UNIVERSIDADE FEDERAL DA BAHIA
FACULDADE DE MEDICINA DA BAHIA

PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA E SAÚDE

TIAGO DA SILVA LOPES

PROTOCOLO PARA CONTROLE DA DOR CRÔNICA EM INDIVÍDUOS COM DOENÇA FALCIFORME AVALIADO POR MEDIDAS SUBJETIVAS, ELETROFISIOLÓGICAS E MOLECULARES

DISSERTAÇÃO DE MESTRADO

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Orientador: Dr. Abrahão Fontes Baptista

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RESUMO

Introdução: A dor crônica é principal comorbidade relacionada a Doença Falciforme (DF). O Sistema Nervoso Central (SNC) pode sofrer alterações mal adaptativas devido ao persistente estimulo doloroso, a qual por sua vez tem importante papel na crônificação da dor. A combinação de técnicas neuromodulatorias, tais como Estimulação Transcranianas com Corrente Constante (ETCC) e Estimulação Eletrica Periferica (EEP), tem se mostrado uma opçao terapêutica promissora no controle da dor e diminuição as alterações mal adaptativas do SNC. Porém, até o momento, poucos estudos avaliaram o SNC de individuos com DF e nenhum estudo buscou controlar a dor desta população com o uso de ETCC e EPP. **Objetivos:** Apresentar uma revisão narrativa destacando as possíveis alterações mal adaptativas do SNC e propor formas de avaliá-las no ambiente clínico. Além disso, propor um protocolo com a combinação de ETCC e EEP para controle da dor crônica em individuos com DF. **Metodologia:** A revisão narrativa foi realizada no período de março a setembro de 2017. O protocolo é um ensaio clínico, paralelo, controlado, randomizado, duplo cego. Neste protocolo, 128 indivíduos com DF e dor crônica secundária a osteonecrose do quadril serão divididos em dois grupos (HbSS n=64) e (HbSC n=64). Os participantes em cada grupo serão randomizados nos seguintes tratamentos combinados: 1) ETCC ativo + EEP ativo (n = 16); 2) ETCC ativo + EEP simulado (n = 16); 3) ETCC simulado + EEP ativo (n = 16); 4) ETCC simulado + EEP simulado (n = 16). A intervenção ETCC ativa consistirá em estimulação anodal com 2mA por 20 minutos sobre o córtex motor primário (M1) contralateral ao quadril mais doloroso. A intervenção ativa de EEP consistirá em estimulação elétrica a 100Hz por 30 minutos no quadril mais doloroso. O efeito desta combinação será avaliado sobre a intensidade da dor, representação cortical do glúteo médio, densidade de potência eletroencefalográfica e niveis sistemicos de BDNF e TNF. **Resultados e conclusão:** A revisão narrativa destacou alterações mal adaptativas, tais como diminuição do controle inibitorio, sensibilização central, reorganização cortical, comprometimento do controle motor e inibição neuromuscular artrogênica. Os resultados do protocolo fornecerão os primeiros dados sobre o uso da neuromodulação não invasiva para o controle da dor nessa condição.

Palavras-Chaves: Anemia falciforme, Neuromodulação, Estimulação periférica, BDNF, TNF, tDCS.

ABSTRACT

Introduction: Chronic pain is the main comorbidity related to Sickle Cell Disease (SCD). The Central Nervous System (CNS) may undergo poorly adaptive changes due to persistent painful stimulation, which in turn plays an important role in chronic pain. The combination of neuromodulatory techniques, such as Transcranial Direct Current Stimulation (tDCS) and Peripheral Electrical Stimulation (PES), has been shown to be a promising therapeutic option for pain control and to decrease the CNS maladaptive changes. However, to date, few studies have evaluated the CNS of individuals with SCD and no study sought to control pain in this population with the use of tDCS and PES. **Objectives:** To present a narrative review highlighting the possible maladaptive changes in the CNS and propose ways of evaluating them in the clinical setting. In addition, to propose a protocol with the combination of tDCS and PES for the control of chronic pain in individuals with SCD. **Methodology:** The narrative review was conducted from March to September 2017. The protocol is a parallel, controlled, randomized, double blind clinical trial. In this protocol, 128 individuals with SCD and chronic pain secondary to hip osteonecrosis will be divided into two groups (HbSS n = 64) and (HbSC n = 64). Participants in each group will be randomized to the following combination treatments: 1) active tDCS + active PES (n = 16); 2) active tDCS + simulated PES (n = 16); 3) Simulated tDCS + active PES (n = 16); 4) Simulated tDCS + Simulated PES (n = 16). Active tDCS intervention will consist of 2mA anodal stimulation for 20 minutes on the primary motor cortex (M1) contralateral to the most painful hip. The active intervention of PES will consist of electrical stimulation at 100Hz for 30 minutes in the most painful hip. The effect of this combination will be evaluated in pain intensity, cortical representation of the gluteus medius, electroencephalographic power density, and systemic levels of BDNF and TNF. **Results and conclusions:** The narrative review highlighted maladaptive changes, such as decreased inhibitory control, central sensitization, cortical reorganization, impaired motor control, and arthrogenic neuromuscular inhibition. The results of the protocol will provide the first data on the use of noninvasive neuromodulation to control pain in this condition.

Keywords: Sickle cell anemia, Neuromodulation, Peripheral electrical stimulation, BDNF, TNF, TMS.

LISTA DE ABREVEATURAS E SIGLAS

AMI	Arthrogenic muscle inhibition
ANOVA	Repeated measure analysis of variance
BDNF	Brain-derived neurotrophic factor/ fator neurotrófico derivado do cérebro
CAR	Central activation rate
CNS	Central nervous system
CPM	Conditonal pain modulation
CSI	Central sensitization inventory
DF	Doença falciforme
DIRES	Regional Health Directorate
DN-4	Douleur Neuropathique 4 questionnaire
EEG	Eletroencefalografia/ Electroencephlography
EEP	Estimulação elétrica periférica
EMG	Electromyographic
EMT	Estimulação magnética transcraniana
ETCC	Estimulação transcraniana com corrente constante
HADS	Hospital anxiety and depression scale
HbS	Hemoglobin S
HbSC	Heterozigotos para os alelos S e C / Heterozygous for S and C alleles
HbSS	Homozigotos para os alelos S / Homozygous for S allele
ICC	Intra-session reliability
KVIQ	Kinesthetic and visual imagery questionnaire
M1	Primary motor cortex
MI-kinesthetic	Kinesthetic motor imagery
NMDA	N-methyl-D-aspartate

PAG	Periaqueductal gray matter
PAN	Primary afferent nociceptor
PCST	Painful conditioning stimulus
PDI	Pain Disability Index
PES	Peripheral electrical stimulation
PRS	Protocol Registration and Results System
PST	Painful stimulus test
QST	Quantitative sensorial test
ROI	Regions of interest
RT	Room temperature
RVM	Rostral ventromedial medulla
SDC	Sickle cell disease
SNC	Sistema nervoso central
tDCS	Transcranial direct current stimulation
TMS	Transcranial magnetic stimulation
TNF	Tumor necrosis factor/ fator de necrose tumoral
VAS	Visual Analogue Scale

LISTA DE SÍMBOLOS

µV	Microvolts
kΩ	Quiloohms

SUMÁRIO

1. INTRODUÇÃO

A doença falciforme (DF) é a mais conhecida das alterações hematológicas hereditárias no homem. Estimativas sugerem que no mundo nasçam 250 mil crianças por ano com DF. O estado da Bahia possui a maior prevalência da doença no Brasil e em Salvador 1 a cada 655 crianças nascidas tem DF. Nesta doença, em condições de baixas concentrações de oxigênio, a hemácia sofre uma deformação estrutural que dificulta o fluxo sanguíneo nos vasos de pequeno calibre, ocasionando vaso-oclusão que resulta em hipoxemia local, lesão tecidual e dor. O genótipo é um importante fator de risco para a gravidade clínica da doença e indivíduos homozigotos para o alelo S (HbSS) exibem manifestações clínicas mais intensas. Entretanto, indivíduos heterozigotos para os alelos S e C (HbSC) apresentam maior risco para complicações tromboembólicas, retinopatia e necrose papilar renal quando comparados com individuos HbSS.

Dentre as manifestações clínicas relacionadas à DF, destaca-se a dor crônica, a qual pode ser causada por osteonecrose, e também por outras condições musculoesqueléticas, tais como osteomielite, osteoartrite séptica, osteoporose e osteopenia. No entanto, não há uma boa correlação entre as lesões estruturais e a intensidade da dor. Grande parte dos indivíduos com DF são refratários aos recursos farmacológicos e cirúrgicos utilizados para controlar a dor. Isto faz com que a avaliação e manejo da dor crônica seja um grande desafio tanto para clínicos quanto para pesquisadores.

Estudos têm mostrado de maneira consistente que, em condições de dor crônica, o sistema nervoso central (SNC) sofre importantes alterações mal adaptativas, tais como, diminuição da inibição ou aumento da facilitação endógena descendente, sensibilização central, alterações na organização motora cortical e inibição neuromuscular artrogênica. Estas alterações mal adaptativas têm um importante papel na manutenção da dor crônica e possivelmente podem explicar a pouca resposta dos pacientes com DF aos tratamentos com finalidade analgésica. Até o momento, poucos estudos avaliaram as alterações mal adaptativas no sistema nervoso dos indivíduos com DF. Além disso, no ambiente clinico, os instrumentos disponíveis para avaliar estas alterações ainda são pouco utilizados por profissionais de saúde, e isto possivelmente contribui para o uso menos eficiente das abordagens terapêuticas.

A estimulação transcraniana com corrente continua (ETCC) é um recurso eletroterapêutico que tem se mostrado eficaz no controle das alterações mal adaptativas em diversas condições neurológicas. Trata-se de uma técnica de estimulação cerebral não invasiva, que se baseia na alteração do potencial de repouso da membrana neuronal para induzir modulação da excitabilidade cortical. Esta técnica já foi utilizada em diversas condições dolorosas e apresentou bons resultados. Entretanto, a ETCC possui um tamanho de efeito considerado pequeno para o controle da dor. Diante disto, a combinação de ETCC com Estimulação Elétrica Periférica (EEP), um outro recurso eletroterapêutico não invasivo, tem se mostrado promissora no manejo da dor lombar crônica, possivelmente devido a um sinergismo entre as técnicas neuromodulatórias. Porém, o efeito da combinação destas técnicas ainda precisa ser avaliado em outras amostras, tais como indivíduos com DF.

Com base neste racional teórico, foi realizado uma revisão narrativa da literatura com o objetivo destacar as potenciais alterações mal adaptativas do SNC dos indivíduos com doença falciforme que sofrem de dor articular crônica, e apresentar métodos confiáveis de avaliação neuromusculoesquelética. Em seguida, foi proposto um protocolo de ensaio clínico com o objetivo principal de avaliar se a combinação de ETCC e EEP tem efeito superior sobre a intensidade da dor articular crônica de indivíduos falcêmicos com genótipo HbSS e HbSC comparado com o uso individual destas técnicas. Além disso, este protocolo se propõe a avaliar os efeitos desta combinação sobre desfechos eletrofisiológicos e moleculares, tais como representação motora cortical avaliada por Estimulação Magnética Transcraniana (EMT), densidade de potência de frequências Eletroencefalográficas (EEG) e níveis sistêmicos de Fator Neurotrófico Derivado do Cérebro (*BDNF*, do inglês *brain-derived neurotrophic factor*) e Fator de Necrose Tumoral (*TNF*, do inglês *tumor necrosis factor)*.

Os assuntos abordados na revisão narrativa contribuirão no levantamento de hipóteses de pesquisa para futuros estudos e darão suporte ao profissional de saúde em relação a escolha de instrumentos e métodos eficientes para a avaliação da dor crônica de indivíduos com doença falciforme. O protocolo de ensaio clínico proposto é o primeiro a buscar a combinação terapêutica de ETCC e EEP para o controle de dor crônica em indivíduos com DF. Além disso, este protocolo é o primeiro a usar resultados eletrofisiológicos e moleculares para avaliar os efeitos neuromodulatórios desta combinação.

Os resultados do protocolo fornecerão os primeiros dados sobre o uso da neuromodulação não invasiva para o controle da dor nessa condição. Além disso, os resultados secundários fornecerão dados importantes sobre segurança, tolerabilidade e possíveis mecanismos de ação através de medidas eletrofisiológicas (EEG e EMT) e moleculares (BDNF e TNF).

2. OBJETIVOS

2.1 Objetivos da revisão narrativa:

Primário:

Destacar as possíveis alterações mal adaptativas do SNC na dor articular crônica relacionada a DF

Secundário:

Descrever métodos confiáveis de avaliação neuromusculoesquelética

2.2 Objetivos do artigo protocolo:

Primário:

Avaliar se uma única sessão de ETCC anódica combinada com EEP sensorial tem efeitos superiores sobre a intensidade da dor de indivíduos com DF HbSS e HbSC comparado com o uso individual das técnicas em indivíduos com DF

Secundários:

Avaliar os efeitos de uma única sessão de ETCC anódica combinada com EEP sensorial sobre variáveis neurofisiológicas, tais como: densidade de potência eletroencefalográfica, representação cortical do músculo glúteo médio e níveis sistêmicos de BDNF e TNF em indivíduos com DF

Avaliar se o genótipo HbSS ou HbSC está associado com a resposta terapêutica em indivíduos com DF

3. REVISÃO DE LITERATURA NARRATIVA

Artigo n°1

Assessment of central nervous system maladaptive changes related to chronic joint pain in sickle cell disease

Ainda não submetido

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Abstract

Chronic joint pain in individuals with sickle cell disease (SCD) is associated with various musculoskeletal conditions. The poor correlation between structural injury and the intensity of joint pain reported suggests the involvement of maladaptive changes in the central nervous system (CNS). The impairment of pain control mechanisms, central sensitization, primary motor cortex reorganization, motor control deficits and arthrogenic muscle inhibition

are possibly related to chronic joint pain in the SCD. Understanding CNS mechanisms related to joint pain in SCD is very important to guide treatment of individuals with this condition. Our objective is to highlight maladaptive changes in the CNS related to SCD joint pain. A secondary objective is to present to general practioners, haemathologists and other health professionals that do not have strong background on musculoskeletal assessment reliable methods of clinical musculoskeletal examination. This review highlights that the use of methods of musculoskeletal assessment in conditions of chronic pain not related to SCD, has shown several maladaptive changes. It is possible that these alterations are also present in subjects with SCD, so it is necessary for health professionals to evaluate these changes in the population with sickle cell disease.

Introduction

Sickle cell disease (SCD) is a set of hereditary diseases caused by substitution of glutamine acid by the valine at the sixth position of the hemoglobin β chains, which leads to the presence of hemoglobin S (HbS). Conditions such as low oxygen concentration, hypovolemia and others can precipitate `fiber formation` twisting HbS molecules forming the sickle-shaped red blood cell membrane causing vaso-occlusive crises that over time favor the onset of chronic pain conditions (Ballas 2015).

Joint pain is a common condition in SCD that may be also associated with several musculoskeletal problems such as osteomyelitis, dactylitis, arthritis and osteonecrosis (Caracas Mda et al. 2013; Hernigou et al. 2006; Hughes et al. 2016). These conditions have a higher incidence in SCD, are usually chronic, and may play an additional role for chronic pain generation. (Caracas Mda et al. 2013; Hernigou et al. 2006). They may be focal, when involving a single joint, or multifocal, when they involve more than one joint (Flouzat-Lachaniette et al. 2016).

The poor correlation between structural lesions and the intensity of self-reported pain, as well as the diffuse nature of the symptoms, makes chronic joint pain a challenge for SCD patients, clinicians and researchers (Bedson and Croft 2008). As in other joint diseases (Arendt-Nielsen et al. 2010; Kim et al. 2015; King et al. 2009; Schabrun et al. 2015; Shanahan et al. 2015), the role of the central nervous system (CNS) in the perpetuation of

symptoms should also be considered in SCD. CNS maladaptive changes in chronic joint pain can lead to peripheral and central sensitization, as well as a deficiency in the nociceptive modulation systems, which may contribute in perpetuating pain cycle (Arendt-Nielsen et al. 2015).

To date there are no consistent data demonstrating the influence of maladaptive change in the CNS in the maintenance of chronic joint pain in individuals with SCD. However, it is possible that those neural changes occur, once these individuals have neuropathic pain characteristics when evaluated with quantitative sensory tests (Ezenwa et al. 2016), which may suggest the presence of CNS (central) sensitization. In addition, those with central sensitization have had more episodes of pain crisis and frequent hospitalizations (Campbell et al. 2016). However, none of these studies was specifically for chronic joint pain in SCD.

Considering the high frequency of chronic joint pain in individuals with SCD and the potential relation to central maladaptive changes, it is important to highlight the mechanisms behind those changes, as well as how to assess them in clinical and research settings. Therefore, the purpose of this review is to highlight the maladaptive changes of the CNS in SCD related joint pain, and to describe reliable methods of clinical musculoskeletal evaluation.

Methods

This study is a narrative review of the literature conducted from March to September 2017, through a systematic search in the PubMed and ScieLO databases using the following eligibility criteria: a) Relevant reviews explaining the physiological mechanisms of pain; b) Studies evaluating instruments for the evaluation of chronic pain.

There was no limit placed on the publication year and no language restrictions. We did not include as relevant papers to be read letters to the editors, clinical trials, and article commentary. Key words used were “chronic joint pain AND sickle cell disease, chronic joint pain AND central sensitization; chronic pain AND cortical reorganization; inventory central

sensitization, quantitative sensory test, arthrogenic inhibition AND joint pain". In addition, there was search in the list of references of articles found in the main search strategy

Results

Joint Pain: *Problem overview*

Pain is defined as an unpleasant sensory and emotional experience, associated with real or potential lesion or described as such, and plays a very important role in the organism's defense reaction to a hostile environment. In individuals who are born with pain insensitivity, injuries are not perceived as such, which decreases life expectancy (Cox et al. 2010). The primary afferent nociceptors have on their membrane a wide variety of transient receptor potential ion channels that are responsible for the transduction of a wide variety of noxious stimuli of high magnitude of the mechanical, thermic or chemical type (Julius 2013; Schaible et al. 2006).

The primary afferent nociceptor (PAN) is responsible to mediate nociceptive information to the dorsal horn of the spinal cord, where they form synapses with projection neurons that ascend contralaterally via the anterolateral system. Nociceptive information reaches higher brain areas that process and modulate pain sensation. Among these areas, it is possible to highlight the rostral ventromedial medulla (RVM), periaqueductal gray matter (PAG), thalamus, amygdala and primary and secondary somatosensory cortical areas (Ossipov et al. 2010).

Nociceptive information received by the thalamus is processed and redirected by thalamocortical and thalamus-amygdala connections to cortical areas of the primary and secondary somatosensory cortex (Ossipov et al. 2010). The PAG, in turn, receives inputs from these superior centers and sends it to the RVM, which through axonal fibers of ON and OFF cells form the dorsolateral funiculus and reach back the dorsal horn of the spinal cord. This is one of the endogenous mechanisms of pain inhibition, considered a 'descending modulatory mechanism of pain control'.

Insufficiency of descending inhibitory control in persistent joint pain

The descending modulation of pain is related to the activity of ON and OFF cells, which respectively modulate neuronal activity facilitating or inhibiting the transit of nociceptive information in the dorsal horn of the spinal cord both at the presynaptic and post-synaptic levels (Millan 2002; Vanegas and Schaible 2004). Malfunctioning of this mechanism may be an important triggering factor for central sensitization and chronic pain (Bouwense et al. 2013).

Malfunctioning of the descending inhibitory control has been evidenced in other similar chronic joint pain conditions, such as hip and knee osteoarthritis (Kosek and Ordeberg 2000). Regarding SCD chronic pain, an imaging study with functional magnetic resonance imaging associated coupled with electroencephalography showed increased resting state functional connectivity at the PAG, consequently affecting RVM, ON/OFF cells activity. This finding was interpreted as indicating the presence of a central mechanism (Case et al. 2017) related to SCD chronic pain. So far, data is still scarce to consistently understand CNS changes in SCD chronic joint pain.

The CNS has various ways of inhibiting the input of pain information to higher processing centers. Descending inhibitory control is a mechanism of diffuse inhibition of pain. One of the classic ways of assessing descending pain inhibitory system is through the paradigm of Conditioned Pain Modulation (CPM), previously known as “counter-irritation”, “pain inhibits pain”, “heterotopic noxious conditioning modulation”, and “diffuse noxious inhibitory control” (Yarnitsky 2010). The evaluation of descending inhibitory control by CPM in subjects with SCD should be encouraged as malfunctioning of this mechanism is closely related to the persistence of joint pain in musculoskeletal conditions such as osteoarthritis and temporomandibular dysfunction (Arendt-Nielsen et al. 2010; King et al. 2009; Kosek and Ordeberg 2000).

In the clinical setting, CPM can be assessed by subjecting the patient to a painful stimulus test (PST) before and after a painful conditioning stimulus test (PCST). Commonly, pain threshold is assessed in the non-dominant side of the body (usually the thenar eminence) and then a painful stimulus is administered in the other side. After a sufficient period so that the pain caused by the PST has ceased, a PCST is made in a heterotopic region distant from the initially stimulated region and preferably on the contralateral side of the body, lasting 1 minute. This stimulus can be done with cold or hot water, however, immersion of the dominant hand in a water basin with a temperature of 46.5 °C is more recommended with ICC = 0.79 (Kennedy et al. 2016). The use of a thermometer is important to verify the heat dissipation and ensuring the ideal temperature during immersion.

The use of a pressure threshold meter for applying painful mechanical stimulus in the thenar region of the non-dominant hand has a coefficient of intra-session reliability (ICC> 0.75) and seems to be a reliable method for performing a PST. The pain caused by mechanical stimulus must be of moderate intensity and the kilograms-force generated by the pressure threshold meter during the painful stimulus test must be the same generated before and after the PCST (Kennedy et al. 2016; Tousignant-Laflamme et al. 2008).

Immediately after the PCST, a PST is again applied, and the patient will indicate the intensity of perceived pain through the visual analog scale. The final calculation of the CPM is done according to the following equation:

$$CPM = PST1 - PST2$$

Where, PST1 corresponds to the first painful stimulus test and PST1 to the second painful stimulus test. A negative result indicates the presence of a preserved descending inhibitory control (Kennedy et al. 2016).

Central sensitization in joint pain

The perpetuation of joint pain can be favored by poor descending inhibitory control, which over time causes phenotypic alteration of Aβ fibers specialized in conducting non-painful stimuli (Millan 2002). In addition, nociceptive information is not properly inhibited, but rather it is facilitated in the dorsal horn of the spinal cord, which advances freely until it reaches higher brain areas causing a sensitization of pain processing regions (Schaible et al. 2009).

Central sensitization is associated with decreased pain threshold, expansion of pain receptive field to further regions unrelated to pain, and interpretation of non-painful stimuli as painful (Lluch Girbes et al. 2016). Individuals with central sensitization due to chronic pain secondary to osteoarthritis of the knee are five times more likely to have pain refractory to surgical treatment of total knee arthroplasty (Kim et al. 2015). Central sensitization is also related to other non-musculoskeletal symptoms, such as photophobia, phonophobia, bowel diseases and others (Caumo et al. 2017), which start to change the clinical status from a musculoskeletal disease to a multi-systems disease. Typically, those symptoms are under-evaluated by clinicians and not related to the presence of persistent pain. Central sensitization is also present in individuals with SCD, which is associated with an increase in vasoocclusive crises, poor sleep quality and psychosocial disorders (Campbell et al. 2016). For this reason, this should be considered during the evaluation since this is probably one of the main causes of refractory joint pain (O'Leary et al. 2016).

Some methods are essential in the evaluation of central sensitization characteristics in the clinical and research settings, such as the central sensitization inventory (CSI) (Caumo et al. 2017) and the quantitative sensorial test (QST) (Campbell et al. 2016). These methods can help to evaluate whether chronic joint pain in SCD is being influenced and/or supported by central sensitization. The CSI is divided into parts A and B. In part A there are 25 descriptive alternatives of multidimensional symptoms associated with central sensitization. Each alternative has a score varying from 0 (never) to 4 (always), with a maximum total score of

100 points. In part B, there are 10 alternative clinical conditions recognized as central sensitivity syndromes (Neblett et al. 2015). The cut-off at 40 points has excellent levels of sensitivity (81%), specificity (75%), positive predictive value (2.93), and negative predictive value (0.52) to recognize central sensitization (Neblett et al. 2015).

Due to the need for severity ratings of central sensitization, a 10-point classification with severity intervals was created, consisting on the following cathegories: subclinical (≤29), mild (30-39), moderate (40-49), severe (50 - 59) and extreme (≥60) (Neblett et al. 2017). This allows better utilization of CSI in the clinical practice setting, and may help as a parameter of the therapeutic response. This instrument has been culturally translated and validated in numerous languages, including the Brazilian Portuguese (Caumo et al. 2017).

Sensitivity deficits, such as allodynia or hyperalgesia to thermic and vibratory stimuli, as well as mechanical and thermal temporal summation have been associated with the presence of central sensitization in individuals with chronic pain. QST are also a way of assessing central sensitization (Campbell et al. 2016; O'Leary et al. 2016; Walk et al. 2009).

All systematic forms of sensory evaluations that allow quantified responses can be viewed as a QST. However, a set of quantitative sensory tests (mechanical, thermal and vibratory) was standardized to evaluate the integrity of the somatosensory system and to guarantee the accuracy and reproducibility of the findings (Walk et al. 2009). QST protocols in the clinical setting can be performed in both bedridden and non-bedridden individuals. These proposed protocols take into consideration several sensory parameters, as well as biological aspects ranging from body temperature to trophic changes in the musculature (Starkweather et al. 2016; Walk et al. 2009).

The safety of the QST protocol in the clinical setting has previously been tested in subjects with SCD and there was no perpetuation or worsening of pain after its application. However, caution is needed during the test because the sensitivity threshold of this population

with SCD is below the expected normal values (Ezenwa et al. 2016). Therefore, if the test temperature is very high, there may be tissue injury and possibly perpetuation of pain after QST. In patients with SCD, cold pain thresholds <17.01°C are indicative of impaired nerve sensitivity. Also heat pain threshold <43.91 °C and mechanical pain threshold <4.42g are indicative of the existence of altered sensory function (Brandow and Panepinto 2016). These reference values can be used as therapeutic markers.

Another way of assessing central sensitization in individuals with SCD uses its typical clinical characteristics through a checklist developed by a consensus of experts, which contain signs and symptoms characteristic of central sensitization (Smart et al. 2010) (see box 01). The presence of these discriminative items indicates the presence of central pain sensitization with excellent accuracy values, (sensitivity 91.8%, specificity 97.7%, positive predictive value 91.8 and negative predictive value 97.7) (Smart et al. 2011).

The use of these instruments during the evaluation of individuals with SCD and chronic joint pain may help in the more precise knowledge of the mechanism underlying the patient's pain. This provides a basis for better clinical decision making and possibly less chance of non-adherence to the proposed treatment.

Modifications of motor control and cortical reorganization in joint pain

In face to pain, the neuromusculoskeletal system undergoes adaptive motor modifications that affect motor control and consequently joint mechanics. These modifications have been studied over time due to the importance of its understanding for the clinical and research environment. Therefore, one theoretical model (Hodges 2011) was established with the purpose of clarifying the interaction between pain and motor control changes making the following propositions:

Firstly, the adaptation of the motor control to pain is a consequence of the redistribution of the activity within and between muscles. Secondly, the change in mechanical

behavior initially has a protective function to prevent further pain or injury, however, in the long term it involves changes in various levels of the nervous system, which lead to increasing joint load, decreased mobility and variability of movement and muscle weakness (Hodges 2011).

In the presence of chronic joint pain, there is a motor and sensory primary cortical reorganization, associated with motor control impairment. This has been demonstrated in individuals with low back pain (Tsao et al. 2008; Tsao et al. 2011), chronic lateral epicondylalgia (Schabrun et al. 2015), osteoarthritis of the knee (Shanahan et al. 2015) and chronic patellofemoral pain (Te et al. 2017). This cortical reorganization is expressed through the overlap, retraction or lack of clear limits (blurring) in the areas of somatotopic representation of the motor and sensory homunculi. The greater the cortical reorganization, the greater the perpetuation of the pain (Shanahan et al. 2015).

The intracortical inhibitory system, modulated by tonic GABAergic activity, plays an important role in the formation of cortical somatotopic representations. This is due to mechanisms that differentiate cortical efferent motor actions, either by facilitating muscle activation during a motor task or by inhibiting undesirable muscular activations (Liepert et al. 1998). Although changes in intracortical inhibition are not a consensus, dysfunction of GABAergic connections, such as intracortical disinhibition, has been demonstrated in individuals with chronic pain (Parker et al. 2016; Schwenkreis et al. 2010).

In individuals with SCD, joint pain is possibly associated with maladaptive changes both in motor behavior and cortical representation due to their chronic and disabling pain (Flouzat-Lachaniette et al. 2016). Considering the changes in functional connectivity of the structures involved in the descending inhibitory control of nociceptive information in individuals with SCD (Case et al. 2017), and the fact that this mechanism is negatively correlated with intracortical inhibition (Tarrago Mda et al. 2016), it is necessary that investigators and clinicians can investigate and evaluate these possible alterations of these individuals.

Arthrogenic muscle inhibition and joint pain

It is common that after joint injuries there is presence of weakness in the adjacent involved musculature. The possible cause for muscle weakness is the presence of a central reflex inhibition, which prevents complete activation of the surrounding musculature to the injured joint during a maximal voluntary muscular contraction. This phenomenon has been called arthrogenic muscle inhibition (AMI) (Rice and McNair 2010).

AMI can be interpreted as a mechanism of physiological protection to prevent new lesions and potentiation tissue repair (Hopkins and Ingersoll 2000). However, it is possible that the AMI persists for several months, or even years, after injury (Becker et al. 2004). This may compromise the rehabilitation process, through a negative impact on strengthening protocols, and lead to injury progression (Hopkins and Ingersoll 2000). Joint conditions such as joint pain, ligamentous laxity and joint effusion are potential factors that facilitate the establishment of AMI (Rice and McNair 2010).

In the AMI there is an alteration of the firing of the joint receptors that send signals for the spinal cord inhibitory interneurons, causing inhibition of the activity of the alpha motoneurons and consequently the musculature involved in the affected joint (Hopkins and Ingersoll 2000). Joint pain may contribute to the AMI due to the alteration of the excitability of the flexor reflex pathway (Rice and McNair 2010), which has the characteristic of facilitating the flexor and inhibiting the extensor muscles in the region surrounding the painful joint (Lundberg et al. 1987). In addition, joint pain in the knee has been associated with decreased muscle activation of the quadriceps (Hart et al. 2010; Stevens et al. 2003).

Although a systematic review has shown that the mechanisms of AMI are mostly studied in knee joint injury (Hart et al. 2010), it may be also observed in individuals with pathologies in the hip due a decrease in maximal gluteus activation during extension activity in pronation (Freeman et al. 2013). The most affected joint in SCD is the hip due to avascular

osteonecrosis (Flouzat-Lachaniette et al. 2016), and AMI may be a pain related mechanism in this condition. However, no studies have evaluated this mechanism in individuals with SCD.

At the clinical setting, AMI can be evaluated by the central activation rate (CAR) method, which quantifies the rate of muscle inhibition by a superimposition of an electric current pulse train applied to the muscle target during the maximal voluntary contraction (Hart et al. 2010) (see box 02).

Conclusion

The results of this review were described in a way comprehensive starting from a physiological view of joint pain and evolving to maladaptive nervous system changes in conditions of chronic joint pain not related to SCD. However, the chronic joint pain in patients with SCD might present potential to modify various neurophysiological mechanisms that continue persistent pain. These changes are characterized by poor descending inhibitory control, in addition the central pain sensitization, motor control impairment, reorganization of cortical motor representation and inhibition of induced maximal voluntary contraction.

Some instruments and methods such as, Conditional pain modulation, Central Sensitization Inventory, Quantitative sensorial test, the Central Activation Rate method, and a checklist containing the characteristic signs and symptoms of central sensitization must be used in clinical practice, to evaluate the possible changes in the nervous system in individuals with SCD.

Future studies should be performed with the objective of elucidating and confirming these possible changes in the nervous system in individuals with SCD related to chronic joint pain, to understand and threat the pain on those patients with better results.

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Box 1 Clinical indicators that are related to the presence of central sensitization

A) Pain disproportionate to the magnitude of injury;

B) Unpredictable pain that does not have a provocative pattern proportional to the provocative stimuli, be they thermal, mechanical or chemical;

C) Pain diffuse to palpation and reaching healthy areas unrelated to injury (oil spot phenomenon);

D) Maladaptive psychosocial characteristics such as:

- history of sexual abuse
- catastrophis
- erroneous beliefs
- pain-related moodiness
- medical conflict
- change in social performance in the family
- work
- social life.

Box 01. Clinical indicators related to central sensitization

Box 2 Evaluation by the central activation rate (CAR) method

1) The muscles evaluated must be in resting and the skin region cleansed with dermoabrasive gel to decrease the impedance of the skin and ensure an adequate electromyographic record during the evaluation;

2) The electric stimulus uses trains of 10 pulses, in 100Hz, with 200ms of pulse duration and 400V (51). The evaluator should give stimuli in steps of 100mA in ascending order with intervals of 10 seconds inter stimulus in order to reach the ideal intensity to generate a greater peak of torque (51). The torque evaluation can be performed using a dynamometer;

3) Next step, the individual performs a maximal voluntary contraction. When the muscle contraction reaches the plateau of torque, one electric stimulus pulse is applied at a maximum supra value (120% of the amplitude that generated the greatest muscle torque);

4) The quantification and interpretation of AMI is done through the equation:

$$AMI = \left[\frac{a}{b}\right] x100$$

Where "a" corresponds to the plateau of torque before the electric stimulus is applied, and "b" corresponds to the peak of torque generated by the electric stimulus associated with the maximum voluntary contraction (51). The greater the peak torque generated by the electrical stimulus superimposed to muscle contraction, the greater the AMI.

Box 02. Evaluation of Arthrogenic muscle inhibition by central activation rate (CAR) method

4. METODOLOGIA

O artigo principal trata-se de um protocolo de ensaio clinico, paralelo, controlado, randomizado, dublo cego. Neste protocolo à randomização será em bloco de acordo com uma web-ferramenta especifica (www.randomization.com). Um pesquisador assistente não envolvido em qualquer outra etapa de execução do estudo gerará uma planilha de alocação com quatro grupos para cada tipo de genótipo (HbSS e HbSC). A ocultação de alocação será feita com o uso de envelopes opacos lacrados e enumerados em ordem crescente e o sigilo de alocação será mantido até o fim das análises. O envelope de alocação será aberto no dia da intervenção de acordo com a ordem de inclusão do participante do estudo, e dentro do envelope poderá haver apenas um dos seguintes grupos de tratamento: 01) ETCCa ativa + EEP sensorial ativa; 02) ETCCa ativa + EEP sensorial simulada; 03) ETCCa simulada + EEP sensorial ativa; 04) ETCCa simulada + EEP sensorial simulada.

Neste protocolo de ensaio clinico está previsto a participação de indivíduos com DF cadastrados nas Unidades Básicas de Saúde dos municípios da 31ª Diretoria Regional de Saúde (DIRES - BA) e nos Centros de Referência de Atendimento às pessoas com DF dos municípios de Salvador - BA e Feira de Santana - BA. Todos os procedimentos de coleta de dados antes e após o tratamento serão realizados no Núcleo de Estudo em Saúde e Funcionalidade (NESF) do Instituto de ciências da Saúde na Universidade Federal da Bahia.

Os indivíduos deverão ser submetidos aos seguintes critérios de inclusão: Ter dor crônica no quadril de no mínimo 6 meses de duração, ter idade entre 18 anos e 50 anos. Os excluídos serão todos os que tiverem contraindicação para o uso de estimulação magnética transcraniana (EMT) como: Possuir implante coclear, marcapasso cardíaco ou implante metálico no crânio/encéfalo. Estar em uso de droga que modifique o limiar de ativação neuronal (ex: antidepressivo, anticonvulsivante e antipsicóticos). Histórico de convulsão ou epilepsia e estar em período gestacional. Ter dor do tipo neuropática confirmada pelo Douleur Neuropathique de 4 questões (DN-4). Ter implante metálico no local de aplicação da estimulação periférica.

Por fim, como o produto final dessa dissertação tratou-se da publicação deste protocolo de ensaio clinico todos os aspectos metodológicos e operacionais estão descritos detalhadamente no artigo de protocolo.

5. RESULTADO:

Artigo n°2

Does Transcranial Direct Current Stimulation combined with Peripheral Electrical Stimulation have an additive effect in the control of hip joint osteonecrosis pain associated with sickle cell disease? A protocol for a one-session double blind, randomized clinical trial

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ABSTRACT:

Chronic pain in sickle cell disease (SCD) is probably related to maladaptive plasticity of brain areas involved in nociceptive processing. Transcranial direct current stimulation (tDCS) and peripheral electrical stimulation (PES) can modulate cortical excitability and help to control chronic pain. To date, no study investigating the conjoint effects of both neuromodulatory techniques on chronic pain among patients with SCD. This protocol aims to assess whether the combined application of tDCS and PES would be more effective for alleviating pain in patients with SCD than the single application of each technique. The protocol consisted of one-session double blind, randomized clinical trial (NCT02813629) in which 128 patients with SCD and femoral osteonecrosis will participate. Stepwise procedures will occur on two independent days: On 1st day the participants will be assessed for eligibility criteria. On 2nd Day the procedures will begin in the morning and will be divided into four stages: 1) Sample Characterization; 2) baseline assessment; 3) Intervention; 4) Post-

intervention assessment. Participants will be divided into two groups according to homozygous for S allele (HbSS) (n=64) and heterozygous for S and C alleles (HbSC) (n=64) genotypes. Patients in each group will be randomly assigned to one of the following combined interventions: 1) active tDCS + active PES (n=16); 2) active tDCS + sham PES (n=16); 3) sham tDCS + active PES (n=16); 4) sham tDCS + sham PES (n=16). Active tDCS intervention will consist of anodal stimulation at 2mA over the primary motor cortex contralateral to the most painful hip for 20 minutes. Active PES intervention will consist of electrical stimulation at 100Hz over the most painful hip for 30 minutes. The main outcome of the study will be pain intensity measured by visual analogue scale (VAS). In addition, Electroencephalography (EEG) power density, cortical maps of the gluteus medius muscle elicited by transcranial magnetic stimulation (TMS), systemic levels of the brain-derived neurotrophic factor (BDNF) and the tumor necrosis factor (TNF) will be obtained as secondary outcomes. Counteracting measures will have the objective of controlling the potentials pitfalls in sample selection, intervention and outcome measures.

Keywords: Neuromodulation, Electroencephalography, Sickle Cell Disease, BDNF, TNF, tDCS, Peripheral Electrical Stimulation

INTRODUCTION

Sickle-cell disease (SCD) refers to the group of haemoglobinopathies in which haemoglobin S pay a relevant role. The severity of SCD differs depending on the different genotype, of which the most prevalent are homozygous for S allele (HbSS) and heterozygous for S and C alleles (HbSC), what the more severe being the HbSS genotype (1). Pain is the major symptom reported by patients with SCD and it is present throughout all the life of the individual (2). The main cause of pain in SCD is the cyclic presence of ischemic vaso-oclusive events (3) that can provoke relevant damages of bone tissues (4) and may lead to chronic joint pain syndromes (5) such as osteomyelitis, dactylitis, arthritis and osteonecrosis (6-8).

Chronic pain syndromes has a strong impact on quality of life of patients with SCD and lead to significant disability (9). However, as it occurs with many other chronic pain syndromes, radiographic examinations are poorly related with reported pain intensity and structural injuries are unable to fully explain the effects provoked by pain (10, 11). A possible explanation for this disagreement between pain and objective clinical findings is the non-adaptive changes of brain areas involved in nociceptive information processing and maintenance of pain over time (12). This phenomenon is termed maladaptive plasticity (13).

Although little is known about the role of maladaptive plasticity in the maintenance of chronic pain in patients with SCD, several functional brain imaging studies (14-16) have revealed an increased functional connectivity of anterior cingulate cortex, primary and secondary somatosensory cortices (16), as well as the periaqueductal grey matter (PAG) (15). Those findings seem to be related to high frequency of hospital admissions (16), and

enhanced central sensitization in individuals with SCD (14). Thus, it seems that pain in SCD display similar neurobiological characteristics as it occurs in other chronic pain conditions.

Some molecular changes may be related to the presence of chronic pain in SCD. Brain-derived neurotrophic factor (BDNF) is involved in the neural regulation, maintenance and synaptic formation and therefore has an important role in the plasticity of the central nervous system (17). Due to these functions, the increase of BDNF levels in response to inflammatory processes has been interpreted as an adaptive action to neural protection (18, 19). However, increased levels of BDNF may also potentiate N-methyl-D-aspartate (NMDA) receptors in the terminals of the primary afferent nociceptors (20), which may be associated with increased sensitization of dorsal horn neurons in response to nociceptive stimuli (21, 22). In addition, a recent study showed that higher levels of BNDF were associated with higher scores on central sensitization and poor endogenous inhibitory control in individuals with chronic pain (23). Systemic levels of brain-derived neurotrophic factor (BDNF) have been found to be about 130% higher in people with chronic joint pain (24) and positively correlated with levels of tumor necrosis factor (TNF)(19).

In addition to these biomolecular changes, electrophysiological changes in brain activity have also been observed. A systematic review (25) of studies evaluating motor cortex excitability through transcranial magnetic stimulation (TMS) has identified a decrease in primary motor cortex GABAergic intracortical inhibitory connections in individuals with chronic pain, which has been inversely correlated with systemic BDNF levels (26). This appears to potentiate dysfunctional reorganization in the motor cortex (27), either by an overlap (28), "blurring" (29) and / or decreased somatotopic representation in this region (30). In addition, chronic pain can be characterized by the presence of an abnormal EEG pattern, mainly a preponderance of slow brain rhythms such as delta (31), theta and alpha (32, 33). This has been interpreted as the result of significant changes in the thalamocortical loop due to sensitization of the structures involved in nociceptive processing (34).

All these data provide support for the idea that maladaptive plasticity may underlie chronic pain and, therefore it should be also present in patients with SCD. This may help explain why some of these people are refractory to pharmacological and non-pharmacological analgesic treatments for pain management (35, 36). Within this context, the need arises to explore new therapeutic strategies that are intended to reverse or diminish the effects of chronic pain. Transcranial direct current stimulation (tDCS) has the capacity to induce neuroplasticity changes dependent on polarity (anodic pole increasing corticospinal excitability and cathodic pole leading to opposite effect) (37, 38). This neuromodulatory technique has been investigated in several conditions of chronic pain(39-43), and has the potential to influence many of these previously exposed maladaptive changes (44-47).

The duration of effects of a single tDCS session on human cortical excitability is dependent on the intensity and duration of the stimulation (48). A study with healthy subjects showed that 13 minutes of anodic tDCS over the primary motor cortex (M1) caused an increase in cortical excitability that could be monitored in up to 90 minutes after the end of

stimulation (49). The effects of a single tDCS session on systemic levels of BDNF remain unclear. However, animal studies have shown that tDCS is capable to immediately decrease systemic BDNF levels under experimental pain conditions (50, 51). It has been also demonstrated that one session of tDCS can influence EEG brain rhythms(52, 53) and clinical outcomes such as pain (54).

However, a recent systematic review (55) showed that tDCS alone provide little effects on pain control. By contrast, other studies have found contradictory results for the effects of tDCS on chronic pain (56-58), making it necessary to investigate ways to enhance its therapeutic effects. Therefore, studies have proposed its use in association with other therapeutic techniques such as aerobic exercise (59) physical therapy (60) and peripheral electrical stimulation (PES)(61-64). These therapeutic associations assume that brain responsiveness to a particular therapy may be facilitated by techniques that increase or decrease the cortical excitability (65). PES is a neuromodulatory technique that can also induce transient changes in corticospinal excitability and this is dependent on intensity (66, 67) and time (68) parameters. PES with intensity at the sensory threshold decreases excitability, while at the motor threshold the effect is opposite (66).

PES may have synergistic effects with tDCS favoring long-term potentiation, or long-term depression, depending on how they are combined (69). It has previously been shown that if two excitatory stimuli are associated, a null result occurs (62). However, when the association is made between an inhibitory and an excitatory stimulation, the result is a synergistic summation effect (61, 64). Therefore, when a PES at the sensory threshold is associated with anodic tDCS, the expected result is a sum of the analgesic effects of both techniques. To corroborate this, one study showed that the association of these two neuromodulatory techniques reduced pain intensity by 36.5%, while tDCS alone reduced pain by 15% in persons with chronic pain (64). In addition, it has also been shown that the association of these two techniques, generated better immediate effects on the restoration of the cortical representation of paraspinal muscles(63), and was more effective than their isolated administration in the control of chronic low back pain (61).

These evidences suggest that the association of tDCS and PES in the sensory threshold is a promising therapeutic strategy for the control of chronic pain. However, so far, specifically in subjects with SCD of HbSS and HbSC genotypes, no study has tested the effect of this therapeutic association on pain intensity. In addition, little is known about the mechanisms related to the maladaptive plasticity in the central nervous system of individuals with SCD and the effects of this combination on neurophysiological mechanisms in this painful condition.

OBJECTIVES

Primary objective:

- Evaluate whether a single session of anodal tDCS associated to sensory PES has superior effects on pain intensity of individuals with SCD in HbSS and HbSC compared to individual use of techniques.

Secondary objective:

- Evaluate the effect of a single session of anodal tDCS associated with sensory PES on neurophysiological variables such as: electroencephalographic power density, TMS cortical mapping of the gluteus medius muscle and systemic levels of BDNF and TNF.

- Evaluate whether the genotype type HbSS or HbSC is associated with a therapeutic response.

HYPOTHESIS TO BE TESTED

Hypothesis to primary outcome:

- A single session of anodal tDCS associated with sensory PES should provide a greater analgesic effect compared with the exclusive use of one of the techniques.

Hypothesis to secondary outcomes:

- The association of anodal tDCS and sensory PES should cause a greater effect of decreasing the systemic levels of BDNF and TNF and increasing cortical representation of the gluteus medius muscle of participants with SCD compared to the individual use of the techniques

- It is also expected that due to the analgesic effects promoted by this therapeutic association there will be a rebalancing in the electroencephalographic power density of participants with SCD.

METHODOLOGY

Study design, description of allocation and blinding:

This is a clinical, parallel, controlled, randomized, double blind trial. This study will be carried out in two independent days and all pre – and post intervention assessment will be performed at the Laboratory of Clinical Electrophysiology at Federal University of Bahia, Brazil.

The randomization will be placed in the block according to a specific web-tool (www.randomization.com). An assistant researcher who does not take part in any other stage of the study will generate an allocation sheet in four intervention groups for each genotype

(HbSS and HbSC), totaling eight groups. Allocation concealment will be done with the use of sealed opaque envelopes listed in ascending order and the allocation secrecy will be maintained until the end of the analyses. The allocation envelope will be opened on the day of the intervention according to the order of inclusion of the study participant. The envelope will contain one of the following combined interventions: 1) active tDCS + active PES (n = 15); 2) active tDCS + sham PES (n = 15); 3) sham tDCS + active PES (n = 15); 4) sham tDCS + sham PES (n = 15) (see Figure 1).

Participants and Eligibility and Discontinuity Criteria

Participants in this study will be individuals with SCD enrolled from the health units of the municipalities of the 31st Regional Health Directorate (DIRES - BA) and in the Reference Centers of attention to patients with SCD of the municipalities of Salvador - BA and Feira de Santana - BA. The enrollment of participants will take place between March 2016 to December 2019, and will be conducted through advertisements in the reference centers. In order to improve adherence to the intervention protocol, all participants in this study will receive financial aid for round-trip transportation, as well as a meal voucher. In addition, all will receive care recommendations regarding SCD, and at the end of the study will receive the most effective intervention.

To homogenize the sample, only participants with SCD of HbSS and HbSC genotypes who suffer from chronic pain secondary to femoral head osteonecrosis will participate in this study. In addition, the eligibility and discontinuity criteria of this study are:

Inclusion:

A) Have chronic pain secondary to hip osteonecrosis of at least six months of duration.
B) Pain intensity above 3 in a 11 points visual analogic scale (VAS)

C) Be 18 to 50 years old.

Exclusion:

A) Any contraindication to the use of TMS and tDCS such as: cochlear implant, cardiac pacemaker or metallic implant in the skull / brain. Being under a drug treatment that modifies the threshold of neuronal activation (i.e. Antidepressant, anticonvulsant and antipsychotic). History of seizure or epilepsy and gestational period.

B) Neuropathic pain screened by the Douleur Neuropathique 4 questionnaire (DN-4) (70).

C) Metal implantation at the site of peripheral stimulation

D) Occurrence of infectious disease in less than one week before inclusion in the study.

Discontinuity:

There will be discontinuity if:

A) In the event of a moderate adverse effect (i.e. discomfort caused enough to interfere with the patient's usual activities) or severe (i.e. significant impairment of the patient's usual activities or even total disability, life threatening, or death) during the intervention protocol or neurophysiological evaluation.

B) If the participant withdraws consent at any stage of the study.

Interventions:

Interventions protocols will be managed by an experienced physiotherapist. During intervention, participant will be sitting comfortably in a chair in silence and will be encouraged to make no cognitive effort such as mathematical calculations or complex reading. tDCS will be applied with an electrostimulation device (tDCS stimulator – TCT, China) connected with two silicon 5x7 (35 cm^2) electrodes embedded in saline solution (0.9%) with anodic pole placed in the region of the primary motor cortex (M1) contralateral to the painful hip (or more painful in cases of bilateral symptoms) and cathodic pole placed in the contralateral supra-orbital region. The duration of the stimulation will be 20 minutes with 2 mA of intensity, ramped up and down for 30 seconds at the initial and final stages of stimulation.

PES will be administered through a clinical pulse generator (Endophasys, KLD Medical Products, Brazil) using 35 cm^2 dischargeable electrodes located in the gluteus medius muscle on the side of the most painful hip. The stimulation will be held for 30 minutes and the intensity maintained at the sensory level, characterized as a comfortable intensity just under motor threshold, with pulse rate of 100 Hz and pulse duration of 200 μs. Participants will be asked about their perception every five minutes during intervention. In the sham procedures for both, tDCS and PES, the devices will be ramped up for 45 seconds and then down until no electric current is delivered. To induce blinding, participants will receive information that they may or may not feel the stimulation. At the end of each intervention protocol, the potential adverse effects and quality of blinding will be assessed by a self-report questionnaire.

Clinical and sociodemographic characterization of the sample

The sample characterization will be performed by collection of socio-demographic (i.e. gender, age, education, profession, marital status and race) and clinical data (i.e. anxiety and depression symptoms, and disability index related to pain). The socio-demographic data will be collected by self-report questionnaire designed specifically for this study by the researchers. Clinical data will be collected through the following instruments:

Hospital anxiety and depression scale (HADS): HADS comprises two 7-items subscales. Subjects will rate each item using an ordinal scale varying from 0 (non-existent symptom) to 3 (very severe symptom) (71).

Pain Disability Index (PDI): PDI is composed of seven items: family and domestic obligations, recreation, social activities, profession, sexual life, autonomy and elementary activities indispensable to life. Subjects will rate each item using an ordinal score ranging from 0 (no disability) to 10 (total disability) (72).

Outcomes measures:

Primary outcome measure:

- Intensity of pain:

This evaluation will be performed by using a VAS pre – and post intervention.

Secondary outcome measures:

- Systemic levels of BDNF and TNF.

Systemic levels of BDNF and TNF will be measured pre – and post intervention. Approximately 5 mL of blood from all study participants will be collected and stored in test tubes with anticoagulant EDTA (0.03%). The blood sample will be centrifuged at 2500 rpm for 10 minutes and the plasma stored at a temperature of -40° C.

BDNF and TNF will be quantified using enzyme-linked immunosorbent assay kits (DuoSet, R&D Systems, Minneapolis, MN). A volume of 100 µL of the capture monoclonal antibody will be added to a 96-well plate, which will be incubated for 12 hours at room temperature (RT). The wells will be washed with wash buffer (PBS/Tween) and incubated with a blocking solution (300 µL) containing PBS and bovine serum albumin for 1 hour at RT. Samples and standards will be plated and incubated for 2 hours at RT. After washings, the detection monoclonal antibody will be added to the plate and incubated for 2 hours at RT and a streptavidin-peroxidase solution added and incubated for 1 hour at RT.

Finally, the substrate solution (H_2O_2 and tetramethylbenzidine) will be added to the plate and a blue color will develop within a period of 20 minutes. The staining reaction will be stopped by adding H_2SO_4 2N and the reading will be made on a microplate reader at 450 nm. Levels of BDNF and TNF will be expressed in pg/mL and calculated from the reference values obtained with a standard curve built with known concentrations of recombinant BDNF and TNF. Concentrations of BDNF and TNF in plasma will be quantified using commercially

available antibody pairs and recombinant cytokine standards (DuoSet, R&D System, Minneapolis, MN), by sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

- Power density of electroencephalographic frequencies.

Relative power density will be measured pre– and post–intervention. An electroencephalograph (EEG Brainet36, EMSA, Brazil) with 30 electrodes arranged according to the international system of electroencephalography 10/10 will be used in the following electrode locations: F7 T3 T5 Fp1 F3 C3 P3 O1 F8 T4 T6 Fp2 F4 C4 P4 O2 Fz Cz Pz Oz FT7 FT8 TP7 CP3 FC3 CPz FCz CP4 FC4 TP8. EEG data will be collected at a sampling rate of 600 Hz and referenced to Cz. Impedance will be maintained below 5kΩ for all electrodes. The recording environment will be kept in subdued light and protected by a Faraday cage. Participants will be instructed to sit in a comfortable chair and keep their eyes closed during EEG recording pre – and post intervention. EEG record will be recoded under the following conditions:

A) **Resting state:** This condition will last 4 minutes, and the participant will be asked to focus on no specific cognitive activity.

B) **Kinesthetic motor imagery (MI-kinesthetic):** MI - Kinesthetic will start after the recording at rest and will be subdivided into two distinct stages. 1) *MI - kinesthetic of a movement in the non-painful region of the body:* After 10 seconds of recording the participants will hear a standardized command requesting to mentally simulate closing and opening the contralateral hand to the most painful hip. The command will be displayed 8 times, being 5 seconds of closing movements and 10 seconds of opening movements of the hand, totaling 120 seconds. 2) *MI-kinesthetic of a movement in the painful region of the body*: The same protocol described above will be repeated, but this time the command will request to mentally simulate the abduction and adduction of the most painful hip. Electromyographic (EMG) data of the gluteus medius muscle on the most painful side and flexor muscle of the fingers contralateral to the hip will be also recorded to ensure the absence of real movement. After each recording, the quality of the kinesthetic IM will be rated by the kinesthetic and visual imagery questionnaire (KVIQ).

The processing of EEG data will be carried out in MATLAB software (version 2015) and EEGlab *toolbox* (version 14). The signals will be filtered offline with a bandpass between 0.5 and 45Hz. The EEG data will be segmented into epoch of 1.71 seconds to allow an analysis of power densities at frequencies ranging from 1.2 – 30 Hz. EEG artifacts with minimum amplitude below -750 or maximum amplitude above 700 μV will be rejected by a semi-automated rejection protocol. EEG data with more than 33% of rejected epochs will be excluded from further analyses. After the artifact rejection protocol, all EEG data will be adjusted to the same percentage of epochs.

Power density will be calculated by fast Fourier transform in each epoch and electrodes separately for each participant. The average power densities will be grouped in delta (1.2 – 3.5Hz), theta (4 – 7Hz), alpha (8 – 12Hz), and beta (13 – 30Hz) frequency bands. Regions of interest (ROI) chosen for analysis will be computed by averaging power densities at the four frequency bands for the following groups of electrodes: frontal (F7, F3, Fp1, Fz, Fp2, F4, F8), central (FC3, C3, FCz, Cz, C4, FC4), parietal (CP3, P3, CPz, Pz, CP4, P4) temporal (FT7, T3, TP7, T5, FT8, T4, TP8, T6) and occipital (O1, Oz, O2). After obtaining absolute power density calculation, the relative power density will be calculated dividing electrode's values in each one of the analyzed frequencies by their values in the total power spectrum.

- TMS cortical mapping

Cortical mapping will be quantified pre– and post–intervention. After EEG recording, each participant will be asked to lie down comfortably in a supine position on an examination table with head and neck resting on a support. The TMS cortical mapping will be done using a single-pulse transcranial magnetic stimulation (Bi-Stim; Magstim Co. Ltd, Dyfed, UK) delivered over M1, contralateral to the hip with the worst pain in the participant with SCD. An eight-figure coil will be positioned with the handle oriented backwards and aligned to the sagittal suture, inducing a postero-anterior flow of current. One cap marked with an 8 x 7 cm grid and oriented to the vertex will be placed on the head of the participants and regularly checked to guarantee placement consistency. The vertex will be marked on the intersection of interaural and nasion to inion lines according to the 10/20 international EEG system.

The stimulus intensity for mapping will be set at 120% of active motor threshold for the gluteus medius muscle. Active motor threshold will be defined as the minimum intensity at which a TMS stimulus evoked a response of 200 μV while the gluteus medius muscle is contracted under a bridge position in supine, at 10% of maximum voluntary contraction force and determined using the TMS motor threshold assessment tool (MTAT 2.0). The pulse of TMS will be applied every six seconds, with a total of five stimuli at each site on the 8x7 cm grid.

Surface electrodes Ag/AgCl (Noraxon, USA) will be used to record EMG activity at the gluteus medius muscle. Placement and positioning of the electrode will be determined after palpation of the gluteus medius muscle during a moderate voluntary contraction. The reference electrode will be placed on the anterior superior iliac spine. The EMG signals will be amplified 3000 times, filtered, bandpass between 1 Hz and 2 kHz with sampling rate maintained at 4 kHz using Signal v.06 software (Cambridge Electronic Design, UK). EMG data elicited by TMS will be monitored in real time to ensure the consistency of responses evoked (Signal, Cambridge Electronic Design, UK).

TMS map volume and center of gravity (Cog) of the gluteus medius muscle will be used as dependent variables. These parameters will be calculated respectively with the sum of normalized MEP amplitudes at each site and the formula:

$$CoG = \sum V_{iX} X_i / \sum V_i ; \sum V_{iX} Y_i / \sum z_i$$

Where V_i= mean MEP amplitude at each site with the coordinates X_i, Y_i.

Potential for adverse effects and harm:

The tDCS has relatively minimal adverse effects, which includes mild tingling, itching, burning and mild pain sensation under the surface of the electrodes, fatigue, and somnolence (73). These potential adverse effects may be avoided by appropriate training in the handling of the technique. The use of TMS to evaluate the motor cortical representation of the gluteus medius muscle also has some potential risks (74), but all these are rare and will be duly clarified to the participant at the moment of recruitment and declaration of free and informed consent. The Health and Functionality Study Group is made up of physiotherapists and physicians and all will be accessible to assist in case of any risk of harm to the participant of this study.

Sample Size:

The sample size was calculated using the Gpower software version 3.1.9.2. The overall study objective is to show that association of anodal tDCS and sensory PES will reduce pain intensity by a large percentage as opposed to use of isolated tDCS or PES in individuals with the two SCD genotypes. We assume that sample will be equally randomized to four intervention groups: active tDCS + active PES; active tDCS + Sham PES; Sham tDCS + Active PES; and Sham tDCS + Sham PES. We now proceed with the desired sample size assuming no financial or logistic limitations. To achieve 80% power at 5% Type I error, based on an effect size of 0.35 (75) on the intensity of pain evaluated by the VAS, four intervention groups, two genotypes (HbSS and HbSC), and two repeated measures (pre– and post–intervention). Using these parameters yield to an estimated 64 participants for each genotype, a total n = 128. To reach the maximum sample size, this clinical trial will be publicized through social media ads and posters explaining the purpose and benefits of the study.

Statistical Analysis:

To ensure the impartiality of the results, researchers who did not participate in any stage of evaluation and interventionwill perform the statistical analysis. Descriptive statistic will be used to summarize demographic and clinical sample characteristics. Shapiro-Wilk test will be performed to test normality of the data. Chi-square test will be used to compare frequency distributions and t-test, or Mann-Whitney test will be used to compare means among intervention groups. The main outcome of the study is pain rating measured by VAS. For that, we will run a repeated measure analysis of variance (ANOVA) to evaluate differences among interventions (active tDCS + active PES; active tDCS + Sham PES; Sham

tDCS + Active PES; and Sham tDCS + Sham PES), between genotypes (HbSS and HbSC), and time (pre- and post-intervention). For most of the secondary outcomes (TMS volume map; TMS center of gravity; BDNF systemic level; and TNF systemic level) the repeated measures ANOVA will have the same three factors. Relative EEG power will further include in the ANOVA the factors ROI (frontal, temporal, central, parietal, and occipital) and EEG condition (resting, kinesthetic motor imagery of painful, and kinesthetic motor imagery of non-painful region of the body). All analysis will be controlled for anxiety and depressive symptoms. Bonferroni test will be used to correct for multiple comparisons. An α value of 5% (P <.05) will be used to accept statistically significant differences for all analyses.

STEPWISE PROCEDURES

The procedures of this study protocol will occur on two independent days (see figure 2).

1st Day: On this day, all the enrollment procedures will be done. A trained assistant researcher will visit the reference centers or basic health units to interview face to face a candidate study participant. At this time, complete information on this protocol will be provided and all eligibility criteria will be registered. If the candidate is suitable for the trial and willing to participate, then the informed consent form will be filled ou and the participant allocated to an intervention group. After enrollment, the participant will be called upon to go on another day to the Clinical Electrophysiological Laboratory at Federal University of Bahia to continue the study protocol.

2nd Day: the procedures should begin in the morning and will be divided into 4 stages:

- Stage 1: Sample Characterization
- Stage 2: Baseline assessment
- Stage 3: Intervention
- Stage 4: Post-intervention assessment

POTENTIAL PITFALLS AND COUNTERACTING MEASURES

This study protocol was designed with the main purpose of answering the question "Does tDCS combined with PES have an additive effect in the control of joint osteonecrosis joint pain associated with sickle cell disease?". However, some considerations related to presence of potentials pitfalls should be considered due to their capacity to interfere in the answer to this question.

- Potential pitfall in sample selection

According to the Ficat classification (76), the femoral head osteonecrosis has four radiographic stages of progressive severity. In this study protocol, the population will not be stratified according to the degree of femoral osteonecrosis. However, as mentioned earlier, there is no good association between radiographic examinations and pain intensity. Furthermore, the randomization method used in this protocol study avoids selection biases

and allow that sample will be distributed equally between groups (77). In addition, inclusion criteria of only hip joint osteonecrosis also support to decrease biases risk.

- Potential pitfall in intervention

Regarding the intervention, the stimulation time used in this study protocol differs between neuromodulatory techniques, being 20 min for tDCS and 30 min for PES. However, the choice for a longer time of PES compared to tDCS is based on studies demonstrating that its effects on cortical excitability takes at least 30 min to occur (68). In this way, it is probable that this time difference does not interfere in the synergic mechanism between the techniques.

One of the major challenges of clinical trials is to ensure the quality of the intervention blinding protocol. Studies using neuromodulatory techniques such as tDCS and sensory PES have shown that one of the reliable alternatives for performing blinding is to instruct participants that they may or may not feel stimulation (62, 63, 78). A recent study using tDCS for 30 min showed that the erythema caused in the supra-orbital region ranges from mild to moderate, and may interfere with the study blinding(79). In the present study, participants will not be able to observe themselves during the stimulation procedure and it is expected that they will not be able to recognize any erythema over their frontal region. In addition, it has been previously shown that the gradual application of the stimulation intensity is very effective in blinding study participants(80).

- Potential pitfalls in outcomes measures

Pain intensity

Emotional aspects such as anxiety and depression are commonly related to conditions of chronic pain (81). In addition, a recent study showed that anxiety and depressive symptoms are associated with increased sensitivity to experimental painful stimuli in individuals with SCD (82). In order to control the presence of these potentials confounding factors, the symptoms of anxiety and depression will be evaluated in baseline through the HADS (71).

Systemic level BDNF and TNF

Studies have reported that the circadian rhythm has influence on BDNF levels. A study with healthy subjects evaluated BDNF levels at intervals every 4 hours and identified a continuous decrease in BDNF levels throughout the day (83). Similarly, another laboratory animal study demonstrated that levels of TNF also vary according to the circadian rhythm (84). To control these potential pitfalls, the whole experiment will be carried out during the morning. In addition, recent studies have shown that the presence of the Val66Met BDNF gene polymorphism may influence cortical neuroplastic changes and consequently change the response to tDCS (44, 85). However, the population studied in this protocol is mostly Afrodescendant of Yoruba origin, of which only 0.9% is heterozygous for the Met allele (86). Therefore, it will not be possible to evaluate the influence of the val66met BDNF gene polymorphism on the response of this population to neuromodulatory effect of tDCS associated with PES, because due to the sample size there will not be sufficient

representability to guarantee an accurate comparison.

TMS cortical mapping

Gluteus medius muscle TMS mapping may be difficult because of its small and deep representation in the primary motor cortex. We aim to counteract this barrier through using active motor threshold and contraction during TMS assessment, a method that has been used by others successfully (28, 87). One possible aid would be to use neuronavigated TMS, but this resource is not available in our laboratory. However, the original study to validate TMS cortical mapping of the gluteus medius did not use neuronavigation and showed the methodology is valid (88).

ETHICAL ASPECTS:

Volunteers who are involved in the survey will receive guidance regarding their participation in the research and will sign an Informed Consent Form according to Resolution 466/2012 of the Ministry of Health of Brazil. This study has been approved by the Ethics and Research Committee of the Adventist Institution of Bahia under CAAE: 31237514.1.0000.0042 and registered by the Protocol Registration and Results System (PRS) with trial number NCT02813629. Any possible change in this study protocol will be informed to both the ethics committee and the PRS. A written consent to publication will be requested from all participants in this study and will be filed at the Health and Functionality Study Group and accessible for review by the lead editor.

CRITERIA OF AUTHORSHIP

The authors of this study should pass the following criteria of authorship (89):

1. Contribute substantially to the execution of clinical trial protocol; or the acquisition, analysis, or interpretation of data for this study.

2. Participate in the writing of this manuscript or examine it critically the intellectual content.

CONFLICT OF INTEREST:

The authors of this protocol declare that there is not any conflict of interest.

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FIGURE 1. Flow chart of the study based on CONSORT criteria

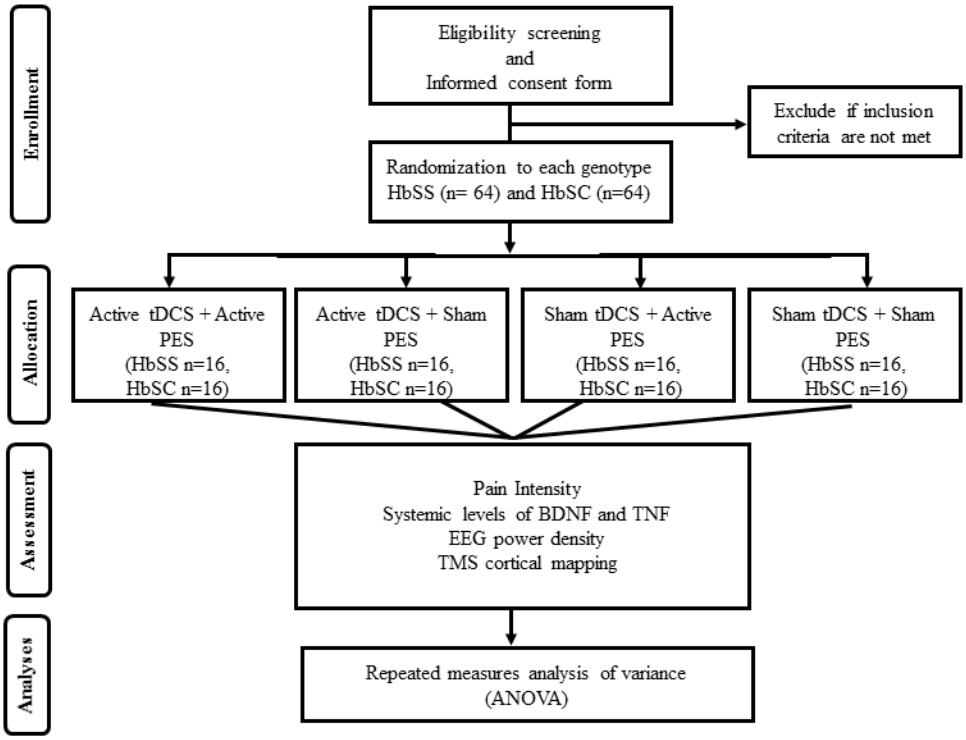
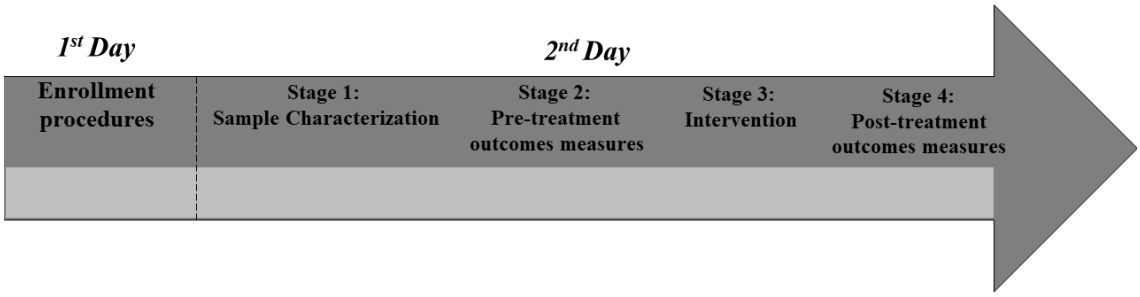


FIGURE 2. Timeline of study procedures



6. DISCUSSÃO

Este protocolo é o primeiro a procurar, por meio da combinação terapêutica de estimulação transcraniana com corrente continua e estimulação elétrica periférica, controlar a dor crônica de indivíduos com doença falciforme (DF). Os resultados deste protocol de ensaio clinico fornecerão os primeiros dados sobre o uso da Neuromodulação não invasiva para o controle da dor nesta condição. Os resultados secundários também oferecerão importantes dados sobre a segurança e tolerabilidade, e possiveis mecanismos de ação via analises eletrofisiológicas, tais como Eletroencefalografia (EEG) e Estimulação Magnética Transcraniana (EMT) e moleculares, tais como, dosagem de Fator neurotrofico derivado do cérebro e Fator de necrose tumoral Alfa.

Esta combinação neuromodulatórias como tratamento para dor crônica já foi testada em outras condições dolorosas, tais como dor lombar crônica. Os efeitos se demonstraram promissores no controle da dor crônica, mas ainda necessita ser replicado em outras amostras.

Uma limitação este protocolo é o fato da amostra não ser estratificada de acordo com o grau de osteonecrose femoral e por isso não será possível mensurar a possível interferência desta variável nos desfechos do estudo. Entretanto, a estratificação da amostra de acordo com o grau de ostenecrose femoral leva a um aumento no número de subgrupos e tamanho amostral. Os autores deste estudo escolheram estratificar a amostra por localização da osteonecrose (isto é, femoral) porque isto possibilita uma amostra mais abrangente e consequentemente facilita o processo de inscrição e recrutamento dos participantes. De todo modo, estas limitações serão controladas ao máximo por todos os pesquisadores envolvidos na execução.

7. CONCLUSÕES E CONSIDERAÇÕES FINAIS

A revisão narrativa mostrou que é possível haver diversas alterações mal adaptativas no SNC de indivíduos com DF. Além disso, alguns métodos de avaliação neuromusculoesqueléticas, tais como, modulação condicionada da dor, inventario de sensibilização central, teste sensorial quantitativo, *checklist* de características clinicas relacionadas a sensibilização central, método de taxa de ativação central tem grande potencial para ajudar o profissional de saúde a avaliar pacientes com DF de uma maneira mais precisa e consequentemente oferecer um tratamento mais adequado.

O protocolo de ensaio clinico, apresenta uma combinação terapêutica (ETCC mais EEP) que tem um grande potencial de suporte aos profissionais de saúde que buscam meios para um melhor manejo a dor articular crônica de indivíduos com DF. Além disso, os resultados deste protocolo fornecerão um entendimento mais claro sobre os efeitos neuromodulatórios desta combinação terapêutica sobre a representação cortical do musculo glúteo médio, oscilações corticais cerebrais e níveis sistêmicos de BDNF e TNF que muito possivelmente podem estar alterados nesta população.

ANEXOS

-ANEXO A – QUESTIONARIOS DE TRIAGEM INICIAL

Identificação do Participante do Estudo

Nome:_____

Sexo: ( ) M ( ) F Idade:_____ Data de Nascimento:__/__/_____

Endereço:_____

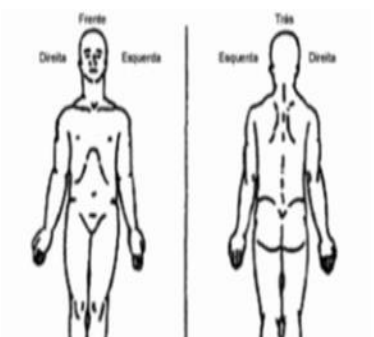
Escolaridade:_____ Profissão:_____ Etnia:_____

Lateralidade:_____ Número de Telefone:_____

CRITERIOS DE ELIGIBILIDADE

- Genótipo: ( )HbSS ( )HbSC
- Há quanto tempo sente dor? _____
- Quadril mais doloroso?

Assinale um X no lugar onde sente dor?



( ) Direito ( ) esquerdo

( ) Ambos os lados

- Fez cirurgia no quadril?

  ( ) Sim ( ) Não

- Lado da cirurgia?

  ( ) Direito ( )Esquerdo

  ( ) Ambos os lados

- Data da cirurgia ___/___/___

Questionário de segurança para uso da estimulação magnética transcraniana (EMT) e estimulação transcraniana com corrente constante (ETCC)

Com sinceridade, responda os itens a baixo:	SIM	NÃO
Você possui histórico de trauma encefálico?		
Você possui histórico de episódio epiléptico pessoal?		
Você possui histórico de episódio epiléptico familiar?		
Você possui algum implante coclear, marca-passo cardíaco ou implante metálico no crânio/encéfalo?		
Você utiliza algum tipo de medicamento para depressão ou convulsão?		
Você está em período gestacional?		
Você está tomando algum tipo de remédio que modifique o funcionamento neural (tais como, antidepressivos e anticonvulsivantes)		

Questionário de diagnóstico de dor neuropática – DN4

A sua dor tem alguma das seguintes características?	SIM	NÃO
Queimação		
Frio doloroso		
Choque elétrico		
Há presença de um ou mais dos seguintes sintomas na mesma área da sua dor?		

Formigamento		
Alfinetada ou agulhada		
Adormecimento		
Coceira		
A dor está localizada em uma área onde o exame físico pode revelar um ou mais das seguintes caraterísticas?		
Diminuição da sensibilidade ao toque		
Diminuição da sensibilidade à picada de agulha		
Na área dolorosa, a dor pode ser causada ou aumentada por:		
Escovação		

CARACTERIZAÇÃO DE DADOS CLINICOS DOS PARTICIPANTES

Medicações em uso continuo:

Nome do medicamento:_____Dose:_____

Uso de medicamento no dia do tratamento:

Nome do medicamento:_____Dose:_____

Horário de ingestão:_____

INTENSIDADE DA DOR

Momento de avaliação	Pontuação EVA
Pré Tratamento (Repouso)	
Pré tratamento (Abertura do quadril)	
Pós Tratamento (Repouso)	
Pós tratamento (Abertura do quadril)	

ESCALA HOSPITALAR DE ANSIEDADE E DEPRESSÃO

A1)Eu me sinto tenso ou contraído: 3( )A maior parte do tempo

2( )Boa parte do tempo
1( )De vez em quando
0( )Nunca

D2)Eu ainda sinto gosto pelas mesmas coisas de antes
0( )Sim, do mesmo jeito que antes
1( )Não tanto quanto antes
2( )Só um pouco
3( )Já não sinto mais prazer em nada

A3)Eu sinto uma espécie de medo, como se alguma coisa ruim fosse acontecer:
3( )Sim, e de um jeito muito forte
2( )Sim, mas não tão forte
1( )Um pouco, mas isso não me preocupa
0( )Não sinto nada disso

D4)Dou risada e me divirto quando vejo coisas engraçadas:
0( )Do mesmo jeito que antes
1( )Atualmente um pouco menos
2( )Atualmente bem menos
3( )Não consigo mais

A5)Estou com a cabeça cheia de preocupações:
3( )A maior parte do tempo
2( )Boa parte do tempo
1( )De vez em quando
0( )Raramente

D6)Eu me sinto alegre:
3( )Nunca
2( )Poucas vezes
1( )Muitas vezes
0( )A maior parte do tempo

A7)Consigo ficar sentado à vontade e me sentir relaxado:
0( )Sim, quase sempre
1( )Muitas vezes
2( )Poucas vezes
3( )Nunca

D8)Eu estou lento para pensar e fazer as coisas:
3( )Quase sempre
2( )Muitas vezes
1( )De vez em quando
0( )Nunca

A9)Eu tenho uma sensação ruim de medo, como um frio na barriga ou um aperto no estômago:
0( )Nunca
1( )De vez em quando
2( )Muitas vezes
3( )Quase sempre

D10)Eu perdi o interesse em cuidar da minha aparência:
3( )Completamente
2( )Não estou mais me cuidando como deveria
1( )Talvez não tanto quanto antes
0( )Me cuido do mesmo jeito que antes

A11)Eu me sinto inquieto, como se não pudesse ficar parado em lugar nenhum:
3( )Sim, demais
2( )Bastante
1( )Um pouco
0( )Não me sinto assim

D12)Fico esperando animado as coisas boas que estão por vir:
0( )Do mesmo jeito que antes
1( )Um pouco menos do que antes
2( )Bem menos que antes
3( )Quase nunca

A13)De repente, tenho a sensação de entrar em pânico:
3( )A quase todo momento
2( )Várias vezes
1( )De vez em quando
0( )Não sinto isso

D14)Consigo sentir prazer quando assisto a um bom programa de televisão, de rádio ou quando leio alguma coisa:
0( )Quase sempre
1( )Várias vezes
2( )Poucas vezes
3( )Quase nunca

INDICE DE INCAPACIDADE RELACIONADA A DOR

1. Responsabilidades familiares/domesticas:

0 1 2 3 4 5 6 7 8 9 10

Sem Incapacidade — Incapacidade Total

2. Tempo de Lazer

0 1 2 3 4 5 6 7 8 9 10

Sem Incapacidade — Incapacidade Total

3. Atividades Sociais

0 1 2 3 4 5 6 7 8 9 10

Sem Incapacidade — Incapacidade Total

4. Ocupação

0 1 2 3 4 5 6 7 8 9 10

Sem Incapacidade — Incapacidade Total

5. Comportamento Sexual

0 1 2 3 4 5 6 7 8 9 10

Sem Incapacidade — Incapacidade Total

6. Cuidados Pessoais

0 1 2 3 4 5 6 7 8 9 10

Sem Incapacidade										Incapacidade Total

7. Atividades Vitais

0 1 2 3 4 5 6 7 8 9 10

Sem Incapacidade										Incapacidade Total

ANEXO B - QUESTIONÁRIO DE AVALIAÇÃO DO CEGAMENTO

Por favor responda às questões abaixo da melhor forma possível:

1- Você recebeu uma estimulação:

( ) ativa na cabeça e ativa na região dolorosa

( ) ativa na cabeça e simulada na região dolorosa

( ) simulada na cabeça e ativa n na região dolorosa

( ) simulada na cabeça e simulada na região dolorosa

2- Com quanta certeza você responde a estas perguntas?

Nenhuma Certeza — Certeza total

0 1 2 3 4 5 6 7 8 9 10

3- Como você avalia o resultado do tratamento?

1. Muito pior ( )

2. Levemente pior ( )

3. Nada mudou ( )

4. Levemente melhor ( )

5. Muito melhor ( )

ANEXO C - QUESTIONÁRIO DE EFEITOS ADVERSOS:

Sessão: _____

Você sentiu alguma das reações abaixo?	Classifique o que sentiu de 0 a 3: 0-ausente; 1-pouco; 2-médio; 3-muito	Se alguma reação apareceu, ela foi devida ao tratamento? 0 – não 1 – dificilmente 2 – possivelmente 3 – provavelmente 4 – com certeza	Notas
ETCC			
Dor de cabeça			
Dor no pescoço			
Dor no couro cabeludo			
Queimaduras			
Coceira abaixo do eletrodo			
Vermelhidão na pele			
Formigamento			
Sonolência			
Dificuldades em se concentrar			
Mudanças agudas no humor			
Outras (especificar)			
ESTIMULAÇÃO			

ELÉTRICA NA REGIÃO DOLOROSA			
Vermelhidão na pele			
Coceira abaixo do eletrodo			
Cansaço muscular			
Dor muscular			
Outro tipo de dor			
Tremor muscular			
Inchaço			

ANEXO D - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

TÍTULO DO PROJETO

Efeitos da neuromodulação no controle da dor crônica e sua influência nas medidas neurofisiológicas e moleculares em indivíduos com doença falciforme

PESQUISADOR RESPONSÁVEL:

Prof. Dr. Wellington dos Santos Silva.

Endereço: Rod. BR 101, Km 197 – Capoeiruçu – Cachoeira, BA – Caixa Postal 18

CEP 44300-000 – Tel. (75) 3425 8157 – 9194 4868

ENDEREÇO DO COMITÊ DE ÉTICA DA FADBA

Endereço: Rod. BR 101, Km 197 – Capoeiruçu – Cachoeira, BA – Caixa Postal 18

CEP 44300-000 – Tel. (75) 3425 8096

PESQUISADORES PARTICIPANTES:

Profa. Ms. Sanzia Bezerra Ribeiro.

Ft. Tiago da Silva Lopes.

Você está sendo convidado (a) para participar como voluntario (a) de um estudo que tem como objetivos analisar os efeitos da técnica de Estimulação Transcraniana com Corrente Continua (ETCC) no alívio da dor e os aspectos bioquímicos e moleculares da dor em

pacientes portadores de doenças falciformes. Os procedimentos que serão realizados neste estudo são:

Triagem sociodemográfica e clínica: Nesta etapa, você receberá uma ficha para ser preenchida com informações pessoais do tipo: nome, idade, sexo, profissão, escolaridade. Na mesma ficha também haverá espaço para preenchimentos de dados clínicos, como: tipo de genótipo, lado do quadril que dói mais, nome de remédio ingerido para dor, quanto tempo sente dor no quadril e características de sensibilização central.

Serão apresentados a você alguns instrumentos específicos para avaliação da dor e suas repercussões na vida diária. Primeiramente você deverá identificar a dor que você sente através do instrumento Escala Visual Analógica (EVA) e do mapa corporal. Em seguida responderá aos seguintes questionários: O Índice de incapacidade relacionada à Dor (PDI), Questionário específico para rastreio de dor neuropática – DN4, Escala de ansiedade e depressão hospitalar (HAD) e Questionário de imagética visual e cinestésica (KVIQ). Para responder a esses questionários serão necessários aproximadamente 30 minutos. Estes instrumentos serão aplicados por avaliadores treinados na aplicação de instrumentos avaliativos de dor. O material será arquivado pelos pesquisadores por um período de (05) cinco anos, sendo posteriormente incinerado.

Avaliação eletrofisiológica:

Registro eletroencefalográfico: Para esta avaliação será colocado na sua cabeça eletrodos, eles ficaram ajustados na cabeça com ajuda de uma pasta. Esta pasta é apropriada para esta avaliação e é dermatologicamente testada para evitar alergias. Em seguida você deverá ficar sentado (a) confortavelmente em uma cadeira, com os olhos fechados durante 4 minutos.

Depois você ouvirá um áudio pedindo para fazer alguns movimentos com a mão e depois com o quadril. Serão colocados eletrodos para captação da atividade muscular no musculo lateral a sua “nádega”, chamado glúteo médio, e no musculo do seu braço, chamado flexor superficial dos dedos. Estes movimentos deveram ser realizados sentado em uma cadeira e de olhos fechados. A duração dessa atividade guiada pelo áudio deverá durar 2 minutos. Esta avaliação será realizada antes e depois do tratamento.

Estimulação Magnética Transcraniana: Primeiro pediremos para que você responda sinceramente as perguntas do questionário de segurança para o uso desta técnica. As respostas honestas afastam os riscos decorrentes desta avaliação. Em seguida limparemos a sua pele com álcool e uma pasta especial e colocaremos dois eletrodos autoadesivos no músculo da sua "nádega" chamado de glúteo máximo, do lado onde você sente mais dor no quadril.

Se você for homem e possuir muito pelo talvez necessitemos raspar a região com um barbeador descartável. Estes eletrodos só irão captar a atividade elétrica do seu músculo. Em seguida aplicaremos uma série de pulsos magnéticos no seu crânio, na área que controla o músculo da "nádega". Ele irá contrair a cada pulso e com isso poderemos compreender como esta conexão funciona. Para se submeter a essa avaliação existem algumas condições que você precisa conhecer:

- Você não deve ter tido em nenhum momento da sua vida convulsões ou epilepsia. Em mais de 250 estudos feitos no mundo, só houve um caso de crise convulsiva provocada por uma técnica parecida, mas com mais riscos do que esta que usamos. Isto aconteceu porque a pessoa possuía uma doença chamada de Doença Bipolar, tinha história de epilepsia na família e estava usando remédios que podem ter facilitado à convulsão. Entretanto, para evitar qualquer risco, não fazemos esta avaliação em quem é epiléptico ou já teve convulsões;

- Você não pode estar grávida, pois não sabemos o que pode acontecer com o bebê que está na sua barriga;
- Você não pode possuir nenhum metal implantado no seu corpo. Isto inclui o uso de marca passos, aparelhos no cérebro, metais no crânio ou na coluna vertebral, fios metálicos implantados, implantes dentro da orelha (implante coclear) e outros que possam ter estas mesmas características. Sugerimos também que durante o exame sejam retirados relógios, óculos, anéis, joias ou quaisquer outros metais que possam ser magnetizados.
- Você não deve usar o seu telefone celular ou portar cartões de crédito no momento da avaliação, pois eles podem se desmagnetizar;
- Você não deve estar usando remédios que interfiram com a atividade elétrica do seu cérebro, incluindo remédios para epilepsia e para algumas doenças mentais incluindo depressão, doença bipolar e esquizofrenia.
- Já foram registrados alguns efeitos indesejáveis deste exame, como desmaios, dor na cabeça, pescoço ou dentes e alterações no estado de humor. Estes efeitos são

extremamente raros e você deve nos informar se eles acontecerem, para que possamos tomar todas as medidas necessárias.

- A máquina de estimulação emite um barulho a cada pulso, que pode ser incômodo e/ou aumentar a sua audição após o exame. Para prevenir estes desconfortos, pediremos que você use um protetor na orelha durante o exame.

Coleta de sangue para análise laboratorial: Nesta etapa você deverá permitir a coleta de sangue para que possamos proceder à realização de exames laboratoriais. Existem alguns riscos de contaminação e desconfortos durante a coleta. Para evitar esses riscos, a coleta será feita com material descartável e por um técnico capacitado. Porém, você estará livre para escolher desistir do processo a qualquer momento de acordo com sua vontade. O DNA extraído das amostras de sangue ficará armazenado em freezer no Laboratório de Genética da Faculdade Adventista de Fisioterapia em microtubos com código de identificação. Existe a possibilidade de este material ser utilizado em pesquisas futuras, porém isto só será feito com sua autorização e a aprovação do projeto por um Comitê de Ética registrado na Faculdade Adventista da Bahia.

Tratamento com estimulação elétrica para controlar a dor: Após as etapas de avaliação, terá início o tratamento utilizando a técnica de estimulação na cabeça associada com estimulação no quadril onde você mais sentir dor. Esta técnica é feita com dois eletrodos que serão colocados em sua cabeça e dois no seu quadril, os quais emitiram uma corrente de estimulação muito fraca.

Em alguns trabalhos realizados anteriormente, algumas poucas pessoas apresentaram sensação de formigamento e/ou ardor no local abaixo dos eletrodos, coceira, fadiga, leve sensação de dor sob os eletrodos. É possível que você também possa sentir algum destas sensações, no entanto, nenhuma destas é grave e todas são de duração curta. O uso de conta gotas será utilizado para umedecer com água as esponjas dos eletrodos. Você ficará será sentado (a) em uma poltrona confortável com o intuito de diminuir o desconforto gerado pelo tempo de duração da técnica.

Em caso de eventuais micros lesões dermatológicas teremos à disposição uma pomada para auxiliar no tratamento deste micro lesão. Para prevenir qualquer um destes efeitos, todos os pesquisadores e auxiliares receberam um treinamento sobre a operacionalidade do

aparelho. Mesmo assim, caso você venha a sentir algum desconforto, a estimulação será imediatamente interrompida.

A aplicação da estimulação ocorrerá em apenas 1 dia com avaliação dos resultados após a sessão. Após a estimulação, você será orientado (a) para não fazer nenhum esforço mental (ex. Leitura e cálculos matemáticos). Ao final do tratamento, você responderá um questionário relacionado à sua percepção sobre o resultado do tratamento. Também serão respondidas questões sobre a segurança e efetividade da técnica.

Como benefício de sua participação nesta pesquisa, você será informado (a) sobre os resultados laboratoriais do seu exame de sangue e orientação sobre seu estado de saúde. Serão preservados a privacidade e o anonimato, bem como o direito de aceitar ou recusar a participação nesta pesquisa e poder desistir ou anular este consentimento em qualquer fase da pesquisa. Os resultados serão publicados em artigos científicos. Não haverá qualquer despesa pela sua participação voluntaria neste estudo. Qualquer dúvida ou desistência a respeito da pesquisa você poderá entrar em contato com os pesquisadores responsáveis.

Eu, _____

Entendo que, qualquer informação obtida sobre mim, será confidencial.

Eu também entendo que meus registros de pesquisa estão disponíveis para revisão dos pesquisadores.

Esclareceram-me que minha identidade não será revelada em nenhuma publicação desta pesquisa; por conseguinte, consinto na publicação para propósitos científicos.

Eu entendo que estou livre para recusar minha participação neste estudo ou para desistir a qualquer momento e que minha decisão não afetará adversamente meu tratamento.

Eu certifico que li ou foi lido para mim o texto de consentimento e entendi seu conteúdo. Uma cópia deste formulário ser-me-á fornecida. Minha assinatura demonstra que concordei livremente em participar deste estudo.

Assinatura do participante da pesquisa: _____.

Data: ___/___/___.

Eu, _____ certifico que expliquei o (a) Sr (a) _____, acima a natureza, propósito, benefícios e possíveis riscos associados à sua participação nesta pesquisa, que respondi todos as questões que me foram feitas e testemunhei a assinatura acima

---

Assinatura do pesquisador responsável

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Eletroterapia no Controle da Dor Crônica e Análise de Marcadores Neurofisiológicos em Indivíduos com Doenças Falciformes.

Pesquisador: Wellington dos Santos Silva

Área Temática:

Versão: 7

CAAE: 31237514.1.0000.0042

Instituição Proponente: FACULDADES ADVENTISTAS DA BAHIA

Patrocinador Principal: FACULDADES ADVENTISTAS DA BAHIA
Fundação de Amparo a Pesquisa do Estado da Bahia - FAPESB

DADOS DO PARECER

Número do Parecer: 2.175.645

Apresentação do Projeto:

O projeto intitulado "Eletroterapia no Controle da Dor Crônica e Análise de Marcadores Neurofisiológicos em Indivíduos com Doenças Falciformes" se refere há protocolo já analisado e aprovado por este comitê que se propõe a realizar um estudo do tipo Ensaio Clinico Randomizado duplo cego no qual utilizará uma intervenção através da técnica de estimulação transcraniana com corrente contínua associada à estimulação periférica
(ETCC/EP). A amostra será composta por 120 indivíduos de ambos os sexos, escolhidos de forma sequencial entre os pacientes com DF cadastrados nas Unidades Básicas de Saúde dos municípios que fazem parte da 31ª DIRES da Secretaria de Saúde do Estado da Bahia, no centro de referência de atendimento as pessoas com doença falciforme - Feira de Santana, Centro de referencia Carlos Gomes e Vale das Pedrinhas - Salvador, Ambulatório Magalhães Neto - Salvador e por meio de divulgação em redes sociais. 20 indivíduos controles saudáveis pareados por sexo e idade que apenas participarão das medidas eletrofisiológicas também farão parte deste trabalho. Na primeira etapa da pesquisa serão aplicados os seguintes instrumentos de coleta de dados: Ficha de triagem sociodemografica e clinica, Escala Visual Analógica de Dor, Índice de incapacidade relacionada a Dor (Pain Disability Index - PDI), Questionário Específico Para Rastreio de Dor Neuropática – DN4 e Escala de Ansiedade e depressão Hospitalar (HAD). Em seguida serão coletadas amostras de

sangue para a realização de exames laboratoriais e a determinação do perfil eletroforético de cada paciente. Após a determinação do perfil eletroforético, os pacientes serão submetidos ao eletroencefalograma seguindo a classificação internacional 10/10 para colocação dos eletrodos.Em seguida a excitabilidade cortical de todos será avaliada com a estimulação
magnetica transcraniana (EMT).Todos os que realizarem avaliação com EEG e EMT de ambos os grupos seguirão para o tratamento utilizando ETCC com estimulação de 2mA, com eletrodos de 35cm2 sendo o anodo na região C3 do córtex motor e o catodo na região supra orbital
contralateral. A estimulação transcraniana ocorrerá em uma unica sessão de 20 minutos. Para a aplicação de TENS os grupos receberão uma corrente com intensidade de acordo com o limiar sensorial, caracterizado como confortável, frequência de pulso de 100Hz e duração de pulso de 200s com uma aplicação de 30 minutos. Os participantes serão perguntados sobre a sua percepção a cada 5 minutos de intervenção (Hazime et al, 2015). Os eletrodos serão de 35cm² colocados no local de maior intensidade de dor. A dor será reavaliada logo após o termino da intervenção. O grupo sham receberá a mesma montagem, mas somente os 30 segundos iniciais de estimulação (NITSCHE, 2008). Ao final o participante responderá a um questionário para que descreva se a estimulação a que foi submetido é ativa ou simulada e um questionário de efeitos adversos especifico para neuromodulação cerebral não-invasiva associado com estimulação periférica. Ao final do tratamento todos serão submetidos a
responder um questionário avaliando o sigilo de alocação e segurança da técnica. As avaliações de EEG e EMT serão feitas novamente após o tratamento.

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar se a associação de ETCC com EEP tem efeito superior sobre a intensidade da dor de indivíduos com DF comparado ao uso individual das técnicas.

Objetivo Secundário:

Avaliar o efeito de uma única sessão de ETCC associado com EEP sobre variáveis neurofisiológicas tais como: densidade de potência eletroencefalográfica, representação cortical do glúteo médio e níveis sistêmicos de BDNF e TNF alfa. Avaliar se o tipo de genótipo, HBSS ou HBSC, é associado com a resposta terapêutica.

Avaliação dos Riscos e Benefícios:

Há uma análise de riscos e benefícios adequada elaborada pelos pesquisadores que aponta as medidas necessárias para a minimização dos riscos e as medidas a serem tomadas caso haja alguma improvável lesão cutânea decorrente da estimulação proposta. Os benefícios apontados

superam os riscos minimizados pelas ações propostas pelos pesquisadores.

Comentários e Considerações sobre a Pesquisa:

A pesquisa proposta é relevante e encontra-se devidamente fundamentada bibliograficamente e com metodologia proposta adequada aos objetivos expostos. A emenda apresentada amplia a amostra de 80 para 120 participantes, altera objetivos primários e secundários o que permitiu a retirada de um tipo de teste que seria utilizado anteriormente (a genotipagem do polimorfismo Val66Met do gene BDNF).

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos necessários à apreciação de acordo com a Res. CNS 466/12 foram apresentados devidamente preenchidos, atualizados e assinados.

Conclusões ou Pendências e Lista de Inadequações:

Considerando os pareceres anteriores a este projeto de pesquisa e que as alterações ora propostas não alteram a relação risco/benefícios, bem como não implicam em nenhuma repercussão ética segundo as diretrizes da Res. CNS 466/12, recomendo que esta emenda seja aprovada.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_957338E3.pdf	06/07/2017 16:40:40		Aceito
Projeto Detalhado / Brochura Investigador	Projeto_detalhado.docx	06/07/2017 16:27:26	Wellington dos Santos Silva	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_NOVO.docx	29/01/2017 14:12:40	Tiago da Silva Lopes	Aceito
Declaração de Instituição e Infraestrutura	Anuencia_LEF.bmp	10/08/2016 18:14:47	Tiago da Silva Lopes	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_CONTROLE.docx	04/08/2016 10:59:20	Tiago da Silva Lopes	Aceito
Folha de Rosto	Folha de Rosto.PDF	20/05/2015 17:51:51		Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CACHOEIRA, 17 de Julho de 2017

Assinado por:
Wilma Raquel B. Ribeiro
(Coordenador)