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Electroacupuncture and acupuncture in the treatment of anxiety - A double blindered randomized parallel clinical trial

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ABSTRACT

Background: The estimated number of people living with anxiety disorders worldwide is around 264 million and is estimated to have worsened with the recent pandemic of COVID-19. Acupuncture has shown to have excellent therapeutic effects in reducing anxiety.

Design: Double-blinded randomized controlled clinical trial with 56 participants (21-82 years) with anxiety diagnosed by 3 different anxiety scales (BAI, GAD-7 and OASIS). A 30-min acupuncture session was applied once a week for 10 weeks.

Aims: Evaluate the effectiveness of acupuncture and electroacupuncture in the treatment of anxiety to verify if: (1) People with high anxiety report reduced scores after 5 and 10 sessions; (2) Salivary cortisol levels accompanied the reduced scores; (3) Electroacupuncture treatment is more effective than acupuncture; (4) the treatments is independent of anxiolytic medication.

Methods: Volunteers were randomized into 3 groups (control, acupuncture, and electroacupuncture). The results were analyzed by anxiety scales and salivary cortisol tests.

Results: The findings show an improvement in anxiety, assessed by BAI, GAD-7 and OASIS, after the 5th session of acupuncture (p < 0.05) and electroacupuncture (p < 0.05) and the 10th session for both techniques (p < 0.001). The salivary cortisol values measured in the morning followed this pattern (p < 0.05), although the reduction of the night cortisol values was not statistically significant. Electroacupuncture and acupuncture show similar efficacy. The positive effect after the treatments is independent of anxiolytic medication (p < 0.001).

Conclusion: Acupuncture and electroacupuncture are effective in treating anxiety on their own or as adjuncts to pharmacological therapy.

Trial registration number: NPI445-08/2017 (Unidade de Investigação em Ciências da Saúde);

1. Introduction

Anxiety disorders are the 6th leading cause of disability for productive activity, accounting in 2015, for 3.4% of all years lived with disability, or DALYs (disability-adjusted life-years, the total years of life lost and the number of years lived with disability) [1]. According to the latest WHO World Mental Health Survey, in 2015, the proportion of the global population with anxiety disorders was estimated at 3.6% being more common among women (4.6% compared to 2.6% in men). The estimated number of people living with anxiety disorders globally is approximately 264 million, representing an increase of 14.9% (from 2005 to 2015) as a result of population growth and aging and is

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estimated to have worsened worldwide with the recent pandemic of COVID-19 [2–4]. Anxiety is the most common mental disorder in Portugal with an estimated population of 16.5% suffering from this condition in 2010–2011, the last registered study carried out in the country [5]. In the DMS-5 (Diagnostic and Statistical Manual of Mental Disorders, 5th Edition) a mental disorder is classified as a syndrome characterized by clinically significant disturbances in an individual’s cognition, emotion regulation, or behavior that reflects a dysfunction in the psychological, biological, or developmental processes underlying mental functioning [6]. The terminology “Anxiety Disorders” is a general term that encompasses various conditions such as panic disorder, social anxiety disorder, separation anxiety, phobias, selective mutism, substance use-induced anxiety, anxiety associated with a medical condition, and generalized anxiety disorder [6]. Anxiety refers to a powerlessness feeling, a conflict characterized by neurophysiological processes existing between the person and the threatening environment. Anxiety arises to the extent that the individual, faced with a certain situation or event, cannot cope with the demands of his environment and feels as if his existence or the values he considers essential are being threatened [7,8]. Mackenzie [9] adds that anxiety is composed of emotional and physiological factors. Physiologically, the human body’s acute stress reaction system is activated, with the “fight or flight” response speeding up the heart rate, dilating the bronchi and constricting the blood vessels, to increase blood flow and oxygenation to the muscles to prepare the person to flee/fight a certain threat. It is an important mechanism in terms of response to the environment but can lead to symptoms such as: insomnia, tachycardia, pallor, sweating, muscle tension, tremor, dizziness, nervousness, difficulty concentrating, bowel disorders and epigastric discomfort, and changes in social interactions. Regarding the emotional state, the individual can manifest several sensations, such as: fear, insecurity feelings, apprehensive anticipation, catastrophic thinking, and increased alertness [9–12]. Anxiety can impact mentally (attention, learning, cognitive processes, work capacity), physically (blood pressure, migraines, skin conditions, reduced immune system activity) and even at a sociocultural level by influencing alcohol consumption, decision making, negative post-event rumination, rest or eating habits [13]. In Western medicine the use of pharmacological therapies has been considered as the first-line of treatment for this ailment [14]. There are, however, some disadvantages and limitations to a purely pharmacological treatment, such as low efficacy, side effects, slow response to the drug (after only 6–12 weeks), and a high percentage of recurrence [15]. Psychotherapy is another option for the treatment of anxiety and is effective because it eliminates the root of the problem and allows the individual to resume life more safely, however, it is usually a long-term solution. It has been shown that more and more patients prefer complementary and alternative medicines (CAM) such as relaxation techniques, nutritional supplements, massage, and acupuncture to avoid the long-term side effects of medications [16]. Errington-Evans [17] demonstrated the effectiveness of acupuncture in the treatment of chronic anxiety unresponsive to conventional treatments, including medication. The attempt to provide effective responses in the treatment of anxiety and depression has led recent research to the application of therapies such as acupuncture as an adjunct and as the first line of treatment of signs, symptoms and even diseases. Acupuncture is an ancient energy-based technique of traditional Chinese medicine. The technique aims to redirect and harmonize the flow of energy along 14 major energy channels called meridians. The technique consists of inserting an extremely thin needle into the skin and muscles. Currently, it is known that acupuncture has profound effects at all levels of the nervous system, from the peripheral nerves, spinal cord to the brain, namely the limbic system, hypothalamus and pituitary gland, as well as the cerebral cortex [19,20]. From the literature review we identified about 300 articles on human clinical trials using the keywords “acupuncture” and “anxiety” and using the filters “clinical trials” and “human”. The number of studies on the application of acupuncture and electroacupuncture for the treatment of anxiety is still scarce.

There are studies that seem to indicate a positive effect of acupuncture on anxiety with fewer side effects compared to drug therapy [21,22]. However, the great heterogeneity of methods, small experimental groups or poor experimental design does not allow to infer any further conclusions [22]. Another limitation is the use of different anxiety scales that, despite being a valid tool, end up being relatively subjective, with few studies using measurable physiological indicators. In fact, Yang et al. [23] reported a study which verified a greater effect on reducing the anxiety symptoms after acupuncture in the group that used the self-assessment anxiety scale (SAS) than in the groups that used the HAMA scale, suggesting a subjective assessment by the patients. In fact, some studies show that there may be a significant “underdiagnosis”, with 50% of people not being identified with anxiety disorders [24]. In Yang’s study, it was also found that regarding the duration of the therapy, acupuncture has a rapid effect, achieving positive responses within the first 6 weeks of treatment, relieving the effects of anxiety faster than other anti-anxiety interventions that usually only show long-term effects.

Biological markers have been detected that may help demonstrate how acupuncture can regulate anxiety, such as: by altering the prefrontal cortex activity, the plasma corticosteroid level, the adrenocorticotrophic hormone level, and the platelet 5-HT level [15]. These changes occur through activation of the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS), with effects that are especially evident in symptoms of chronic anxiety [25]. The use of biological markers is an attempt to approach the diagnosis of mental disorders in a more objective way. These biomarkers can be detected in saliva, blood (plasma), the cerebrospinal fluid and by neuroimaging. Salivary tests are the most popular ones because they are quick, inexpensive, and non-invasive. Saliva has many components that can be identified by different biochemical techniques such as immunological tests. The use of saliva for the detection of markers must be taken with care as the salivary composition can vary depending on many factors like the circadian rhythms, gender, age, diet, tobacco usage and medication. The literature shows a relationship between anxiety and the levels of molecules like cortisol, immunoglobulin A, lysozyme, melanin, alpha-amylose, chromogranin A, and fibroblast growth factor 2 in saliva [26].

Cortisol is a hormone produced by the adrenal cortex commonly used as a biomarker of stress. When a person is exposed to physical stress, the adrenal glands produce increasing amounts of cortisol. Cortisol activates the metabolism to supply the body with energy (for example, by releasing glucose into the bloodstream to enable the body to cope with a stressful situation) and interferes with the Central Nervous System and our mental state by enhancing the action of adrenaline and noradrenaline [26]. Previous studies have successfully correlated anxiety levels with cortisol values, showing that salivary cortisol was an effective biomarker to test anxiety [27,28].

The goal of this study is to evaluate the effectiveness of acupuncture and electroacupuncture in the treatment of anxiety through a score system to determine the anxiety levels by the application of the anxiety scales BAI (Beck Anxiety Inventory) [29], GAD-7 (Generalized Anxiety Disorder questionnaire of 7 items) [30] and OASIS (Overall Anxiety Severity and Impairment Scale) [31] as well as through the measurement of salivary cortisol values. With this study it is expected to find out if: (1) People with high anxiety reported reduced scores after 5 and 10 acupuncture treatments; (2) Salivary cortisol levels follow the reduced scores in the 2 study groups; (3) Electroacupuncture treatment is more
effective than acupuncture alone; (4) A positive effect after 10 acupuncture treatments is independent of anxiolytic medication.

2. Methods

2.1. Design

This was a randomized controlled double-blind study for the experimental groups, performed with a control group in parallel. This clinical study was reviewed and approved by the ethics committee of the Portuguese Health Sciences Research Unit (No. P445-08/2017) and authorized by the national Data Protection Commission (Authorization No. 10300/2017). The study was conducted at the premises of a properly equipped clinic. Participants could voluntarily withdraw from the study at any time or if indicated by the researcher in charge in the case of dangerously high levels of anxiety were detected and were unresponsive to the therapy, in which case they would be referred to the specialist in the area. The control group was offered 10 therapy sessions for their participation in the study, and there was no cost involved for the volunteers, who were offered travel by public transport.

2.2. Participants

2.2.1. Recruitment

Recruitment was carried out by invitation using two strategies: online dissemination through advertisements and on the clinic’s website. Individuals interested in participating in the study could access a link in the form to submit their participation and participation details, benefits, and risks. Those who were interested signed an informed consent, and to ensure each participant’s anonymity they were assigned a random number after submission of the form.

2.2.2. Eligibility and exclusion criteria

Inclusion Criteria: Participants over 18 years of age of both genders with a diagnosis of anxiety validated by 3 scales, having at least 2 scales with the following values (BAI, value greater than 8/63 points; GAD-7 value greater than 5/21 points; and OASIS value greater than 10/20 points).

Exclusion criteria included: (1) People who have previously experienced electroacupuncture (2) People who had received acupuncture treatment in the past 3 months (3) People living with psychiatric disorders on the spectrum of schizophrenia and other psychotic disorders, bipolar disorder, obsessive-compulsive disorder substance-related disorders, and addictive disorders (4) People living with any unstable psychiatric condition or physical illnesses deemed by the researcher to be serious and unsuitable or unsafe to participate in the study (5) People living with valvular heart defects bleeding disorders or those medicated with anticoagulants (6) People carrying any implanted electrical device such as pacemaker, implantable cardioverter defibrillator (ICD), brain or spinal cord neurostimulator (7) People with infection or abscess located at any of the selected acupuncture points and which in the opinion of the acupuncture researcher was unsafe (8) Pregnant woman.

2.2.3. Groups

The volunteers who met the inclusion criteria were distributed in three groups (acupuncture, electroacupuncture or control) through a table of random numbers and given a code number to guarantee anonymity. Everyone was informed of the importance to adhere to the complete study: 10 consecutive weekly treatment sessions lasting 30 min each, saliva collection and questionnaire completion before the first, after the fifth and after the tenth treatment sessions.

The sample was stratified with regard to gender, and anxiety level, so that all groups had the same representation of men and women, and similar anxiety levels. The groups were further homogeneously distributed about whether they took anxiolytic and/or antidepressant medication.

The sample was then randomly distributed into 3 study groups: (1) Control group or group 1: group with participants only monitored for salivary cortisol level, and anxiety scales, without any acupuncture intervention; (2) Acupuncture group or group 2: group consisting of participants treated with acupuncture according to the established protocol; (3) Electroacupuncture group or group 3: group consisting of participants treated with electroacupuncture at specific points according to the defined protocol. Although people were equally distributed in the 3 groups, there were dropouts. Therefore, we obtained 23 participants in the acupuncture group, 20 in the electroacupuncture group, and 13 in the control group (Fig. 1).

2.3. Procedure and intervention

In the first session, the researcher responsible for the randomization received the participant’s informed consent and handed out a data collection instrument (digital questionnaire) to be filled out with the measurement scales. The first salivary cortisol collection was performed the day before the first session. Participants were told that two people would enter the room for the session and that the person who would start the treatment would be different from the person who would finish it, without ever mentioning what kind of treatment they would have or how they would feel about it. It was also explained how to proceed when entering the treatment room: undress and lie down in dorsal decubitus and to cover themselves with the available sheet.

The experimental groups were randomly distributed by rooms (4 rooms). Each room had similar decoration and preparation, with equal brightness and temperature control and equipped to receive any of the 2 experimental groups. The acupuncture researcher was initially allocated to a room and subsequently rotated between rooms according to directions from the randomization researcher. Two researchers were allocated to each room, and they were never in the same room simultaneously. The first researcher assigned to the room was the researcher responsible for the puncturing protocol and connecting the needles to the electrodes of the electroacupuncture machine, leaving the room without ever knowing which treatment was performed next. Communication between the puncture researcher and the subjects was restricted, and the person was only told that the treatment was going to start. The second researcher entered the room and turned on the electroacupuncture machine or pretended to turn it on and left it off, as indicated by the researcher responsible for randomization. The second researcher was also responsible for removing the needles and putting the equipment away at the end of the session.

Communication between the participant and the researchers was restricted and kept to a minimum level: at the beginning of the session to escort him/her into treatment room and to inform that the treatment was going to start; at the end of the session to inform the participant that the treatment had finished and to escort him/her out of the session.

In the 2nd to 4th and 6th to 9th sessions, the participant was simply told which room to go to. On the day before the 1st session (T0), and the day after the 5th (T5) and 10th (T10) sessions, the digital questionnaire with anxiety scales was administered, and salivary cortisol was collected in the morning and evening.

Both the participants and the puncturing researchers who participated in the treatment were blinded to the type of therapy applied (acupuncture or electroacupuncture).

Each session lasted 30 min, once a week (Friday or Saturday, preferably on the same day, at the same time) for 10 weeks. Each 30-min session had a maximum time of 15 min for needle application and 30 min for the remaining therapy, totaling 60 min (30 min session + 15 min for needle application and removal + 15 min of formal processes).

2.3.1. Acupuncture and electroacupuncture protocol

After disinfecting the puncture area with alcohol, the needles were
placed in the acupuncture points in the following order: Yintang (Ex-HN-3), Sanyinjiao (SP-6) on the right, Zusanli (ST-36) on the left, Hegu (L.I.-4) on the right, Taichong (LIV-3) on the right, Neiguan (P-6) bilateral, Shenmen (HE-7) bilateral, Danzhong (Ren-17), Baihui (Du-20), and the auricular points, She Men, Antidepressant 1, Heart, Master Cerebral. For electroacupuncture, electrodes were connected at the Yintang (Ex-HN–3) and Baihui (Du-20) points, She Men and Antidepressant 1 points, and Heart and Brain Master points. Direct current was used at a 2 Hz frequency, 250 μs, for 30 min. The needles used were disposable stainless-steel needles with size 0.25 × 30mm, and 0.20 × 15mm for the auricular points.

2.3.2. Data collection and processing

Each participant was initially scheduled on an agreed time and day of treatment so that he/she would always perform the treatments at the same time as well as the cortisol collections. Care was also taken to leave adequate time between sessions so that volunteers would not cross paths and share experiences. The data collection instruments consisted of: 1) Primary online questionnaire for randomization; 2) Log grid with salivary cortisol values for the 6 evaluations performed; 3) Digital questionnaire with the anxiety assessment scales BAI (Beck Anxiety Inventory), GAD-7 (Generalized Anxiety Disorder Assessment), and OASIS (Overall Anxiety Severity and Impairment Scale).

2.3.3. Salivary cortisol collection

Patients were provided with instructions and appropriate material for saliva collection. The collection was done at home, always at the same time, and then frozen upon arrival at the facility. The instructions given to the volunteers were as follows: (1) avoid physical exertion for 3 h before collecting the sample; (2) The participant should not eat, drink, chew gum, or brush their teeth in the 30 min prior to collecting the saliva sample; (3) Inform the researchers if they were taking any medication; (4) Not to collect samples in case of oral diseases, inflammation or injuries, or recent tooth extraction to avoid contaminating the samples with blood; (5) Not to use any substance that would increase saliva production when collecting the sample; (6) To rinse/wash the mouth, drink half a glass of water before collecting the sample and wait for 5 min (7) Before starting the collection, hands should be washed with soap and water and use a clean towel to dry the hands; (8) Start the 1st saliva collection 30 min after waking up using the container provided by the researchers; (9) Accumulate small amounts of saliva in the mouth and transfer to the collection container, collecting at least 2.5 mL of

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Fig. 1. Flow chart of the stages of the double-blinded parallel-group randomized clinical trial.
saliva. (10) Place the samples in the refrigerator (2–8 °C) until delivered to the researchers on the agreed date. (11) Record the time of sample collection on the label of the collection container; (12) Perform the 2nd saliva collection 12 h after the first collection; (13) Notify the researchers if they deviated from any of these instructions.

2.3.4. Salivary cortisol measurement

Salivary cortisol concentration was analyzed with ELISA (range 0.005–3 μg/dl, IBL International Cortisol Saliva ELISA, RES2611, Hamburg, Germany) according to the manufacturer’s procedure. Samples were tested in duplicates and the mean coefficient of variation was <10%. The samples obtained from one subject were analyzed in one batch to avoid inter-run variation.

2.3.5. Statistical analysis

The data collected were processed and analyzed by the Statistical Package for the Social Science (SPSS), Windows version 27.

In order to check the consistency of the 3 scales used to assess anxiety levels, their reliability was verified. It was verified that Cronbach’s Alpha values for the scales were excellent: 21-item BAI was 0.917; 7-item GAD was 0.932; 5-item OASIS was 0.911.

Next, the same test was applied to the total values between the same scales, for the 3 measurements in the 56 cases analyzed. Cronbach’s Alpha value was acceptable in the first measurement (0.601) and excellent in the following evaluations (0.834 and 0.861, respectively in the second and third measurement) [32].

The difference in means between the experimental group and the control group at the initial evaluation was analyzed. It was found that only in the case of the BAI test there was a statistically significant difference between the study groups (p < 0.05). Since with the remaining scales and cortisol tests there was no such difference, it was assumed that there was sufficient consistency to test the effectiveness of the treatment, given that several measures would be used. Regarding data analysis, a Mann-Whitney U test, a one-way ANOVA and a MANOVA using F of Wilks’ Lambda for the longitudinal analysis were performed [33,34].

3. Results

In total, 56 persons participated in the study, of which 14.29% were male and 85.71% were female. The control group was composed of 13 participants (23.21%), 23 participants received acupuncture treatment (41.07%), while 20 participants (35.71%) received electroacupuncture. Of the 56 participants, 33 (58.93%) were taking medication for anxiety.

3.1. Anxiety scales results

The results obtained for the three anxiety scales used are presented in Fig. 2. As can be seen, for all scales there is a marked decrease in the anxiety scores as soon as after the fifth treatment (T5) for both acupuncture and electroacupuncture. The decrease is less marked from the fifth to the tenth session (T10), however, it continues to decrease. Regarding the control group the anxiety level remains stable or continues to increase over time. All results have statistical significance. The longitudinal study between groups along time showed a p-value < 0.0001 for all scales applied. These results validate the prediction that people with high anxiety would report improvement after 5 and 10 acupuncture treatments.

3.2. Analysis of salivary cortisol results

The analysis of the salivary cortisol levels is presented in Tables 1 and 2. The number of participants analyzed for the salivary cortisol was lower than those for the anxiety scales as there were samples that were not viable for analysis. Of the samples obtained from the acupuncture group (n = 23), three samples had to be disregarded at T5 timepoint (n = 20) and 2 samples at T10 (initially with n = 20) as they were not viable as well (n = 18). Regarding the electroacupuncture group (n = 20), at the T5 timepoint 3 samples were not viable and therefore not considered for the analysis. Relative to the T10 timepoint analysis (n = 18), two samples were disregarded. Table 1 shows that there is a clear statistical difference between morning (M) and night (N) cortisol levels for all groups, as expected, with night cortisol levels being substantially lower (about 10 times) than the morning levels, for all groups.

In Table 2, both morning and night salivary cortisol mean values for all groups are presented. When analyzing the values trend for each group, it seems that the general tendency is the decrease of the salivary cortisol levels along time. For the morning cortisol mean value, a MANOVA analysis using F of Wilks’ Lambda test allowed to verify that these values are statistically significant (p < 0.05) for the acupuncture and the electroacupuncture groups, but not for the control group (p = 0.259). This outcome accompanies the anxiety surveys effect lowering tendency which showed decreased anxiety within each own group along time, with a more pronounced decrease after the 5th treatment of electroacupuncture and after the 10th treatment of acupuncture.

Regarding the night cortisol mean values trend for each group, it was observed that the decrease along time was not significant (p > 0.05).

**Fig. 2.** Longitudinal study with the anxiety scales BAI, GAD-7 and OASIS.
Graphic for the BAI (Beck Anxiety Inventory) [29], GAD-7 (Generalized Anxiety Disorder questionnaire of 7 items) [30] and OASIS (Overall Anxiety Severity and Impairment Scale) [31] anxiety scales at T0 (before any treatment), T5 (after 5 treatments) and T10 (after 10 treatments).
To find out whether taking medication influences the effect of acupuncture, the data was further analyzed, comparing the subgroups of people taking anxiolytic medication with those who were not medicated, through a Mann-Whitney U test (Table 3). The possible impact of the medication on the variation of morning cortisol was assessed and no significant differences were found when analysing the results along the different time points adjusted by group \((p > 0.05)\). In respect to the anxiety scales (BAI, GAD-7 and OASIS) globally, the results were also not verified to be statistically different. It seems that both acupuncture and electroacupuncture are effective whether patients are taking anxiolytic medication or not.

### 4. Discussion

Regarding the results obtained from the anxiety surveys a clear reduction of the patients’ anxiety levels has been verified. This effect is greater from the first to the fifth session (T0 – T5) for both acupuncture and electroacupuncture and the scores continue to lower until T10 in contrast with the control group which even seems to have a slight increase of anxiety levels, regarding all scales. This is in accordance with the results obtained by Yang’s study \([23]\), that found that regarding the duration of the therapy, acupuncture has a rapid effect, achieving positive effects within the first 6 weeks of treatment.

The use of three different anxiety scales in our study, recognized as valid tools, end up being relatively objective due the correlated scores in-between scales, confirming, and validating each other and the obtained results, overcoming any doubts of a subjective evaluation.

Regarding the salivary cortisol levels, no statistically significant results could be found when comparing values between different groups, only when comparing the results within the same group, along time. This is expected as cortisol is a biological biomarker presenting a high variability between individuals, therefore the variation along time within the same group is more reliable and logical to compare. The variations in the morning cortisol levels show the same general tendency for all groups, with higher variations for the acupuncture group at the T0-T5 time frame and for the electroacupuncture group in the T0-T5 time frame.

Regarding the night salivary cortisol, the results are more difficult to compare, as the night collection with the “N” letter. The p-value was calculated using the T of Wilcoxon test.

## Table 1

| Differences between morning (M) and night (N) samples of salivary cortisol levels for all groups. |
|-----------------------------------|-------|-------|-----------------|-----------------|
| **Groups**                        | **N** | **Mean** | **Std. Deviation** | **Average Difference** | **p-value** |
| Acupuncture                       |       |         |                  |                   |            |
| T0 Cortisol – 1 M                 | 20    | 0.681   | 0.405            | 0.619             | <0.00001   |
| T0 Cortisol – 1 N                 | 20    | 0.062   | 0.088            |                   |            |
| T5 Cortisol – 2 M                 | 20    | 0.529   | 0.369            | 0.465             | <0.00001   |
| T5 Cortisol – 2 N                 | 20    | 0.064   | 0.036            |                   |            |
| T10 Cortisol – 3 M                | 18    | 0.400   | 0.250            | 0.352             | <0.00001   |
| T10 Cortisol – 3 N                | 18    | 0.048   | 0.039            |                   |            |
| Electroacupuncture                |       |         |                  |                   |            |
| T0 Cortisol – 1 M                 | 17    | 0.643   | 0.395            | 0.562             | <0.00001   |
| T0 Cortisol – 1 N                 | 17    | 0.081   | 0.075            |                   |            |
| T5 Cortisol – 2 M                 | 17    | 0.402   | 0.152            | 0.337             | <0.00001   |
| T5 Cortisol – 2 N                 | 17    | 0.065   | 0.085            |                   |            |
| T10 Cortisol – 3 M                | 16    | 0.518   | 0.330            | 0.454             | <0.00001   |
| T10 Cortisol – 3 N                | 16    | 0.060   | 0.023            |                   |            |
| Control                           |       |         |                  |                   |            |
| T0 Cortisol – 1 M                 | 13    | 0.614   | 0.243            | 0.552             | 0.001      |
| T0 Cortisol – 1 N                 | 13    | 0.061   | 0.022            |                   |            |
| T5 Cortisol – 2 M                 | 13    | 0.463   | 0.247            | 0.406             | 0.001      |
| T5 Cortisol – 2 N                 | 13    | 0.057   | 0.027            |                   |            |
| T10 Cortisol – 3 M                | 13    | 0.493   | 0.211            | 0.433             | 0.002      |
| T10 Cortisol – 3 N                | 13    | 0.060   | 0.023            |                   |            |

Salivary cortisol results for the three groups before treatment (T0) after the 5th treatment (T5) and the 10th treatment (T10). Morning cortisol samples are defined by the “M” letter and the night collection with the “N” letter. The p-value was calculated using the T of Wilcoxon test.

## Table 2

| Variation of the absolute values for morning and night salivary cortisol during time for each group. |
|-----------------------------------------------|-------|-------|-----------------|-----------------|
| **Groups**                                    | **N** | **Mean** | **Std. Deviation** | **Average Difference** | **p-value** |
| Cortisol – T0M                                |       | 0.689   | 0.658            | 0.614             |            |
| Cortisol – T5M                                |       | 0.392   | 0.402            | 0.243             |            |
| Cortisol – T10M                               |       | 0.552   | 0.403            | 0.463             |            |
| Cortisol – T0N                                |       | 0.379   | 0.157            | 0.247             |            |
| Cortisol – T5N                                |       | 0.400   | 0.518            | 0.493             |            |
| Cortisol – T10N                               |       | 0.250   | 0.230            | 0.211             |            |
| Cortisol – T0M                                |       | 0.066   | 0.084            | 0.061             |            |
| Cortisol – T5M                                |       | 0.092   | 0.076            | 0.022             |            |
| Cortisol – T10M                               |       | 0.064   | 0.067            | 0.057             |            |
| Cortisol – T0N                                |       | 0.037   | 0.087            | 0.027             |            |
| Cortisol – T5N                                |       | 0.048   | 0.064            | 0.060             |            |

Morning (M) salivary cortisol data at T0M (Morning cortisol before any treatment), at T5M (Morning cortisol after the 5th treatment) and at T10M (Morning cortisol after the 10th treatment) and Night (N) salivary cortisol data at T0N (Night cortisol before any treatment), at T5N (Night cortisol after the 5th treatment) and at T10N (Night cortisol after the 10th treatment). For Morning cortisol MANOVA with F of Wilk’s Lambda test (W-L) showed for the acupuncture group along time: W-L = 6.072; p-value = 0.011; Eta^2_partial = 0.431; Electroacupuncture group along time: W-L = 4.323; p-value = 0.035; Eta^2_partial = 0.382 and control group along time: W-L = 1.534; p-value = 0.259; Eta^2_partial = 0.218.

For Night cortisol MANOVA with F of Wilk’s Lambda test (W-L) showed for the acupuncture group along time: W-L = 10.06; p-value = 0.388; Eta^2_partial = 0.112; Electroacupuncture group along time: W-L = 7.55; p-value = 0.488; Eta^2_partial = 0.097 and control group along time: W-L = 0.074; p-value = 0.929; Eta^2_partial = 0.013.

### 3.3. Analysis of the impact of anxiolytic medication on results

Electroacupuncture are effective whether patients are taking anxiolytic medication.
The main conclusion of this work is that both acupuncture and electroacupuncture have a similar effectiveness on reducing the anxiety levels and that only 5 sessions are needed to reduce anxiety to a lower degree, however, until the 10th session the anxiety levels continue to decrease.

**Ethical approval**

The present study was approved by the ethics committee of the Portuguese Health Sciences Research Unit (No. P445-08/2017) and authorized by the national Data Protection Commission (Authorization No. 10300/2017).

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No.

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**Author’s contribution**

Diogo Amorim: participated in the project design, project administration, intervention, supervision, writing original draft; data gathering, data analysis, results interpretation, writing review & editing, Irma Brito: participated in the project design, supervision, data gathering, data analysis, results interpretation, writing review & editing, Armando Caseiro: participated in the project design, methodology, data creation, data analysis and results interpretation and writing review & editing, João Paulo Figueiredo: participated in the data creation, data analysis and results interpretation, review & editing, André Pinto: participated in the intervention and methodology, Inês Macedo: participated in the intervention, methodology and review & editing, Jorge Machado: participated in the project design, supervision, results interpretation, review & editing.

**Declarations of competing interest**

No.

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**Table 3**

|                      | Acupuncture + Electroacupuncture |                      |
|----------------------|----------------------------------|----------------------|
|                      | Without anxiolytic medication    | With anxiolytic medication |
|                      | N  Mean  Std. Deviation | p-value   | N  Mean  Std. Deviation |
| BAI T5               | 19  9.63  6.56                |           | 24  11.33  8.40            | 0.641 |
| T10                  | 18  4.72  3.16                |           | 20  9.95  7.25            | 0.007 |
| GAD-7 T5             | 19  5.84  5.06                |           | 24  5.50  4.10            | 0.902 |
| T10                  | 18  3.61  3.36                |           | 20  4.60  3.98            | 0.470 |
| OASIS T5             | 18  4.53  3.26                |           | 24  5.79  3.20            | 0.093 |
| T10                  | 18  3.28  2.40                |           | 20  4.30  3.08            | 0.408 |
| Cortisol T5          | 18 .509 .356                  |           | 19 .434 .224              | 0.466 |
| T10<sup>M</sup>      | 17 .404 .285                  |           | 17 .507 .299              | 0.326 |

Anxiety results obtained with the anxiety surveys BAI (Beck Anxiety Inventory) [29], GAD-7 (Generalized Anxiety Disorder questionnaire of 7 items) [30] and OASIS (Overall Anxiety Severity and Impairment Scale) [31] anxiety scales and morning cortisol salivary levels at T5 (after 5 treatments) and T10 (after 10 treatments). T5<sup>M</sup> (Morning cortisol after the 5th treatment) and at T10<sup>M</sup> (Morning cortisol after the 10th treatment); the p-value was calculated using a Mann-Whitney U test.
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