EFFECT OF ACUPUNCTURE ON HEART RATE VARIABILITY IN INDIVIDUALS WITH MULTIPLE SCLEROSIS

1. Introduction

Multiple sclerosis (MS) is a severe autoimmune demyelinating disease that affects nervous system, has high morbidity and mortality and no effective targeted therapies are available. There are currently about 3 million people who are affected by MS worldwide. The disease typically begins between the ages of 20 and 50 and is twice as common in women as in men. (Lozano-Quilis *et al.*, 2014). This equates to 1 in 3,000 people in the world living with MS, and in countries with the highest prevalence as many as 1 in every 300 people have MS (The Multiple Sclerosis International Federation, Atlas of MS, 3rd Edition, 2020).

The correlations between clinical presentations and pathological changes are extremely complex (Obert, D. et al, 2016). The clinical subtypes of Multiple Sclerosis (MS) are determined to be: Relapsing-remitting, Secondarily Progressive and Primarily Progressive. These names were quickly incorporated into clinical practice, being used for better communication with the patient in relation to the course of the disease and to define specific populations in research. So far, no biomarkers or MRI signals have been identified which can distinguish between them. (Lublin, F.D, 2014; Lucchinetti, C., Bruck, W, 2004).

Relapsing-Remitting type is defined by clinical outbreaks or increased lesions in the central nervous system (CNS), which generate major phenotypic changes. In turn, the Primarily Progressive type also shows alterations, generating lesions in the CNS, but there is no inflammatory activity, that is, outbreaks (Lublin, 2014; Lucchinetti and Bruck, 2004). Progression in this subtype occurs within months or years, with a deterioration process which occurs in a slowly and irreversibly way (Pender, M. P., 2004). The Secondarily Progressive type is an evolution of the RR phase, promoting a progressive and continuous neurological disability, and this transition mechanism is still poorly described (Trapp, B.D et al., 1999). One of the existing explanations is the scarcity of outbreaks in the RR phase with a poor response to corticosteroid treatments and a consequent increase in disabilities (Palace, J., 2003).

Up to now there is no cure for MS, but there are several therapies aimed at improving function after an outbreak and preventing disability. Therapies, including medications and neuro rehabilitation can improve some symptoms, but cannot change the course of the disease (Lozano-Quilis *et al.*, 2014).

Autonomic impairment, including cardiovascular autonomic dysfunction, is not uncommon in patients with MS. Cardiovascular involvement may be expressed as poor physical fitness, fatigue, orthostatic hypotonia, or cardiac arrhythmias. The autonomic dysfunction observed in MS is believed to be associated with plaques located in the brainstem as well as spinal cord which affect autonomic areas and their connections (Cygankiewicz.and Zareba, 2013).

Autonomic Nervous System (i.e. sympathetic and parasympathetic) is responsible for involuntary control of vital physiological functions, such as heart rate at rest and in response to stress. Therefore, adequate responses depend on the balance between the sympathetic and parasympathetic system (Tracy et al., 2016; Valenti et al, 2012; Vanderlei et al., 2009; Appelhans et al., 2006).

To evaluate the activity of the ANS (Autonomic Nervous System) under physiological and pathological conditions, HRV measurement is a non-invasive technique capable, providing important information about the individual's physical and mental health (Vanderlei et al., 2009; Gamelin et al., 2006). The presence of a high HRV represents efficient autonomic mechanisms, while low HRV may indicate abnormal adaptation of these mechanisms and insufficient ANS response (Khaled et al., 2006; Vanderlei et al., 2009), and it is a widely accepted means of assessing cardiac autonomic regulation (European Society of Cardiology and North American Society of Stimulation and Electrophysiology, 1996).

Some studies have documented abnormal HRV in patients with MS, and it showed a significant loss of vagal and increased sympathetic tone (Brezinova et al., 2004; Tombul et al., 2011). Dysautonomia in MS patients was more likely to occur in patients with a greater extent of physical disability (Merkelbach et al., 2001) and most pronounced in the progressive variant of MS disease (Zawadka-Kunikowska et al., 2020). In its turn Studer et al., (2017) and Shirbani et al., (2018) showed autonomic balance appears to be intimately linked with both the inflammatory activity of multiple sclerosis, which is featured by an overall hypoactivity of the sympathetic nervous system, and its compensatory plastic processes, which appear inefficient in case of worsening and progressive multiple sclerosis.

Neuronal stimulation is a promising emerging field in modern medicine for the control of organ function and reestablishing physiological homeostasis during illness. Multiple recent studies show the potential of nerve stimulation to control inflammation and improve organ function in MS disorders. Acupuncture is a practice of Traditional Chinese Medicine and aims to both treat and prevent diseases through various methods, including the insertion of needles in specific points of the body, and should be analyzed as a complementary therapeutic measure in the treatment of MS. The location of the points used in Acupuncture is found in channels or medians through which the qi (energy) circulates, which

in this objective way, aims to regulate the functions of the organism and allows the free flow of qi. The 1997 National Institutes of Health consensus considers the use of acupuncture as a complementary therapy in the management of painful conditions (Stall et al., 2015).

Besides that, neuromodulation with acupuncture can provide vital information about the functional organization of the nervous system (Ulloa, Quiroz-Gonzalez and Torres-Rosas, 2017). In this way Quispe-Cabanillas et al., (2012) evaluated thirty-one patients with Relapsing-Remitting Multiple Sclerosis undergoing treatment with immunomodulators, and they conclude that Electroacupuncture improved various aspects of quality of life, including a reduction in pain and depression. In addition, according to Anderson et al., (2014), evaluate the ability of acupuncture to modulate HRV could imply that acupuncture may function by regulating and integrating the various physiological systems in the body. In their review all randomized placebo-controlled trials (a total of 16) suggest that acupuncture improves HRV, but 12 of these studied healthy subjects, four studied subjects with medically diagnosed conditions, and none of these have studied these effects in people with MS.

Given the above, the Objective of this study is to evaluate the heart rate variability in individuals with MS during the applicability of Acupuncture, to analyze the behavior of the autonomic nervous system before, during, and after therapy and the changes of the condition. It is necessary to emphasize the need for studies that prove the benefits of the adopted procedures, to be better understood and consequently better applied, expecting better results, and assisting rehabilitation professionals in choosing the therapeutic program based on scientific evidence. Considering the deliberations presented, as a hypothesis, we believe that individuals with MS will present greater sympathetic modulation that will tend to balance during and after the applicability of Acupuncture.

2. Methods

2.1. Trial Design

A double-blinded randomized sham-controlled crossover trial with a 1:1 allocation ratio will be conducted, 40 individuals without previous illness will be evaluated, who will constitute the control group and 40 individuals with MS will constitute the experimental group, paired by age and gender. All participants will undertake active or sham acupuncture sessions. Groups A–S will start with 5 sessions (1 per week) of active acupuncture combined with HRV evaluation for 20 min. After a 2-week washout, this group will be reallocated to another 5 sessions (1 per week) of sham acupuncture for 20 min combined with HRV evaluation. Meanwhile, Group S–A will carry out the opposite protocol, participants will start an allocated 5 sessions (1 per week) of sham acupuncture combined with HRV

evaluation, and after a 1-week washout period will be reallocated to 5 sessions (1 per week) of active acupuncture combined with HRV evaluation.

As previously described by Shen et al. (2009), Tseng, Tseng and Chang (2015), Li et al. (2020) and Shi et al. (2013), a 2-week washout period has been used and was shown to be sufficient to reset the effects of the first 5 sessions. Figure 2 summarizes the planned experimental design. This research protocol follows the SPIRIT recommendations. 40 participants with MS will be recruited through referral by the coordinators of Brazilian multiple sclerosis association (ABEM), located in São Paulo state.

Those interested in participating will undergo a detailed screening using the eligibility criteria for enrolment in the study. The sample size was calculated using statistical software (GPower 3.1.5) on the main outcome measure (i.e., the motor score). This calculation was based on data from one study with a group of individuals with CP who received tDCS (32). The power was 0.80; the alpha was 0.05; and the effect size was 0.65 (Cohen's d). The sample estimation indicated that 30 participants would be necessary (i.e., 15 per group). With an adjustment to allow for a withdrawal rate (20%).

This article has been reported in accordance with the Standard Protocol Items Recommendations for Interventional Trials (SPIRIT) (Chan et al., 2013a,b; Tables 1, 2).

TABLE 1 | SPIRIT: description of the study protocol, schedule of enrolment, interventions, and assessments.

STUDY PERIOD													
	Enrolment	Allocation					Post-allocation						close-out
TIMEPOINT	t^1	0	t^1	t^2	t^3	t^4	t^5	t^6	t^7	t^8	t^9	t^{10}	t^{11}
			1 day	7 day	14day	21 day	28 day	42 day	49 day	56 day	63 day	77 day	84 day
ENROLMENT													
Eligibility screen	x												
Informed consent	x												
Assessment scales and tools	x												
Allocations		x											
INTERVENTIONS													
Group S-A			←	—	—	—	→	←	—	—	—	→	
Group A-S			←	—	—	—	→	←	—	—	—	→	
ASSESSMENTS													
EDSS	x												x
MoCA	x												x
BRUMS			x				x	x				x	
BDI-II	x												x
EVA (pain)			x	x	x	x	x	x	x	x	x	x	
PHYSIOLOGICAL ASSESSMENT													
Ryodoraku			xx				xx	xx				xx	
Thermography			xx				xx	xx				xx	
Heart Rate Variability			x	x	x	x	x	x	x	x	x	x	

S-A: Sham-Acupuncture; A-S: Acupuncture – Sham; EDSS: Expanded Disability Status Scale; MoCA: Montreal Cognitive Assessment; BRUMS: Brunel Mood Scale; BDI-II: Beck Depression Inventory Second Edition; EVA: Visual Analogue Scales.

TABLE 2 | Trial characteristics based on WHO Trial Registration Data Set.

DATA CATEGORY	TRIAL INFORMATION
PRIMARY REGISTRY AND TRIAL IDENTIFYING NUMBER	ClinicalTrials.gov, ID:
DATE OF REGISTRATION IN PRIMARY REGISTRY	August 2022
SECONDARY IDENTIFYING NUMBERS	Ethical Committee of the University – January 18th, 2019 CAAE: 01515118.3.0000.5505
SOURCE(S) OF MONETARY OR MATERIAL SUPPORT	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES)
PRIMARY SPONSOR	Federal University of São Paulo– UNIFESP
SECONDARY SPONSOR(S)	NA
CONTACT FOR PUBLIC QUERIES	TDS, CF
CONTACT FOR SCIENTIFIC QUERIES	TDS, CF
PUBLIC TITLE	EFFECT OF ACUPUNCTURE ON HEART RATE VARIABILITY OF PEOPLE WITH MULTIPLE SCLEROSIS: A STUDY PROTOCOL FOR A DOUBLE-BLINDED RANDOMIZED CONTROLLED TRIAL
SCIENTIFIC TITLE	ANALYSIS OF AUTONIMIC CARDIAC MODULATION DURING ACUPUNCTURE IN INDIVIDUALS WITH MULTIPLE SCLEROSIS.
COUNTRY OF RECRUITMENT	Brazil
HEALTH CONDITION(S) OR PROBLEM(S) STUDIED	Multiple sclerosis

INTERVENTIONS	For data collection, the following instrument will be used: Visual Analogue Scale of Pain. This scale will be applied at the beginning of each intervention day. In sequence, the participants will perform the Thermography protocol, which comprises capturing images in various postures in the orthostatic position (in the inicial and final assessments). Afterwards, the participants will remain in the supine dorsal position on a stretcher at rest for 10 minutes to start the SNA analysis with the Ryodoraku (device that measures the electroconductivity of certain points on the skin) by the digital reading of the 24 points called ryodos (SCILIPOTI, 2007). Continuing the procedure, immediately after the placement of the Polar V800, an instrument for analyzing HRV, the placing of needles at the points carefully selected for this research begins. The needling will proceed for the experimental group using specific Traditional Chinese Acupuncture points: E36, BP6, F3, IG4, for 20 minutes while the control group will receive needling in sham points (points not recognized as Acupuncture points), with the duration of 20 minutes. Final phase, new reading with Ryodoraku and Thermography.
KEY INCLUSION AND EXCLUSION CRITERIA	**Inclusion Criteria** This study will include individuals diagnosed with MS, aged over 18 years, with motor and intellectual capacity to understand the evaluations, with light and moderate functional classification levels (EDSS scale) and who accept to participate in the study through agreement with informed consent form. **Exclusion Criteria** Participants will be excluded if they (1) do not understand the evaluations; (2) cardiac diseases that impede the assessment of HRV; (3) have a cardiac pacemaker.
STUDY TYPE INTERVENTIONAL ALLOCATION:	Randomized
MASKING:	Double-blind
ASSIGNMENT:	Crossover
PRIMARY PURPOSE:	Treatment

DATE OF FIRST ENROLMENT	September 2021
TARGET SAMPLE SIZE	80 participants
RECRUITMENT STATUS	Recruiting
PRIMARY OUTCOME(S)	Heart Rate Variability improvement
KEY SECONDARY OUTCOME(S)	Ryodoraku and Thermography improvement

2.2. Study Location and Period

Randomization

To perform the allocation procedure, the encoded groups will be placed inside a closed opaque envelope, which will be labeled with the code for each participant. Envelopes will be opened only during the first time of acupuncture or sham intervention.

The results assessors to are blinded, will receive results without group description.

2.3. Study Population and Eligibility Criteria

Inclusion Criteria

This study will include individuals diagnosed with MS, aged over 18 years, with motor and intellectual capacity to understand the evaluations, with light and moderate functional classification levels (EDSS scale) and who accept to participate in the study through agreement with informed consent form.

Exclusion Criteria

Participants will be excluded if they (1) do not understand the evaluations; (2) cardiac diseases that impede the assessment of HRV; (3) have a cardiac pacemaker.

Withdrawal Criteria

Participants will be withdrawn from the study if they are not willing to continue, cannot be present on the day of the experiment, or miss one treatment sessions out of 10.

Screening tools

Functional assessments

Expanded Disability Status Scale (EDSS)

The Expanded Disability Status Scale was developed by Kurtzke (1983). It is an assessment scale evaluating the functional systems of the central nervous system, it is used to describe disease progression in patients with MS and to assess the effectiveness of therapeutic interventions in clinical trials. It is subdivided into eight functional systems: pyramidal, cerebellar, brainstem, sensitive, bladder, intestinal, visual and mental functions. Confirmation of EDSS increase at 12 or 24weeks or a later time point reduces the likelihood of capturing events that may subsequently revert (Kappos et al., 2017). It consists of ordinal rating system ranging from 0 (normal neurological status) to 10 (death due to MS) in 0.5 increments interval (when reaching EDSS 1). The lower scale values of the EDSS measure impairments based on the neurological examination, while the upper range of the scale (> EDSS 6) measures handicaps of patients with MS. The determination of EDSS 4 – 6 is heavily dependent on aspects of walking ability (Meyer-Moock et al., 2014).

Cognitive assessments

Montreal Cognitive Assessment, MoCA

The MoCA is a screening test, useful tool for the detection of mild dementia or mild cognitive impairment. Its cover 10 cognitive domains using rapid, sensitive, and easy-to-administer cognitive tasks. (Smith & Holmes, 2007). It is a 10-minute test, in there patients answer items with the following : the short-term memory recall task, Visuospatial abilities, a three-dimensional cube, Executive functions, a phonemic fluency task, a verbal abstraction task, sustained attention task, a serial subtraction task, and digits forward and backward, language, orientation to time and place (Nasreddine, 2005). Each domain generates a different score, totaling 30-point cognitive screening test designed in a one-page, the suggested cut-off point on the MoCA is 26 (Smith & Holmes, 2007).

Emotional and behavior assessments

Brunel Mood Scale (BRUMS)

The Brunel Mood Scale was developed to allow a quick measurement of the mood state of populations composed by adults and teenagers. It consists of 24 items to assess mood change in the six mood dimensions - tension, depression, anger, vigor, fatigue, and confusion. The question used is “How do you feel now”. The items are assessed on a 5-point scale anchored from “not at all” (0)

to “extremely” (4). Each subscale contains four items. With the sum of the responses of each subscale, a score can be obtained that can vary from 0 to 16. The scale takes about one to two minutes to be answered (Rohlfs et al., 2008), it was presented as a tool for detection of the over-training syndrome, besides that it has been used in different populations (Silva et al., 2021) and contexts in Brazil (Brandt et al., 2016) and other countries (Lan et al., 2012). Including for evaluations in populations with cardiac disorders (Sties et al., 2014).

Beck Depression Inventory Second Edition (BDI-II)

The BDI-II is a relevant psychometric instrument, showing high reliability, and capacity to discriminate between depressed and non-depressed subjects. (Wang & Gorenstein, 2013). The main factors of the inventory are a cognitive and a somatic factor, and that some items can shift between factors depending on which population samples represent (Arnarson et al., 2008). Examples of these items include questions regarding changes in sleep patterns, difficulty concentrating, sadness, self-dislike, crying, loss of energy, and suicidal thoughts (Strunk & Lane, 2016). BDI-II is a self-report measure of depression. Twenty-one symptoms of depression are rated on a 4-point scale (0 to 3) scale, within the time frame of the past 2 weeks. The total scores are obtained by summing the ratings for all items. Scores ranging between 0 and 13 are indicative of minimal depression; scores that fall between 14 and 19 are considered to reflect a mild level of depression; scores of 20 to 28 are considered moderate; and a score ranging from 29 to 63 is labeled severe (Dozois, 2010).

Evaluation analysis tools

Visual Analogue Scales (VAS)

The VAS is by far the most frequently used assessment instrument (Reed e Van Nostran, 2014). Visual analogue scales are psychometric measuring instruments designed to document the characteristics of disease-related symptom severity in individual patients and use this to achieve a rapid (statistically measurable and reproducible) classification of symptom severity and disease control (Klimek et al., 2017). In our study, we will use it for pain mensures. It consisted of 100 mm lines giving a score ranging from 0 up to 10, whose endpoints were designated as ‘no pain’ and’ the worst pain imaginable’. Although some pain sensations may be equally intense and unpleasant, the patient need to judge the two aspects independently, to indicate the relative intensity of your pain sensation: the higher number, the greater the intensity (Heller, Manuguerra and Chow, 2016).

Blinding

The participants and assessors of results analysis will remain blind to group allocation during the study. To ensure proper blinding, participants will receive the same care, but with points outside the area of the correct acupuncture points. Considering that the needle applicator must be a qualified professional, it is not possible that he is blind.

Intervention

For data collection, the following instrument VAS Scale will be used. This scale will be applied at the beginning of the data collection. In sequence, the participants will perform the Thermography protocol, which comprises capturing images in various postures in the orthostatic position.

Afterwards, the participants will remain in the supine dorsal position on a stretcher at rest for 10 minutes to start the SNA analysis with the Ryodoraku (device that measures the electroconductivity of certain points on the skin) by the digital reading of the 24 points called ryodos (SCILIPOTI, 2007).

Continuing the procedure, immediately after the placement of the Polar V800, an instrument for analyzing heart rate variability, the placing of needles at the points carefully selected for this research begins. The needling will proceed for the experimental group using specific Traditional Chinese Acupuncture points: E36, BP6, F3, IG4, for 20 minutes while the control group will receive needling in sham points (points not recognized as Acupuncture points), with the duration of 20 minutes. Final phase, new reading with Ryodoraku and Thermography.

Ryodoraku

Based on the Ryodoraku (meridian) theory, developed by Dr. Yoshio Nakatani (Nakatani, 1956), there are 12 meridians on the right and left side of the human body respectively. The property of Ryodoraku can reflect the condition of certain organ(s) by analyzing and comparing their mutual relations and changes with micro-electrical current. The electrical state of the acupuncture points of the human subject is measured by a computerized testing instrument with a very low electrical current. From the traditional theory, Ryodoraku is a pathological phenomenon. The mechanism can be explained by the viscera-skin sympathetic nerve reflex. The impulses from the viscera radiate to the spinal cord. The reflex zones are then reflected onto the skin surface via the efferent sympathetic nerves and appear as a longitudinal connecting system, just like meridian lines (Weng et al., 2004).

Electrodermal screening measures either skin impedance or conductance at acupuncture points as diagnostic aids for planning treatment strategies. Skin impedance is the skin's opposition to the flow of current. The practice of measuring skin impedance is based on the widely held assumption that acupuncture points are loci of decreased impedance compared to skin sites where there are no known acupuncture points (Pearson et al., 2007).

Recently, researchers have begun to explore it at acupoints as a potential physiological outcome measure for use in clinical trials of acupuncture. The technique consists of measuring electrical conductance at representative points for each meridian, generally the Yuan points on the wrists and ankles. Because of the high variability in skin conductance measurements, readings are never taken as absolute but rather as relative to all other measurements on the same patient and reported on a normalized scale. Increased conductance (decreased resistance) is believed to represent an excess in the meridian being measured, and decreased conductance (increased resistance) represents a deficiency (Colbert et al., 2011).

Thermography

Infrared cameras measure the wavelength of infrared radiation an object emits and converts it to electrical energy, which is then used to measure surface temperature. As an imaging technology, has numerous advantages resulting from its non-invasive and non-contact approach. In this way, thermal information can be assessed from a distance, a valuable feature for field biology, without the need for surgical interventions or handling that have prolonged effects on body temperature (Tattersall, 2016). It is one of the most promising methods of probing the psychological status of human beings (Hong e Hong, 2015).

The human skin acts as a black-body radiator operating at the body temperature generating an infrared radiation spectrum with a broad peak at around λ= 9.5um. The body's core temperature is developed in the central part of the body where the major organs are located (heart, lungs, liver, etc.) and heat is transported by the blood through the arteries to the rest of the body. Because the environment temperature is usually lower than the core temperature (37 °C), there is a cooling effect from the skin to the environment and heat transfer from the blood into the surrounding tissue and to the environment takes place (De Graaf et al., 2019).

With the aid of thermometry infrared electromagnetic waves of 0.7–1000 M (which the human eye is unable to identify) can be visualized, healthy human bodies emit waves with a length of 3–10 M. Temperature differences as low as 0.1°C can be pursued and may help track response to acupuncture as opposed to needling of non-acupoints to objectively estimate effects of needling (Agarwal-Kozlowski, Lange e Beck, 2009).

Lastly, changes in temperature detected will be related to changes in blood flow when the external environment is stable (no changes in irradiative, conductive, or convective heat transfer), and thus sensitive to acute changes in sympathetic activity which would induce vasoconstriction as the vasculature is not parasympathetically innervated. This temperature rise under extreme stimuli may be due to increases in cardiac output, which may increase perfusion pressure or the release of local factors such as nitric oxide from nearby endothelial cells, and it may be sympathetically mediated but could relate to parasympathetic withdrawal that induces cardiac acceleration, suggesting a complex interplay of autonomic control (Huggins e Rakobowchuk, 2019).

Analysis of Heart Rate Variability

After the initial evaluation, the capture strap will be placed on the volunteers' chests, and the heart rate receiver (V800, Polar) will be placed on their wrists, previously validated equipment for capturing the heart rate, beat by beat, and the use of their data for HRV analysis (GAMELIN et al., 2006; KINGSLEY et al., 2005).

After placing the strap and the monitor, the individuals will be positioned in a sitting position and will remain at rest, breathing spontaneously for 20 minutes. Following that, the analysis will be made during the performance of Acupuncture.

or analysis of HRV data in the sitting position, 1000 consecutive RR intervals will be used, and during the tasks, 256 consecutive intervals will be used. Then, a manual filtering will be performed through the *Microsoft Excel* program, to eliminate premature ectopic beats and artifacts, and there will be no substitution, only the elimination of artifacts. After manual filtering, the first 1000 beating intervals are chosen and only series with more than 95% of sinus beats will be included in the study (GODOY et al., 2005). The software used to assess HRV will be the Kubios HRV.

HRV analysis will be performed using linear methods (time and frequency domains) and non-linear methods that will be analyzed using the softwares: HRV analysis, CDA_Pro.

Linear Methods (Time and Frequency domain)

In the time domain, the RMSSD, pNN50 and SDNN indexes will be used. The RMSSD which is Square root of the mean of squared differences between successive beat intervals (Marães et al., 2003; Batista et al., 2019). The pNN50 index, in turn, is a sensitive and easily interpretable marker

of the parasympathetic SNA modulation, defined as the percentage of successive differences in the R-R interval whose absolute value exceeds 50ms. SDNN, on the other hand, reflects the participation of both branches of the SNA and corresponds to the standard-deviation of the average of all normal RR intervals, expressed in ms (Pumprla et al., 2002; Aubert et al., 2003; Bittencourt et al., 2005; Ribeiro e Moraes Filho, 2005).

For the analysis of HRV in the frequency domain, low frequency spectral components (LF – range between 0.04 to 0.15 Hertz) will be used in absolute units and high Frequency (HF – range from 0.15 to 0.4Hertz), in normalized units, and the ration between these components (LF/HF), which represents the relative value of each spectral component in relation to the total power, minus the exceptionally low frequency components (VLF). The algorithm used for the spectral analysis will be the fast Fourier transform - FFT (256 s window with 50% overlap) (Vanderlei et al., 2009; Martinelli et al., 2020; Carvalho, 2009).

Nonlinear Methods (Fluctuation Analysis, Depuration of Trends and Poincaré Graph)

For HRV analysis using nonlinear methods, the DFA quantifies the presence or absence of fractal correlation properties of RR intervals and has been validated by time series data. This method calculates the fluctuation of the mean square root of the integral and depurates the time series, allowing the detection of intrinsic self-similarity embedded in the non-stationary time series. The DFA graph is not strictly linear, but consists of two distinct regions of distinct curves, separated at one point, suggesting that there is a short-term factal scale exponent (a1) during periods of 4-11 beats (or 4 to 13), and a long-term exponent (a2), for longer periods (greater than 11 beats) (Carvalho et al, 2009; Moraes et al., 2019; Godoy et al., 2007).

The Poincaré graph is a quantitative method of analysis, based on the changes in the sympathetic or parasympathetic modulation of the heart rate over the subsequent intervals, without the need for the property of data stationarity (Godoy et al., 2007; Guerra et al., 2019). The following indexes will be calculated: SD1 (standard deviation of instantaneous beat-to-beat variability in the short term), SD2 (long-term standard deviations of continuous R-R intervals) and the SD1/SD2 ratio (BRUNETTO et al., 2005).

Primary Outcome

We will observe changes in the ANSs after intervention with active acupuncture and sham acupuncture in patients with MS, during a period of 28 days and after 2-weeks follow-up, following by more 28 days of intervention.

Secondary Outcome

We will analyze changes in Ryodoraku and Thermography regarding both interventions (active and sham acupuncture).

DATA ANALYSIS

If the data meet the assumptions for using parametric analysis, analysis of variance (ANOVA) will be performed to identify intra and intergroup differences. The differences, if any, will be detected by Bonferroni's post-hoc test. If the assumptions of ANOVA are not met, non-parametric analyses will be used to identify and locate the differences: Friedman -post-hoc Wilcoxon as test (intra-group) and Kruskal-Wallis and U of Mann-Whitney as post-hoc test (intergroups). A significant level of 0,05 (5%) will be defined and all intervals built along the work will be with e 95% statistical confidence. The statistical program will be the SPSS (Statistical Package for Social Sciences), version 11.0 (Frankfort-Nachmias and Leon-Guerrero, 2005).

RISKS AND BENEFITS

The research is based on analyzing the variability of SNA – Autonomous Nervous System with the therapeutic use of an Acupuncture protocol and a complementary protocol of Thermography in individuals with Multiple Sclerosis. Therefore, it is considered research with human beings and as such has the duty to safeguard the integrity of those involved. This safeguard will be in relation to the questions of privacy, secrecy, and the minimization of any discomfort.

Risks

The participation in this research does not bring complications. The risks in their participation in the research are minimal and related to the use of acupuncture needles, which are sterile, of single and individual use, discarded at the end of the procedure in a compartment suitable for sharp perforating materials. Related to the use of the camera, the participant does not adapt with the environment acclimatization.

And regarding the use of Ryodoraku – Model NKL, the risks are also minimal, because there may be a slight discomfort in relation to the pressure of the tip with wet cotton on the skin applied on the points to be evaluated during the reading. Therefore, there will always be a responsible person, attentive in the orientations to the participant.

The procedures adopted in this research obey the Criteria of Ethics in Research with Human Beings according to resolution 44/12 of the National Health Council (Conselho Nacional de Saúde). None of the procedures used puts their dignity at risk.

Confidentiality: all information that will be collected is strictly confidential, being restricted only to the researcher (a) and the supervisor (a). Only they have the knowledge of the data collected for the development of the research,

Benefits

Research participants will have indirect benefits. They will benefit from future information that may improve the quality of life of the participants. Therefore, there is no immediate benefit foreseen to the participants of this research, only after the conclusions of this work that can contribute in a significant way to the adequate, organized and effective elaboration of the diagnostic methods and treatment in Acupuncture.

Corroborating, we hope that this study will contribute significantly to the rehabilitation programs, so that knowledge observed and acquired from this research can contribute to the advancement of science and health of these patients. The researcher compromisses to divulge the obtained results.

Trial status

Participant recruitment will start in September 2022, it was stopped due to quarantine in March 2020. Expected to end in May 2023. Study completion is estimated by December 2022.

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