, intensidad ajustada al umbral sensorial del paciente, con una duración de 90 minutos cada sesión de estimulación, dos veces al día, durante siete días consecutivos, lo que totaliza 14 sesiones. Se evaluarán antes y después de la intervención la interleucina-6 (IL-6) y la interleucina-10 (IL-10), el cortisol y la proteína C reactiva (PCR), la presión arterial, la variabilidad de la frecuencia cardíaca por los parámetros de baja frecuencia (BF), alta frecuencia (AF) y razón del baja y alta frecuencia (BF/AF), así como la evolución clínica de los pacientes, incluidos los parámetros de ansiedad y depresión cuyos datos se obtendrán de historias clínicas y cuestionarios. También se realizará un seguimiento de 7 y 14 días tras finalizadas las intervenciones para verificar la evolución clínica, incluidos el nivel de ansiedad y de depresión, y durante seis meses se evaluará la memoria y la atención.

Palabras clave | COVID-19; Nervio vago; Estimulación del Nervio Vago; Inflamación.

INTRODUCTION

COVID-19 is an acute respiratory infection caused by the potentially severe SARS-CoV-2 coronavirus, with high rates of transmissibility and global distribution. The COVID-19 epidemic began in Wuhan, China, in late December 2019[1], and has spread rapidly worldwide, being declared a global pandemic by the World Health Organization (WHO)[2] in March 2020.

The clinical aspect of COVID-19 varies widely, but presents an immunological reaction with high levels of inflammatory cytokines such as interleukin-6 (IL-6), considered an important factor for the increase in COVID-19 mortality[3,4]. Thus, the first treatment protocols for patients with SARS-CoV-2 included steroid administration, which aimed to modulate this strong cytokine response, similarly to the treatment of non-viral acute respiratory distress syndrome[5]. However, the treatments of SARS-CoV-2 infection proved ineffective[6,7]. Thus, to minimize this inflammatory response, alternative treatments have been investigated, such as transcutaneous auricular vagus nerve stimulation (taVNS)

The taVNS is considered a potent modulator of pathological immune reactions, suppressing the levels of inflammatory cytokine by activating the cholinergic anti-inflammatory pathway (CAP)[8-11]. Boezaart and Botha[11] reported the case of two patients with stage three COVID-19 and elevated IL-6 levels, having a drastic reduction in IL-6 levels in the blood in a relatively short period after taVNS. Staats et al.[8]

also described two cases of patients with COVID-19 symptoms who were stimulated with to cervical vagus nerve stimulation and presented relevant results in clinical improvement, namely: discontinuation of using opioid medications and cough suppressants; immediate relief of dyspnea (five minutes after stimulation); and an improved ability to clean their lungs. For these researchers[8], noninvasive stimulation can help patients with respiratory consequences of COVID-19 by two mechanisms: bronchodilation and activation of CAP.

A study indicates that taVNS also decreases sympathetic activity and is associated with the release of norepinephrine, improving autonomic control[12]. This evidence is important since patients with COVID-19 present autonomic dysfunction with decreased heart rate variability values[13].

Thus, given the increasing number of deaths in Brazil due to complications of COVID-19, with negative effect on public health, the possibility of using taVNS for people diagnosed with COVID-19 becomes important, considering its promising effects on the reduction of inflammatory processes with minimal side effects and low cost.

Hence, this study aims primarily to evaluate the effect of taVNS on inflammation and, as a secondary objective, to verify cardiac autonomic modulation, clinical evolution, and levels of depression, anxiety, memory, and attention of patients with COVID-19.

METHODOLOGY

Study design

This is a clinical, sham-controlled, randomized, and patient-blind trial, which will be conducted with 52 patients diagnosed with COVID-19 hospitalized at the Lydia Storópoli Hospital, at the Universidade Nove de Julho, São Paulo (SP), Brazil. Participants will be clarified about the procedure before signing an informed consent form. This study was registered in the Brazilian Registry of Clinical Trials (RBR–399t4g5). Figure 1 shows the Standard Protocol Items: Recommendations for Interventional Trials of the study.

	Study Period																	
											Follow-up							
	Registration	Allocation	Post-allocation							Post-treatment evaluation	7 days	14 days	1 month	2 months	3 months	4 months	5 months	6 months
Time Point**	$-t_1$	0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_{x1}	t_{x2}	$tx3$					
REGISTRATION:																		
Eligibility	X																	
Informed Consent	X																	
Allocation		X																
INTERVENTION:																		
[Active taVNS with usual treatment]			●	—	—	—	—	—	●									
[Sham taVNS with usual treatment]			●	—	—	—	—	—	●									
EVALUATION:																		
/interleukin-6, interleukin -10, cortisol, C-reactive protein, heart rate variability, blood pressure	X									X								
/symptoms evolution and Anxiety and Depression scale (HAD)/	X									X	X	X						
Level of memory and attention											X	X	X	X	X	X	X	X

Figure 1. Standard Protocol Items Schedule: Recommendations for Interventional Trials

taVNS: Transcutaneous auricula Vagus Nerve Stimulation; TX1: 7-day follow-up; tx2: 14-day follow-up; tx3: 1-month follow-up; TX4: 2-month follow-up; tx5: 3-month follow-up; TX6: 4-month follow-up; TX7: 5-month follow-up; TX8: 6-month follow-up.

Participants

This study will include 52 individuals with a confirmed diagnosis of COVID-19 who are hospitalized at the Lydia Storópoli Hospital. The selected patients should be older than 18 years; have a moderate to severe diagnosis of COVID-19[1]; be within the onset period of symptoms from one to ten days; be contactable; and sign the informed consent form. Participants under supplemental oxygen therapy, noninvasive ventilation, but not intubated during the initial evaluation may be included. Patients who use cochlear implants — due to the unviability of taVNS application—and/or have pacemakers will be excluded.

Sampling calculation

For sample calculation, the difference between the levels of IL-6 post- and pre-intervention with taVNS was used, based on a pilot study with 21 participants (11 in the control group and ten in the experimental group). An 80% confidence interval, 5% α, with a $p<0.05$ was considered. Sample calculation was performed using the G*POWER 3 software. Using these parameters, the sample calculation resulted in a sample size of 52 participants (26 per group).

Procedures

Study participants will be evaluated before and after seven days of interventions regarding inflammatory profile (inflammatory indicators), cardiac autonomic modulation, blood pressure, and clinical symptoms—including anxiety and depression levels. The population will be randomized into two groups using the Research Randomizer (www.randomizer.org) website: experimental (active taVNS associated with usual medical treatment) and control (sham taVNS associated with the usual medical treatment). After the end of the treatment, the participants' memory and attention levels will be monitored for six months.

Inflammation panel

Enzyme immunoassay techniques

Some 15ml blood samples will be collected by a nurser, through vein puncture, in a tube containing protease inhibitor and ethylenediaminetetraacetic acid (EDTA), which will be promptly centrifuged (3,000rpm, 15min, 4°C). The plasma of this tube will then be transferred to microcentrifuge tubes (1.5ml) and stored at –80°C. Later, it will be sent to a laboratory specialized in clinical analysis, which will perform IL-6, IL-10, CRP, and cortisol analyses.

Cardiovascular parameters

Blood pressure and heart rate variability (HRV) will be evaluated. Brachial blood pressure will be measured using a monitor (Omron HEM-742, Japan). While the individuals remain for 10 minutes in the supine position, three consecutive measurements will be performed, with a minute of interval, in both arms and with arm cuff sizes appropriate to the arms circumference. The value used will be the average of the last two measures, as recommended by the Brazilian Society of Cardiology[14].

HRV will be evaluated to estimate the autonomic modulation of the cardiovascular system. For that, after 20 minutes of rest, patients will remain 15 minutes in the supine position. RR intervals will be recorded during that time, using a heart rate monitor validated for this function (Polar Electro V800, Finland). Signs with at least five stationary minutes will be considered valid.

After collection, RR intervals will be exported to the Kubios HRV program (Biosignal Analysis and Medical Imaging Group, Version 2.0, Finland), and the analyses will be performed in the frequency domain. The parameters of the frequency domain will be obtained by spectral analysis, using the autoregressive method, with 12 models compared following Akaike Information Criterion (AIC). Frequencies ranging from 0.04 to 0.4Hz will be considered as physiologically significant. The low frequency component (LF) is represented by oscillations from 0.04 to 0.15Hz and high frequency component (HF) from 0.15 to 0.4Hz.

Disease evolution

To evaluate the clinical symptoms, a questionnaire will be applied before, immediately after, and seven and 14 days after the end of the interventions, with questions about the symptoms of COVID-19 and their intensity, which may be none (N), light (L), moderate (M),

or accentuated (A). The Hospital Anxiety and Depression scale (HAD)[15]—which consists of a questionnaire with a score from zero to three for each question, totaling 31 points—will also be applied. The HAD result may be: normal (0–7 points), borderline abnormal (8-11 points), and abnormal (12-21 points).

In addition, information on demographic data, health history, onset of symptoms of COVID-19, data on vaccination, comorbidities, medications, complications data, specific parameters related to COVID-19, including blood tests, imaging tests, and disease time, will be obtained by analysis of medical records and telephone calls (in case of hospital discharge), but the patient's name will be kept confidential.

Attention and memory

The quality of attention and memory will be obtained with a questionnaire containing two questions about how memory and attention are after COVID-19, with seven possible answers: (1) much better; (2) better; (3) a little better; (4) no change; (5) a little worse; (6) worse; (7) much worse. This is a version adapted by the authors of the Clinical Global Impression (CGI)[16].

The questionnaire will be applied 7, 14, 30, 60, 90, 120, and 180 days after the end of the taVNS protocol, by telephone contact (in case of hospital discharge).

Interventions

The interventions will be performed during the hospitalization of the patients. To do so, a physical therapist will go to the patient's bed and position the taVNS equipment.

Active transcutaneous vagus nerve stimulation

The active taVNS will be performed by a multifunctional transcutaneous neuromuscular electric stimulator, Dualpex model 071 of Quark Medical Products (Anvisa registration No. 80079190022), with an electrode on the tragus— a human anatomical point where cutaneous distributions of the vagal afferent nerve are present (Figure 2)— on the left side, considered safer[17]. The other electrode will be positioned on the clavicle on the same side.

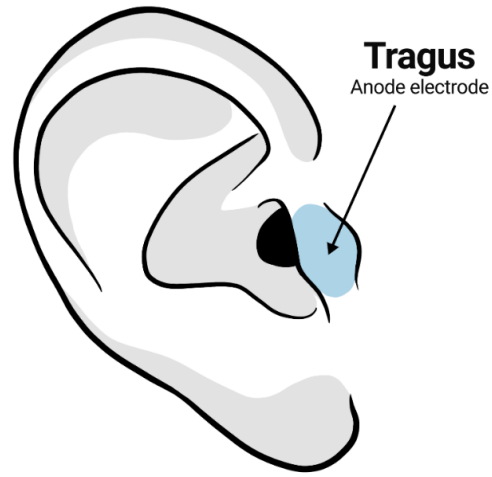

Figure 2. Area of the auricular branch of the vagus nerve where electrostimulation will be applied

The taVNS will be performed with 15mm adhesive electrodes, alternating sinusoidal current with a 30Hz frequency with a variation of 50%, for 90 minutes[18], twice per day (in the morning and afternoon or at night, with six to eight hours interval between stimulations) for seven consecutive days, totaling 14 stimulation sessions. The intensity will be maintained between 0.5 and 12mA, according to the patient's tolerance, in order to avoid pain or muscle contraction, within the sensory threshold[19].

Sham intervention

For sham stimulation, the same active taVNS equipment will be used, which will remain switched off during the study period.

Data analysis

The study data for parametric data will be presented in mean and standard deviation. Nonparametric data will be presented in median and interquartile range. Normality will be assessed by the Shapiro-Wilk test. Inflammatory variables (IL-6, IL-10, CRP, and cortisol) and levels of anxiety, depression, memory, and attention will be calculated with paired t-test.

Heart rate variability will be analyzed by the following variables: normalized unit (nu) of LF and HF. The Statistical Package for the Social Sciences (SPSS version 25.0 for Windows) will be used to analyze LF/HF ratio. Heart rate variability data will be analyzed

by generalized estimation equations. For the analysis, the $p<0.05$ and the intention-to-treat will be considered.

DISCUSSION

COVID-19 generated a global pandemic and increased the curve of infected patients and mortality rates in a short period. Therefore, a large negative effect on economic and public health happened, especially in Brazil. Thus, scientists and health professionals mobilize to reduce this impact by using known techniques and resources as well as searching for new treatments.

Although vaccines are now available, improving treatments aimed at reducing mortality and sequelae are still needed, ensuring better quality of life for the patients. Furthermore, the appearance of new strains shows the urgency of effective and alternative treatments in the face of an unknown scenario. Thus, taVNS may be an anti-inflammatory resource to improve clinical conditions related to autonomic control in patients with COVID-19 sequelae, as already shown in the literature in different study groups and in some case reports with patients diagnosed with COVID-19.

CONCLUSION

The taVNS may be a promising treatment for people diagnosed with COVID-19, presenting minimal side effects, cheap, and good efficiency at reducing inflammatory processes, the main characteristic of the disease caused by SARS-CoV-2.

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